PROCEEDINGS
Seventh Meeting of Agricultural Scientists

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Réduit, Mauritius, 4 - 6 May 2005

Organised by

The Food and Agricultural Research Council (FARC)

in collaboration with

The Agricultural Research and Extension Unit (AREU)
The Agricultural Services, Ministry of Agriculture,
Food Technology and Natural Resources
The Albion Fisheries Research Center (AFRC)
The Faculties of Agriculture and Science,
University of Mauritius (UOM)
The Mauritius Sugar Industry Research Institute (MSIRI)

Sponsored by

THE FOOD AND AGRICULTURAL RESEARCH COUNCIL

Edited by

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September 2005
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Agricultural Research and Extension Unit (AREU)
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The Agricultural Research and Extension Unit functions under the aegis of the Food and Agricultural Research Council as from July 1995. The main objective of AREU is to serve its clients through excellence in cost-effective high quality research and extension and to meet the policy requirements of government. AREU has responsibility for livestock and all crops excluding sugarcane.

Agricultural Services, Ministry of Agriculture, Food Technology and Natural Resources
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Telephone (230) 454 1018 Fax (230) 464 8749

The Agricultural Services of the Ministry of Agriculture, Food Technology and Natural Resources started life as the Department of Agriculture in 1913 itself taking over from the Station Agronomique created in 1893. It is the regulatory body of the Ministry and provides a number of services to the agricultural community.

Albion Fisheries Research Centre (AFRC)
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The objectives of the Albion Fisheries Research Centre are to carry out research and development activities with a view to increasing knowledge on fishery resources within the fishing limits of Mauritius and to provide a basis for their sustainable development and management.

Food and Agricultural Research Council (FARC)
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The Food and Agricultural Research Council was created in 1985. Its main objective is to promote, harmonise and co-ordinate research activities in agriculture, fisheries, forestry and food production in line with government policy and to ensure that the farming community draws the maximum benefits from such research.

Mauritius Sugar Industry Research Institute (MSIRI)
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The Mauritius Sugar Industry Research Institute is a statutory body created in 1953 with mandate to promote by means of research and investigation the technical progress of the sugar industry. It also carries out research on foodcrops that are grown in association with sugarcane.

University of Mauritius (UOM)
Réduit Mauritius
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The University of Mauritius was founded in 1965. While training remains one of its important mandates, it also focuses on research in diverse areas which include agriculture and allied subjects.

CIRAD
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## TABLE OF CONTENTS 2005

<table>
<thead>
<tr>
<th>Participating Institutions</th>
<th>Page no</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>v</td>
</tr>
</tbody>
</table>

| Foreword                                                                 | x       |

<table>
<thead>
<tr>
<th>Opening Session</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcoming address by Dr T Bahorun. Chairman FARC.</td>
<td>xi</td>
</tr>
</tbody>
</table>

| Address by Honourable Nandcoomar Bodha Minister of Agriculture, Food Technology and Natural Resources. | xiii    |

| Hommage à Claude Michel by Mr Yvan Martial                                      | xvii    |

| The Claude Michel Memorial lecture Keynote Address: Our Maritime Zone – Revisiting this source of food. By Dr Mitrasen Bhikajee. | xix     |

### Session 1 – Chairperson Dr J Ramkissoon

- A survey of the Mauritian endemic flora for potential prophylaxis.
  *Neergheen VS*, *Bahorun T*, *Soobrattee MA* and *Aruoma OI.*
  1

- Characterization of the phenolic profile of endemic Mauritian Chassalia species and assessment of their antioxidant activities. *Soobrattee MA*, *Bahorun T* and *Thaunoo P.*
  13

- Mauritian exotic fruits and vegetables: antioxidant and pro-oxidant propencies of their prophylactic agents. *Luximon-Ramma A*, *Bahorun T*, *Crozier A* and *Aruoma OI.*
  23

- Spectroscopic quantitative analysis of food: Chemometrics is a vital tool. *Ramasami P* and *Jhaumeer-Lauloo S.*
  35

- Infrared spectroscopy: An analytical tool in food science. *Jhaumeer-Lauloo S* and *Ramasami P.*
  43

### Session 2 – Chairperson Professor A Gurib-Fakim

- Biosensor as a tool for monitoring the status of fruits and vegetables. *Jawaheer S*, *White SF* and *Cullen DC.*
  51

- Quality systems in the food sector in Mauritius. *Mungloo Z* and *Khodabocus F.*
  59

- Innovation en matière de salaison de venaison en milieu tropical. *Santchurn SJ*, *Collignan A*, *Petit T* et *Trystram G.*
  65

### Session 3 – Chairperson Dr Jean Claude Autrey

- Determination of genetic variation among some anthurium cut-flower cultivars. *Nowbuth P*, *Khittoo G*, *Bahorun T* and *Venkatasamy S.*
  71

- Application of microsatellite markers to the sugar cane breeding programme. *Joomun N*, *Parmessur Y* and *Dookun-Saumontally A.*
  77

- *In-vitro* mutation studies of Taro (*Colocasia esculenta* var. *esculenta*) in Mauritius. *Seetohul S* and *Puchooa D.*
  87

- *In-vitro* and molecular studies in *Asparagus officinalis.* *Bojnauth G*, *Puchooa D* and *Bahorun T.*
  95

Session 4 – Chairperson Dr Claude Soopramanien

Mapping the action processes involved in the management of information by farmers – a case of the small-scale cattle keepers in a village in Mauritius. Naidoo G.

Information Technology as a tool to improve the utilisation and management of sugarcane germplasm at the MSIRI. Mundil D, Ramdoyal K, Rivet L, Ng See Cheong FM and Chintaram E.

A Geographical marketing information system for potato. Neeliah H.

Session 5 – Chairperson Mr J Li Yuen Fong

Agronomic performance and tuber characteristics and quality of newly-released local potato clone Belle Isle. Govinden N, Wong Yen Cheong K and Kanhye H.

The efficiency of transplanting sugarcane seedlings directly in the field and its impact on the selection cycle and resources. Mungur H, Ramdoyal K and Santchurn D.

Options for raising radiation use efficiency in the superhumid zone of Mauritius. Koonjah SS and Nayamuth AR.

Microbial bioferilisers: a source of nitrogen for sugarcane in Mauritius? Umrit G and Ng Kee Kwong KF.

Evaluation and potential of biological nitrogen fixation by French bean (Phaseolus vulgaris cv Long Tom). Sunassee S.

Session 6 – Chairperson Mr S Ramtohul

Effects of two commercially available composts on soil properties, and yield and mineral content of bean (Phaseolus vulgaris). Lalljee B.

The development of a Drought-Tolerant maize variety for Rodrigues. Govinden N.

The impact of natural pollinator, Apis mellifera Latreille on onion seed production in Mauritius. Bhunnoo MA and Abeeluck D.

Effect of wind-protection on agronomic performance of banana cv Petite naine. Jhurree-Dussoruth B.

Session 7 – Chairperson Dr C Ricaud

Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (Lactuca sativa var. Salina). Poinapen D, Beeharry GK, Bahorun T, Bunwaree M and Préfumo S.

Early growth and Phyllochron of eight Palm Species at six sites in a Tropical environment. Govinden N and D’Espagnac L.

Phenological, fruit and chemotaxonomic characterization of litchi cultivars in mauritius: preliminary findings. Madhou M, Bahorun T and Ramhurn N.

Agrofotestry - a potential system to poverty alleviation: The case of Calliandra calothyrsus on the slopes of mount Kilimanjaro, Tanzania. Lyamchai CJ and Kingamkono M.

Characterisation of the Creole cattle in Mauritius. Lam Sheung Yuen R.

**Session 8 – Chairperson Dr A Suddhoo**

Agricultural diversification under changing land use: Modelling the Riviere Des Anguilles catchment. *Le Roux JJ, Sumner PD and Rughooputh SDDV.*
Water budget estimation for water basins of Mauritius. *Hossenbux AS, Khayrattee HA, Boojhawon R and Rughooputh SDDV.*
Delineation of Major Drainage Basins of Mauritius. *Nigel R, Rughooputh SDDV and Nathire F.*

**Session 9 – Chairperson Dr R Lutchmeah**

A farmer-participatory approach for the implementation of a developed Integrated Pest Management System against *Plutella xylostella* in crucifer cultivation in Mauritius. *Dunhawooor C and Abeeluck D.*
Biological control of the spiralling whitefly, *Aleurodicus dispersus.* *Gungah B, Seewooruthun SI, Nundloll P and Rambhunjun M.*
Cypress aphid status in Mauritius and trial releases of *Pauesia juniperorum* (Hymenoptera: Braconidae), a promising biocontrol agent. *Alleck M, Seewooruthun SI and Ramlugun D.*
Assessment on the population of *Cryptophlebia Peltastica* Meyrick (Lepidoptera: Tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. *Manrakhan A, Abeeluck D, Gokool A, Rawananshah T and Dobee B.*
Assessment of attractants for fruit fly (Diptera: Tephritidae) management. *Seewooruthun SI, Permalloo S and Sookar P.*

**Session 10 – Chairperson Mr S Naidu**

Field evaluation of synthetic sex attractants of the tomato fruit worm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in Mauritius. *Unmole L and Abeeluck D.*
Systemic and contact effects of some plant extracts on the serpentine leafminer, *L. trifolii,* in potatoes. *Facknath S.*
Phytophthora blight of cucurbits and capsicum in Mauritius. *Vally V and Beni Madhu SP.*
Phenotypic diversity of *Xanthomonas* strains isolated from Onion blight using metabolic fingerprinting. *Nowbuth P, Khittoo G and Venkatasamy S.*

**POSTERS**

Recent agronomic achievements on tobacco research in Mauritius. *Cadersa Y.*
Evaluation of a ration for ruminants using locally available by-products. *Saraye G.*
Heifer live weight and reproductive performance on smallholder farms. *Toolsee P and Saraye G.*
Supporting goats livestock production in réunion island: which product for which market? *Fontaine O, Spalletta V and Choisis JP.*
A socioeconomic, consumer and ecological study of the use of *Pandanus utilis* leaves in Mauritius. *Mangar E and Chellum J.*
Economics of food labelling: a Mauritian perspective. *Neeliah H.*
Control strategies of the melon fly *Bactrocera cucurbitae* (coquillett) (diptera: tephritidae). *Sookar P, Seewooruthun SI and Khayrattee FB.*
Improving the quality of horticulture project. *APEXHOM / NRI.*
Pineapple: effect of different sucker weight on crop cycle length and yield. *Bhugaloo RA.*

**Author and Subject Index**

MAS 2005. Food and Agricultural Research Council, Réduit, Mauritius. ix
FOREWORD

(Under Construction)
WELCOMING ADDRESS

Dr T Bahorun

Chairman, FARC

Honourable Minister of Agriculture, Food Technology and Natural Resources
Excellencies of the Diplomatic Corps
Chairman, Moka-Flacq District Council
The Senior Chief Executive of the Ministry of Agriculture, Food Technology and Natural Resources
Dr. Bhikajee Director of the Mauritius Oceanography Institute
Mr Yvan Martial
Members of the Michel family
Colleagues Scientists
Ladies and Gentlemen

May I on behalf of the Food and Agricultural Research Council and myself, welcome you all to this 7th meeting of Agricultural Scientists. As you may be aware, this meeting would have been difficult to organize without the precious collaboration of the Mauritius Sugar Industry Research Institute and the participation of the Agricultural Research and Extension Unit, the Agricultural Services of the Ministry of Agriculture and the University of Mauritius.

I would like to first extend our apologies for the last-minute inability of Professor Alan Crozier to participate. As a matter of fact, Professor Crozier could not make it to Mauritius because of an extremely serious setback which happened at the last minute concerning his son’s illness. I’m sure, you will all join me to wish him well in these difficult times.

Distinguished guests, as many of you may be aware, this year’s meeting coincides with the 20th anniversary of the Council and I am therefore taking this opportunity to briefly review some of the events that have marked the history of this relatively new institution. Although many had felt that there was perhaps an absence of coordination of research in agriculture, it was in 1983 that the need for a coordinating body for Agricultural Research was expressed in the White Paper on Agricultural Diversification. FARC was thus established by the FARC Act 49 of 1985 with the responsibility of coordinating, promoting and harmonising Agricultural and Food Research.

The first task that the Council undertook during 1986-1987, was to identify research priorities and projects for funding. This was achieved through wide consultation with stakeholders. Between 1989 and 1994, FARC developed its physical infrastructure with recruitment of its core staff and the setting up of its administrative block, together with a conditioning unit for technology transfer aimed mainly towards the utilisation of tissue culture for the introduction of new plant species and varieties.

A National Biotechnology Strategy was defined in 1992 with the collaboration of the World Bank and the ISNAR (International System for National Agricultural Research). One major outcome of FARC’s implication in biotechnology-related activities was the introduction, in the years 1990-1994, of tissue culture disease free ginger and new anthurium varieties, which solved the cyclical problem of ginger unavailability and facilitating the introduction of new varieties in the anthurium industry. This was immediately followed by a project on banana after cyclone Holland in 1994, to the benefit of the planter community. Following this, plantlets and new varieties have been made available at a highly subsidised price.

FARC’s Tissue culture Laboratory became fully operational in 1996, with support of EU funding. This strengthened the council’s capacity to support projects already initiated and opened the door for others.

Moreover, through contractual arrangements with some major anthurium growers, FARC was able to produce tissue-cultured plantlets of selected varieties: some 38 000 plantlets have thus been delivered over the past 2 years or so.
As a coordinating body for agricultural research, FARC organised the first meeting of Agricultural Scientists in 1995. This was followed by 5 other conferences in 1997, 1998, 1999, 2001 and 2003. In 1997 the Agricultural Research and Extension Unit (AREU, formerly DARE) was set up within FARC. After initial teething problems, AREU has become today a performing organisation whose services are continuously solicited at all levels to cater for the benefit of the non-sugar agricultural community. The Council is fully aware of the various challenges awaiting the agricultural sector and has consequently been fully supportive of AREU’s initiative to develop a more professional approach to dispense its services.

Moreover, as a research coordinating agency, FARC has been active in several areas and has been designated as the regional focal point for the Technical Centre for Agricultural and Rural Cooperation (CTA) for information dissemination and other activities. FARC has not only been active in various high-powered committees on Agricultural diversification but also as a research promoting agency, has so far been funding biotechnology related projects to the tune of over Rs 11 million.

FARC is currently reviewing its strategic plan for the next 5 years, with focus on R & D planning and evaluation, in light of the government policies described in the Non Sugar Sector Strategic Plan, and new powers recently conferred to the Council in the area of technology transfer, namely in the sector of Hydroponics. In relation to the Hydroponics Village, we have already set up a Steering Committee for a fast-track implementation of the project at Belle Vue Albion to start with.

FARC has recently formalized a series of documents on the status of Food and Agricultural Research in Mauritius, and on research priorities in the field of traditional agriculture and biotechnology for the period extending to 2007. An exercise in the identification of specific projects under priority “filières” and an assessment of our research infrastructure is ongoing. These baseline data are fully available to other research institutions like the University of Mauritius, the upcoming Mauritius Agricultural Biotechnology Institute, and funding bodies as well, in their endeavour to address priorities in the agricultural sector.

There is an urgent need to develop high quality research in the non-sugar sector in Mauritius. In this context I reiterate that there is therefore a pressing necessity for further capacity building initiatives and the setting up of updated research infrastructure if we are to meet the research challenges. Constructive lobbying exercises should be a continuous process to convince policy makers of the importance of research investment and, more particularly, in the generation of motivated young scientists.

As you have noticed while flipping through the book of abstracts, this forum is also providing the opportunity to a large number of students to present their work and to interact with other fellow scientists. This is clearly indicative of the emergence of a pool of potential scientists who undoubtedly would be active participants in the setting up of sustainable research culture in favour of the agricultural operators in Mauritius.

During this three day conference 42 presentations will be made on many important aspects of current research, in areas including biotechnology, biodiversity, natural products, crop and horticulture, food science, pathology and livestock.

My wish is that you make the most of this opportunity from the fruitful deliberations and interactions at this meeting.

I would like to have a special word of thanks to the members of the organising committee, who have worked very hard to prepare a stimulating mix of plenary lecture, oral presentations and poster sessions.

Minister, ladies and gentlemen, on behalf of the organising committee, I thank you for your presence this morning and I have the great pleasure and honour to now invite the Honourable Minister of Agriculture, Food Technology and Natural Resources to make his address and declare this meeting open.
MINISTER’S ADDRESS

Honourable Nandcoomar Bodha

Minister of Agriculture, Food Technology and Natural Resources

Colleague Ministers & Members of the National Assembly
Excellencies of the Diplomatic Corps
Chairman, Moka / Flacq District Council
The Permanent Secretary, Ministry of Agriculture, Food Technology & Natural Resources
Chairman and Director General, Food and Agricultural Research Council
Director, MSIRI
Dr. Bhikajee
Mr Yvan Martial
Members of the Michel family
Distinguished Guests and Participants

(Under Construction)
TRIBUTE TO CLAUDE MICHEL

Mr Yvan Martial
Rédacteur en chef le journal l'Express

CLAUDE MICHEL, UN VULGARISATEUR ET SON CONTRAIRE

L’offre qui m’est faite par M. Jairaj Ramkissoon, le directeur général du Conseil National de la Recherche Alimentaire et Agricole (Food and Agricultural Research Council), de rendre hommage ce matin à Claude Michel est sans conteste la requête la plus inattendue de toute ma carrière journalistique. Pensez-y, demander à moi, Yvan Martial, incapable de donner la liste exacte des différents éléments constituant notre univers physique ou chimique, de prendre la parole en ce haut lieu du savoir et des connaissances scientifiques qu’est votre salle Philippe-Bonâme. Je vous rassure tout de suite. J’ai accepté une aussi surprenante proposition après m’être assuré de quelque chose de fondamental et pour une raison évidente. L’assurance obtenue, qui m’enchardit à me tenir devant vous et d’ouvrir un large bec, est que le FARC veut saluer à travers mon humble personne l’œuvre de vulgarisation scientifique de Claude Michel. La raison que je mets volontiers en avant pour justifier mon outrance est que je lui dois trop et plus particulièrement cette salutaire intuition que les connaissances scientifiques peuvent comporter des surprises aussi agréables que les recherches littéraires, artistiques et historiques, pour ne pas profiter de cette opportunité unique de lui rendre en partie l’hommage qui lui est du, même si je sais mieux que vous combien je suis indigné d’un tel honneur.

Et comme une confusion ne vient jamais seule voila que les hasards de votre programme de travail me font tenir le crâchoir juste après votre éminent invité, notre ministre de l’Agriculture et juste avant l’éminent Dr Bhikajee du Mauritius Oceanography Institute. De celui-ci, je ne vous dirai rien sinon de l’immense plaisir que nous aurons à l’entendre pour peu que je parvienne à mettre un point final à mes élucubrations. De Nando Bodha, je me contenterai de saluer son immense talent littéraire, preuve s’il en faut que culture et agriculture font bon ménage. Vous me permettrez toutefois d’exprimer publiquement ma perplexité, n’ayant jamais compris comment l’éminence auteur du roman paru chez Julliard à Paris Beaux Songes peut avoir un tel crédit et jouir d’une telle confiance de la part de l’auteur du célèbissime « Arrête rêver Kamarade ! »

Le thème de l’hommage que j’essaye de rendre à Claude Michel - à cœur vaillant rien d’impossible, me disait-on dans mon enfance - sera donc: <<Claude Michel éminent vulgarisateur et son contraire>>. Notre regretté ami, que nous pouvons à coup sûr, classer parmi les sages ayant marqué notre existence d’une empreinte indélébile et parmi les disparus irremplaçables, qui hélàs peuplent nos cimetières, a été de son vivant et demeure dans notre souvenir l’exemple par excellence de ce que pouvait être un humaniste de la Renaissance, à la manière d’un Pic de Mirandole, autrement dit un sujet d’élite capable grâce à ses talents innés et de par son travail et ses recherches, de prétendre à une connaissance universelle. Savant émérite Claude Michel le fut chaque jour de sa vie et dans d’innombrables domaines. Il fut un savant abordable à la présence humble et discrète. Savant jusqu’au bout des ongles au point de pousser la condiscendance à nous faire croire que nous étions en mesure d’éclairer sa lanterne. Ce n’est pas à vous que je l’apprendrai quelque chose sur l’étendue du savoir scientifique de notre ami. Ce matin, je veux simplement rendre hommage à ce souci constant qui ne l’a jamais quitté de vouloir partager avec le plus de personnes possible les merveilles et pas seulement scientifiques de son savoir et de ses connaissances générales. Je m’empresse de vous préciser combien ce terme de vulgarisateur se prête si mal à cet homme si cultivé, à la finesse d’esprit exemplaire. Si courtois et poli dans ses relations avec son entourage, qu’il s’agisse de ses pairs des cercles scientifiques que de l’homme de la rue. Prenons le terme « vulgarisateur » dans son sens le plus noble et le plus enrichissant, à savoir le partage du savoir des savants aux profanes et aux non savants qui souvent restent aux frontières du monde de la connaissance faute d’avoir la chance de pouvoir compter sur un Claude Michel pour leur tendre une main secrable et leur permettre d’aller au-delà de leurs possibilités.

Le directeur de l’institut de Maurice déjà nous comblait d’opuscles les uns plus instructifs que les autres et intitulés «la nature et nous». Lire au hasard une de leurs pages agrémentées d’ilustrations

MAS 2005. Food and Agricultural Research Council, Réduit, Mauritius. xvii
simples mais attrayantes, c'est éprouver tout de suite l'envie de lire la suivante et ainsi de suite jusqu'à la dernière page. Il faut ensuite résister à la tentation de ne pas se plonger dans l'opuscule suivant en se disant : Nous ne pouvons pas passer nos journées à lire et à relire les meilleures pages de Claude Michel. Dans ses écrits scientifiques, on retrouve aisément son sourire coutumier, ses multiples attentions amicales, nous invitant à le suivre dans son exploration de l'univers scientifique même si cela peut ressembler à certains d’entre nous à un labyrinthe particulièrement inextricable.

Une des joies de la succession du Dr Philippe Forget à la tête de *L'Express* à été sans contexte la responsabilité de la page Magazine, au sein de laquelle Claude Michel tenait la dragée haute à Marcelle Lagesse, à Axelle Lamusse, à Patrick Barnwell, à M. Padya de la météo de Vacoas et à tant d'autres vulgarisateurs de haut mérite et co-auteurs avec lui d'une des plus belles pages de toute la presse mauricienne. L'éminent conchyliologue qu'est notre ami n'aimait pourtant pas les coquilles ni les perles. Je veux, bien sûr, parler des fautes typographiques qui parsèment si regrettablement même les pages de nos meilleurs journaux. Prévoyant le moment que son allergie à un tel laisser aller, dû, comme vous le savez, aux distractions des protes au bon dos, risquait de priver les lecteurs de *L'Express* de ses précieux « clins d'œil à la science », je choisis la solution la plus simple mais aussi la plus paresseuse, à savoir de lui confier le soin de confier lui même l'ultime épreuve de sa chronique hebdomadaire. Cet arrangement nous valait des entretiens téléphoniques plus fréquents et plus réguliers. Ils me permettaient de lui exprimer mon appréciation de ses innombrables traits d'esprits et de ses allusions à ses connaissances générales, opéra et opérettes comprises. Je comprenais alors qu'il pouvait multiplier autant de fois qu'il le voulait ses clins d'œil si amusants. Mais il avait le sens de la mesure. Une chronique scientifique même de vulgarisation, ne souffre pas de paraître déséquilibrée. Il savait mieux que nous qu'un grain de sel et une pincée de piment agrémentent avantageusement un curry de crevette. Mais trop de sel ou de ces épices si chères à Pierre Poivre et consorts le rend immangeable. Le croyant en moi a toujours trouvé dans la façon si évangélique de Claude Michel d'être le sel de la Terre, l'inspiration à coup sur d'un esprit sain et même d'un Saint-Esprit.

Je consulte à présent la liste de tous les intervenants de cette septième rencontre d'experts en sciences agricoles et je me dis que vous devez imiter Claude Michel dans son œuvre de vulgarisation et de partage de vos connaissances scientifiques. Les jeunes d'aujourd'hui, l'île Maurice de demain ont besoin de la perche scientifique que vous pouvez leur tendre. Soyez Claude Michel bis et vous l'entendrez vous glisser à l'oreille: *bis repetita* placent. Faisons mieux encore. Mobilisons nos forces et nos énergies pour mettre à leur disposition une édition des œuvres complètes de Claude Michel. Nous ne pouvons ériger plus beau monument à sa mémoire.

J’arrive à présent à la partie la plus importante de mon intervention. Vous savez mieux que moi combien votre temps vous est précieux. Je vous remercie de m'avoir si gentiment accordé ces quelques minutes d'attention. Votre indulgence et votre compréhension me sont alléees droit au cœur.
MR CLAUDE MICHEL MEMORIAL LECTURE
KEYNOTE ADDRESS

OUR MARITIME ZONE- REVISITING THIS SOURCE OF FOOD

Dr. M.Bhikajee

Director, Mauritius Oceanography Institute

It gives me great pleasure to be among you today to deliver the Claude Michel Memorial Lecture. This is the way that the Food and Agricultural Research Council has chosen to pay tribute to a scientist of a rare breed, a man of profound knowledge and who was always ready to share this knowledge with everybody. I had the privilege of personally knowing Mr.Claude Michel; his unassuming ways and his humility was so very characteristic of great people.

Claude Michel spent his entire life in the study of biology. His interest ranged from Common plants of Mauritius to our birds. One of his last publications was “Clin d’œil aux plantes” published by the Old Royals Association. Of course this was in addition to his weekly contribution to l’Express.

But most of his scientific work was on marine organisms. Claude Michel published extensively while he was Director of the Mauritius Institute – better known to most people as the Museum. The issue of the Mauritius Institute Bulletin published in May 1976 entitled “Notes on Marine Biology studies in Mauritius” and edited by Claude Michel is a comprehensive bibliographic study of all Marine Biology studies carried out in Mauritius and is an authoritative reference document. The wealth of bibliographic information available on Mauritius in this document is even now unsurpassed by those in any database in the world.

His commitment to work and his eagerness to share his scientific knowledge to non-scientists was rewarded in 1991 by the United Nations Environment Programme. Mr. Claude Michel is the only Mauritian scientist to have received this award.

Mr. Michel graduated in the UK and taught biology for over five years before working at the Mauritius Institute for some 20 years, finishing as Director. After early retirement he worked at the Mauritius Institute of Education as a curriculum developer and lecturer in science, biology and environment. Over the past 20 years he has been writing a weekly contribution for a local newspaper (“L’Express”) on scientific topics, including plant and animal life, as well as the environment. Written in a light tone, the articles aim at interesting nonscientific readers in the topics covered. These articles have played a major role towards promoting environmental awareness in Mauritius. The individuals and organisations who join the prestigious ranks of global laureates have, in their own distinctive manner, influenced the destiny of life on earth as active participating members of the community. These 'heroes' in the front line of global environmental action realise that global issues are in fact rooted in daily life, and that actions in private life ripple out and affect the larger environment. In honouring them the United Nations Environment Programme seeks inspiration from their extraordinary deeds.

Considering the interest of Mr. Claude Michel for marine resources and the interest of this assembly for food production in general, I have chosen, in this Claude Michel Memorial Lecture, to talk to you about the food resources of our Maritime Zone.

Why “revisiting”. Why revisit this source of food? Simply because this source of food has been visited quite often before and up to now, only a limited amount of effort has been put into exploiting our marine resources for food.

But, what has happened recently that we need to revisit our ocean resources? There are a number of reasons:

1. There is an urgency. With globalisation, our traditional crop, sugar, is going through tough times. Our other foreign currency earner, textile, is not in a more enviable position. We
need new pillars for our economy. It is imperative to explore and exploit new resources and marine resources can be the answer.

2. Land is a resource for which there is a high demand but unfortunately the size of our land is finite. With urbanisation and new infrastructure development, the amount of land which could be used for food production is decreasing. Once land resources are fully used, the oceans will become the evident fall-back option for food production.

3. One more thing has changed over time. Technologies are fast evolving.

   (a) By-catches from the fishing industry used to be considered as trash fish and were very often discarded overboard. But the technology now exists for converting any fish (which does not meet human expectations in terms of taste or texture) into different products which are more acceptable to humans from deflavoured fish flour to fish balls, crab sticks and lobster tail. For example, the horse mackerel which exists in large numbers on the Saya de Malha bank and which can be fished using modern industrial methods has never been exploited because it did not meet consumer acceptance in some countries. However, modern processing technology can give a new life to discarded ideas. With new technologies, old weaknesses turn into new opportunities and trash fish will no longer be considered as trash.

   (b) New fish processing equipment are being developed. Fish filleting equipment is not new. But equipment now exist for fishes to be gutted, deboned, skinned, covered in a batter, cooked, frozen and attractively packed. This gives a new dimension to the industry. A fish which was previously unacceptable by the public because of its colour or skin thickness, now comes as a coated fillet in an attractive box.

   (c) The hit or miss techniques of industrial fishing vessels is something of the past. Through satellite imagery, it is now possible to determine areas of high primary productivity and areas of upwelling which would indicate fish aggregation and potential fishing zones. Satellite sensors provide data on ocean colour, temperature, surface roughness and surface slope. The Mauritius Oceanography Institute is working on a project whereby combinations of these data are now providing new ways of locating fishing grounds.

   (d) The marine resources in our maritime zone have so far been difficult to manage because of the presence of poaching vessels in our waters. With the introduction of the vessel monitoring system or VMS by the Ministry of Fisheries, it is now possible to locate all licensed vessels in our maritime zone and thus have a better control over our resources.

   (e) Aquaculture started way back during the French period. The barachois which are more than two centuries old have not changed much. Some of the more recent aquaculture concerns using land-based ponds have had to close down because of high operating costs, decreased profitability and competition with imported products. The seaweed culture industry which at one time appeared to hold some potential for development is presently suffering from a drop in world market price for seaweeds. Traditional aquaculture systems have not performed well in Mauritius. However, the future seems to be better for new technologies like open-sea culture of fishes. Another potential target organism for aquaculture (though not strictly for food) is the pearl oyster. This may prove to be a new and promising business for Mauritius.

4. The United Nations convention on the Law of the Sea of which Mauritius is a signatory has considerably changed the scenery since 1994. As a result of this convention, Mauritius like all coastal states, can claim an area of 200 nautical miles around its coast as its Exclusive Economic Zone or EEZ. The EEZ of Mauritius, thus covers an area of nearly 1.9 million square kilometres – an area which is about one thousand times our land area. In this zone, Mauritius has exclusive rights over all the marine resources, living or non-living. This new domain opens up new possibilities. Apart from migratory species like tuna and billfishes which are found in deeper waters, the Mascarene Plateau and the shallower Soudan Bank, Nazareth Bank and the water around the cargajos Carajos Shoals may hold the potential for further exploitation.
The seafood hub as proposed by Government can very well become the foundation stone for making marine resources become the next pillar of the Mauritian economy. The seafood hub, as the name implies, acts as a base for import, processing and export of value-added products. Once these transformation industries are well established, the logical step will be for Mauritius to exploit its own fish resources instead of importing them.

Through targeted fishing, through the use of new and more efficient fishing methods, through exploitation of non-conventional species, through exploratory fishing, by increased fish production through open-sea cage aquaculture, Mauritius can supply its own fish to the seafood hub and I am confident that the statement of the President of the Republic will become true when he said:

“With such a large exploitable maritime zone, it is quite probable that ocean resources could become one of the pillars of the Mauritian economy”.

Extract of the address of the President of the Republic of Mauritius on the occasion of the opening ceremony of the National Ocean Science Forum of the Mauritius Oceanography Institute. 18 August 2004.
A SURVEY OF THE MAURITIAN ENDEMIC FLORA FOR POTENTIAL PROPHYLAXIS

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ABSTRACT

There has been a resurgence of interest in natural products with a focus on plants as an alternative source of therapeutic compounds. Bioactive constituents from plant sources include plant secondary metabolites like alkaloids, polyphenolics (flavonoids, phenolic acids, anthraquinones) and terpenoids, many of which are pharmacologically active ingredients of commercially available drugs in particular vincristine, vinblastine, atropine and taxol. These phytochemicals have been identified in a number of traditional medicinal plants and they have also been suggested to account for the protective effects of traditional medicine against a number of diseases. The decline in the use of traditional medicinal plants in occidental societies coupled with a limited detailed scientific scrutiny of traditional treatments have prompted the World Health Organisation (WHO) to recommend that this area be comprehensively investigated. In Mauritius, medicinal plants are an important element of indigenous medical systems with at least 460 medicinal plants being reported. Several endemic plant species have also been described in the traditional pharmacopoeia and many of their uses are widely anchored in the Mauritian culture. This review highlights the potential value of traditionally used medicinal plants in Mauritius and also reports a number of Mauritian endemic plants screened for their phenolic constituents which can be promising sources of novel prophylactic agents, particularly natural antioxidants as determined by their free radical scavenging activity and reducing power. Further evaluation of the phytochemical contents, the pharmacological properties and safety of these plants are warranted to allow the rational use of these endemic plants.

Keywords: Traditional medicine, Mauritian endemic plants, secondary metabolites, phenolics, prophylactics, antioxidants.

INTRODUCTION

The Republic of Mauritius is composed of the islands of Mauritius, Rodrigues, Agalega, St. Brandon and a number of outlying smaller islands, all located in the south of the Indian Ocean between latitudes 10° S and 20° S and longitude 55° E and 65° E. Mauritius is the principal island and is located at latitude 20° South and longitude 58° East, some 800 km from the south east of Madagascar and has a land area of 1,865 km². In isolation the island has evolved a unique flora and fauna with high levels of endemism. Nevertheless the origin of the flora comes from several sources and it is believed that 70% of the phanerogams are derived from Madagascar and the African continent, 8% from Asia, 12% are of pan-indo pacific origin and 8% are endemic (Guého, 1988).

Surveys have revealed that less than 1.9% of the land area supports native vegetation (D’Argent, 1997). The upland rainforest communities make up 1.4% of this total, and are mostly confined to the southwest and eastern mountain ranges while the northern and eastern range support very little rainforest. These areas still harbour a diversity of important indigenous forest trees but the decline in native forest area has significantly reduced the population level of these species. However, the remnant areas of native vegetation still hold a great diversity of plant species that are of great conservation value. About 700 native flowering plants, of which some 300 are endemic, are known to
occur in Mauritius (World Conservation Monitoring Centre 1992; Strahm, 1994). Many of these have become highly endangered, with about 50 taxa being reduced to less than 10 individuals (Strahm, 1994).

Although there has been a decrease in interest in the field of traditional medicine, over 50% of all modern clinical drugs are of natural product origin (Farombi, 2003). Since natural products play an important role in drug development programs, there is an urge to evaluate our green inheritance which represents an enormous reservoir of putative lead compounds. This paper reviews a selected number of commonly used plants in traditional medicine, the bioactive components responsible for such properties and discusses the potential of a number of Mauritian endemic plants with limited traditional uses.

**STATUS OF TRADITIONAL MEDICINE IN MAURITIUS**

Traditional medicine has always had an important place in the therapeutic armoury of mankind. Although folklore medicines have almost disappeared in occidental societies, they continue to be the cornerstone of therapy in several underdeveloped and developing regions. Current estimates from the World Health Organisation suggest that, in many developing countries, a large proportion of populations rely heavily on traditional practices. This is the case in Africa, where 80% of the population depends on traditional medicine for their primary health care due to the severe shortage of qualified personal in modern medicine and the high cost of imported pharmaceuticals (Scott, 1993). Herbal medicine in Mauritius is an important part of the culture and traditions of the Mauritian people and besides this cultural significance; they are generally accessible and affordable.

Plant remedies in Mauritius have long been described in the traditional pharmacopoeias and 460 medicinal plants from 118 families have been described till date (Gurib-Fakim, 1995). Infusions or decoctions of the plant’s various parts are recommended for their antibacterial, antifungal, antihelminthic, anti-amoebic, antischistosomal, antimalarial, anti-inflammatory, anti-tussive, purgative activities, hypoglycemic, laxative, cholesterol-lowering properties (Gurib-Fakim, 1995, 1996). A wide range of exotic and native plants are used by traditional practitioners and these include plants from the Amaranthaceae, Apiaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Bombacaceae, Bromeliaceae, Caesalpinaceae, Celastraceae, Convolvulaceae, Liliaceae, Myrtaceae, Meliaceae, Orchidaceae, Oxalidaceae families. A number of these medicinal plants are part of the diet, in particular mentha (Mentha piperita), cloves (Syzygium aromaticum), onion (Allium cepa), basil (Ocimum gratissimum), thyme (Thymus vulgaris), amongst others (Adjanohoun et al., 1983). Table 1 describes some commonly used traditional plants in Mauritius. Several of the native plants have also found important uses against a range of ailments. For instance, a decoction of the bark of Erythroxylum laurifolium is used as a diuretic and against renal stones (Gurib-Fakim, 1996). Similarly Crinum mauritianum has been found to alleviate rheumatic pain while Cassine orientalis is traditionally used by Mauritian people against hypertension (Gurib-Fakim, 1995). Maytenus pyria is used against dysentery and has anti-tumoral, anti-inflammatory, anti-leukemic properties (Gurib-Fakim, 1995). The Diospyros species mainly D. neraudii, D. tesselaria, D. mellanida, D. revaughanii have been reported against a wide range of pathologies as they possess antibacterial, antifungal, antiviral, antihelminthic, antiprotozoan, and antimalarial properties (Gurib-Fakim, 1996). E. tinifolia and S. glomeratum have also been described in the traditional pharmacopoeia as a purgative and for the treatment of migraine respectively (Gurib-Fakim, 1996).

**SCIENTIFIC VALIDATION OF TRADITIONALLY USED PLANTS**

The increasing trend of integrating traditional medicine with primary health care has led to an upsurge of interest in assessing plant extracts for their pharmacological and genotoxic effects to determine their rational use. For instance, several non-native Eugenia species have been described in the literature for their therapeutic and beneficial uses. The leaves of Eugenia jambos, an Asian folk medicine has been found to induce apoptosis of human leukemia cells suggesting a possible mechanism for their antitumour activity (Yang et al., 2000) while constituents of Eugenia sandwicensis were identified for their potential cancer chemopreventive activity (Gu et al., 2001). Several other Eugenia species particularly E. caryophyllata and E. uniflora have been discussed for their pharmacological uses (Pourgholami et al., 1999; Consolini et al., 1999). Plant species namely Terminalia arjuna, Aegle
marmelos, Sida rhombifolia, Piper longum, Vitex negundo, Cassia fistula, Boerhaavia diffusa, Zingiber officinale and Calotropis gigantea have been described by Sri Lankan traditional medical practitioners for cardioprotection. The proposed mechanisms of cardioprotection by Terminalia arjuna and Cassia fistula is thought to occur through their antioxidant and antilipoperoxidative effects (Munasinghe et al., 2001). The high antioxidative potential of the different plant parts of C. fistula has also been reported (Bahorun and Neergheen, 2005; Luximon-Ramma et al., 2002) suggesting that the antioxidant actions of the bioactive components can be a potential mechanism against ROS mediated diseases. Cassia fistula extract has also been described for its anti-tumour effect by significantly reducing the mitotic activity (Gupta et al., 2000).

Table 1 Some commonly used plants in traditional medicine in Mauritius.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Common name</th>
<th>Medicinal purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apioideae</td>
<td>Foeniculum vulgare</td>
<td>Gros anis</td>
<td>fenouil</td>
</tr>
<tr>
<td>Apioideae</td>
<td>Anethum graveolens</td>
<td>Aneth</td>
<td></td>
</tr>
<tr>
<td>Asclepiadaceae</td>
<td>Tylorophora coriacea*</td>
<td>Ipéca du pays</td>
<td>Treat vomiting</td>
</tr>
<tr>
<td>Asteraeae</td>
<td>Ayapana triplinervis</td>
<td>Ayapana</td>
<td>Against vomiting, diarrhea</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>Psidia viscosa*</td>
<td>Baume de l’île plate</td>
<td>Against asthma</td>
</tr>
<tr>
<td>Bombacaceae</td>
<td>Adansonia digitata</td>
<td>Baobab</td>
<td>Used against diarrhea</td>
</tr>
<tr>
<td>Bromeliaceae</td>
<td>Ananas bracteatus</td>
<td>Ananas sauvage</td>
<td>Against vomiting, also as an abortive</td>
</tr>
<tr>
<td>Caesalpinaceae</td>
<td>Cassia fistula</td>
<td>Fleur cavae de</td>
<td>Laxative</td>
</tr>
<tr>
<td>Celastraceae</td>
<td>Maytenus pyria*</td>
<td>Bois à poudre</td>
<td>Use against dysentery. Anti-tumoral, anti-inflammatory, anti-lemucoidal properties</td>
</tr>
<tr>
<td>Convolvulaceae</td>
<td>Ipomoea indica</td>
<td>Liane bleue</td>
<td>For rheumatic pain and headache</td>
</tr>
<tr>
<td>Ebanacea</td>
<td>Diospyros neraudii*</td>
<td>Ebène</td>
<td>Anti-bacterial, antifungal, antiviral, antihelmintic, antiprotozoan, and antimarial properties</td>
</tr>
<tr>
<td>Erythroxylaceae</td>
<td>Erythroxylum laurifolium*</td>
<td>Bois de ronde</td>
<td>Diuretic, used against renal stones</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td>Plectranthus madagascariensis</td>
<td>Baume du Pérou</td>
<td>Anti-coughing property</td>
</tr>
<tr>
<td>Liliaceae</td>
<td>Aloe barbadensis</td>
<td>Mazambron</td>
<td>Against muscular pain</td>
</tr>
<tr>
<td>Melliaceae</td>
<td>Azadirachta indica</td>
<td>Neem</td>
<td>Anti-diabetic effect, Treatment for hemorroids</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>Eugenia tinifolia*</td>
<td>Bois de néfies</td>
<td>Purgative</td>
</tr>
<tr>
<td>Orchidaceae</td>
<td>Vanilla planifolia</td>
<td>Vanillier</td>
<td>Digestive, aphrodisiac property</td>
</tr>
<tr>
<td>Oxalidaceae</td>
<td>Averrhoa bilimbi</td>
<td>Bilimbi long</td>
<td>Use against hypertension and intestinal infections</td>
</tr>
</tbody>
</table>

Source: Adjanohoun et al., 1983; Gurb-Fakim 1995;1996. * indicates plants that are endemic to Mauritius.

In addition, a wide range of plant species have been described against diabetes mellitus. These include Averrhoa bilimbi, Salacia oblonga, Terminalia chebula, Terminalia becherica and Emblica officinalis which have been described for their hypoglycemic and hypolipaemetic properties thus reducing the incidence of the disease (Pushparaj et al., 2000; Sabu and Kuttan, 2002; Krishnakumar et al., 2000). Eryngium foetidum, traditionally used in Trinidad and Tobago against diabetes has been reported for its hypoglycaemic effects (Offiah et al., 2003). Moreover, the implication of oxidative stress in several pathological conditions including diabetes has suggested the beneficial effects of antioxidative rich extracts on diabetes. A mixture of Phellodendron cortex and Aralia cortex, Trigonella foemum graecum seeds, Tinospora cordifolia roots, Gongronema latifolium leaf extracts have been reported to decrease lipid peroxidation and to enhance the endogenous antioxidant defence mechanisms (Prince and Menon, 1999; Ravikumar and Anuradha, 1999; Pushparaj et al., 2000; Lee et al., 2000). An estimated number of 122 drugs from 94 plants have already been discovered through ethnobotanical
leads and several are in the pipeline being validated by clinical trials (Fabricant and Farnsworth, 2001). Traditional native plants seem to be a promising target for the discovery of novel compounds of pharmaceutical value and in this line careful phytochemical, pharmacological and toxicological analyses are necessary to assess their effectiveness.

**BIOACTIVE CONSTITUENTS ISOLATED FROM MEDICINAL PLANTS**

Considerable attention has been focused on identifying the naturally occurring components in medicinal plants. Characterisation and isolation of the active compounds is of significant interest to the pharmaceutical industry and is also essential to ensure a comprehensive understanding of their overall synergistic action in a complex extract. Alkaloids, terpenoids and polyphenolics are classes of plant secondary metabolites that have gained significant recognition as therapeutic compounds. For instance the alkaloids: vincristine and vinblastine, isolated from *Catharathus roseus* are among the most potent anti-leukemic drug in use while several other alkaloids including quinine (from *Cinchona succirubra*) and atropine (from *Atropa belladonna*) have well established medical applications (Levêque et al., 1996). The terpenoids also comprise some of the very important naturally occurring pharmaceuticals: artemisinin (antimalarial), taxol (anticancer), *Digitális* sterol glycosides (prescribed for congestive heart diseases) and steroidal saponins from yam (precursors for the synthesis of progesterone-like compounds for birth control pills) (Calixto et al., 2000).

Plant phenolics are also considered as valuable prophylactic compounds and are currently sold as dietary supplements and / or herbal remedies (Ferguson, 2001; Erlund, 2004). Epidemiological studies have greatly emphasized the role of phenolics in the prevention of cardiovascular diseases and cancer (Hertog et al., 1993; Keli et al., 1996). Although they have no known role in nutrition, many of them have properties including antioxidant, anti-mutagenic, anti-oestrogenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease (McGregor et al., 1999; Spignoli, 2000). Enzogenol (proanthocyanidin-rich flavonoid extract derived from the bark of *Pinus radiata*), Pycnogenol (standardized extract of proanthocyanidins and phenolic acids), grape seed extract and propolis (mixture of bioflavonoids) are commercially marketed as dietary supplements (Ferguson 2001; Senthilmohan et al., 2003).

Furthermore, several studies have reported the chemopreventive effects of catechins (Lamy et al., 2002), epigallocatechin gallate (EGCG) (Fujiki et al., 2003) and resveratrol (Jang et al., 1997). The possible mechanisms of action of these compounds have been attributed to their ability to modulate cell transduction, inhibit oncogene activation, inhibit polyamine synthesis, enhance gap junctional intercellular communication, inhibit angiogenesis and inhibit aberrant arachidonic acid metabolism (Kelloff et al., 1995; Hou et al., 2004). Flavonoids namely quercitin, fisetin, methyl caffeate, propyl gallate have been reported for their protective effects on neuronal cells implicating this property as a strategy for neuroprotection (Ishige et al., 2001). Moreover because cellular oxidative stress is an important factor in various diseases, including atherosclerosis, ischemia, Alzheimer disease, Parkinson’s disease, AIDS, diabetes, cancer and the process of aging, phenolic antioxidants may have multiple beneficial effects in the treatment of these conditions (Aruoma, 1998; Lefer et al., 2000; Bhatia et al., 2003; Olinski et al., 2003; Peuchant et al., 2004). In addition several of the inherent properties of phenolic compounds namely anti-mutagenic, anti-carcinogenic and anti-inflammatory stem from the antioxidant capacity thus emphasizing the importance of phenolic antioxidants.

Several studies have clearly demonstrated that the biological and pharmacological activities of phenolics are strongly linked to their structures. For instance, Ishige et al., (2001) reported that flavonol, having a C3-OH group, an unsaturated C ring and a catechol structure, was very effective in protecting neuron from excitotoxicity while flavanones and flavan-3-ols, lacking these functional groups were totally inefficient. Studying the redox potential of the catechins, several authors (Lien et al., 1999; Yang et al., 2001; Seeram and Nair 2002) have reported that the antioxidant activity decreases in the following order: (-) EGC > (-) ECG > (-) EGc > (-) EC > (+) C (Figure 1). This antioxidant hierarchy has been attributed to the number of hydroxyl groups attached to the core molecule, which decreases in the same order. In general the following structural features of phenolics are crucial for antioxidant and free radical scavenging activities:

(i) an o-diphenolic group (in ring B),
(ii) a 2-3 double bond conjugated with 4-oxo function, and
(iii) hydroxyl groups in positions 3 and 5 (Bors et al., 1990).
The phytochemical composition of only a minority of the traditional plants in Mauritius has been studied and phenolic acids, tannins, flavonoid glycosides, alkaloids, saponins, terpenoids were the main compounds identified (Table 2).

The Department of Biosciences at the University of Mauritius is actively involved in research based on the extraction of phenolic secondary metabolites from plant sources and systematic screening of the endemic flora has been initiated 8 years ago with the view to providing data on their phytochemical composition and assessing their prophylactic potential. In line with this current research interest, 26 Mauritian endemic plants from the Celastraceae, Ebanaceae, Erythroxylaceae, Myrtaceae, Rubiaceae, and Sterculiaceae families were recently screened for their phytochemical components. Our results showed the significant presence of flavonoids and proanthocyanidins contents. (+)-Catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate and procyanidin dimers were detected in these plant species using high performance liquid chromatography (Table 3). The data indicate that these plants can be promising sources of bioactive compounds like EGC G and oligomeric procyanthocyanidins. The plant extracts also abound in flavonoid glycosides as indicated by the presence of high levels of quercetin and kaempferol aglycones in the hydrolysed extracts (Table 4). Quercetin and flavonoid glycosides have been greatly suggested for their antioxidant and LDL oxidation inhibitory activities and in addition recent epidemiological studies showed that consumption...
Table 2 Phytochemical composition of some endemic plants used in traditional medicine

<table>
<thead>
<tr>
<th>Families</th>
<th>Plant names</th>
<th>Phytochemicals present</th>
<th>Traditional uses *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asteraceae</td>
<td><em>Psideia</em> sp.</td>
<td>Phenols, flavonoids, saponins, tannins</td>
<td>Pulmonary infections, asthma, fever, coughs</td>
</tr>
<tr>
<td>Burseraceae</td>
<td><em>Canarium paniculatum</em></td>
<td>Phenols, anthocyanins, tannins, terpenes, coumarins</td>
<td>Rheumatism, skin ulcerations</td>
</tr>
<tr>
<td>Asteraceae</td>
<td><em>Senecio ambavilla</em></td>
<td>Phenols, flavonoids, tannins</td>
<td>Rheumatism, gout, urinary infections, renal infections</td>
</tr>
<tr>
<td>Chrysobalanaceae</td>
<td><em>Grangeria borbonica</em></td>
<td>Phenols, flavonoids, tannins</td>
<td>Stomach pains, asthma</td>
</tr>
<tr>
<td>Connaraceae</td>
<td><em>Cnestis glabra</em></td>
<td>Flavonoids, tannins, proanthocyanidins, saponins</td>
<td>Fever</td>
</tr>
<tr>
<td>Erythroxylaceae</td>
<td><em>Erythroxylum hypericifolium</em></td>
<td>Flavonoids, saponins, tannins</td>
<td>Fever, renal stones, throat infections</td>
</tr>
<tr>
<td>Erythroxylaceae</td>
<td><em>Erythroxylum laurifolium</em></td>
<td>Phenols, flavonoids, saponins, tannins</td>
<td>Fever, renal stones, throat infections</td>
</tr>
<tr>
<td>Flacouriaceae</td>
<td><em>Aphloia theiformis</em></td>
<td>Phenols, flavonoids, proanthocyanidins, saponins, tannins</td>
<td>Dysentery, fever, rheumatism, gastrointestinal infections, jaundice</td>
</tr>
<tr>
<td>Lecythidaceae</td>
<td><em>Foetidia mauritiana</em></td>
<td>Alkaloids, proanthocyanidins, saponins, tannins</td>
<td>Laxative, emmenagogue, purgative, diuretic</td>
</tr>
<tr>
<td>Leeaceae</td>
<td><em>Leea guinensis</em></td>
<td>Phenols, flavonoids, proanthocyanidols, tannins</td>
<td>Oedemas, antiseptic, colds</td>
</tr>
<tr>
<td>Meliaceae</td>
<td><em>Turraea casimiriana</em></td>
<td>Phenols, flavonoids, proanthocyanidins, saponins, tannins</td>
<td>Boils, hypotensive, emmenagogue</td>
</tr>
<tr>
<td>Monimiaceae</td>
<td><em>Tambourissa sp</em></td>
<td>Flavonoids, proanthocyanidins, saponins, tannins</td>
<td>Dermatitis, emmenagogue</td>
</tr>
<tr>
<td>Moraceae</td>
<td><em>Ficus reflexa</em></td>
<td>Phenols, proanthocyanidins</td>
<td>Throat infections</td>
</tr>
<tr>
<td>Myrsinaceae</td>
<td><em>Embelia angustifolia</em></td>
<td>Alkaloids, phenols, flavonoids, saponins</td>
<td>Liver complaints, dysentery, urinary tract infections</td>
</tr>
<tr>
<td>Rhamnaceae</td>
<td><em>Scutia myrtina</em></td>
<td>Phenols, flavonoids, tannins, proanthocyanidins, saponins,</td>
<td>Diarrhoea, dysentery, astringent, antidote for toxic fish poisoning</td>
</tr>
<tr>
<td>Rubiaceae</td>
<td><em>Chassalia coriaceae</em></td>
<td>Anthocyanin heterosides, saponins, terpenes, iridoids, flavonoids</td>
<td>Astringent</td>
</tr>
</tbody>
</table>

Table adapted from Bahorun et al. 2003. * Source: Gumb-Fakim and Guého, 1995;1996.

Data obtained suggest the potent antioxidant efficacy of plants from the genus *Syzygium* and *Diospyros* (Figure 2), thus indicating that species of these genus hold much promise as new sources of antioxidants. Therefore, the polyphenolic richness and antioxidant propensities of the native plants indicate the potentials of these plant species as a supply of prophylactic constituents.
Table 3 Distribution of flavan-3-ol derivatives in some selected Mauritian endemic species

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Catechins</th>
<th>Procyanidin dimers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+)-C</td>
<td>(-)EC</td>
</tr>
<tr>
<td>Cassine orientalis</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Chassalia grandifolia</td>
<td>(+)</td>
<td>-</td>
</tr>
<tr>
<td>Coffea macrocarpa</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Coffea mauritiana</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Diospyros melanida</td>
<td>(+++)</td>
<td>-</td>
</tr>
<tr>
<td>Diospyros neraudii</td>
<td>Tr</td>
<td>(+)</td>
</tr>
<tr>
<td>Diospyros revaughanii</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Diospyros tessellaria</td>
<td>(+++)</td>
<td>-</td>
</tr>
<tr>
<td>Erythroxylum sideroxyloides</td>
<td>(+)</td>
<td>-</td>
</tr>
<tr>
<td>Eugenia elliptica</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Eugenia orbiculata</td>
<td>(+++)</td>
<td>(+)</td>
</tr>
<tr>
<td>Eugenia pollicina</td>
<td>(++)</td>
<td>(++)</td>
</tr>
<tr>
<td>Eugenia tinfoilia</td>
<td>(+++)</td>
<td>-</td>
</tr>
<tr>
<td>Fernelia buxifolia</td>
<td>(+)</td>
<td>-</td>
</tr>
<tr>
<td>Monimiastrum acutisepalum</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Myonima obovata</td>
<td>(+)</td>
<td>-</td>
</tr>
<tr>
<td>Syzygium commersonii</td>
<td>(+++)</td>
<td>-</td>
</tr>
<tr>
<td>Syzygium glomeratum</td>
<td>(++)</td>
<td>(++)</td>
</tr>
<tr>
<td>Syzygium mauritianum</td>
<td>(++)</td>
<td>(++)</td>
</tr>
<tr>
<td>Syzygium venosum</td>
<td>(+++)</td>
<td>-</td>
</tr>
<tr>
<td>Trochetia boutoniana</td>
<td>(+)</td>
<td>-</td>
</tr>
</tbody>
</table>

(+++)= very prominent; (++)= prominent; (+)= present; tr= trace; (-)= not detected.
Estimated from the peak area of the HPLC profiles.

Table 4 Distribution of flavonol aglycones: kaempferol, myricetin and quercetin in the hydrolysed plant extracts.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Kaempferol</th>
<th>Myricetin</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassine orientalis</td>
<td>(+++)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>Chassalia grandifolia</td>
<td>(+)</td>
<td>-</td>
<td>(++)</td>
</tr>
<tr>
<td>Coffea macrocarpa</td>
<td>(+)</td>
<td>-</td>
<td>(++)</td>
</tr>
<tr>
<td>Coffea mauritiana</td>
<td>(+)</td>
<td>-</td>
<td>(++)</td>
</tr>
<tr>
<td>Diospyros melanida</td>
<td>(+++)</td>
<td>-</td>
<td>(++)</td>
</tr>
<tr>
<td>Diospyros neraudii</td>
<td>(+++)</td>
<td>-</td>
<td>(++)</td>
</tr>
<tr>
<td>Diospyros revaughanii</td>
<td>(+++)</td>
<td>(++)</td>
<td>(++)</td>
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<tr>
<td>Diospyros tessellaria</td>
<td>(+++)</td>
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<tr>
<td>Erythroxylum sideroxyloides</td>
<td>(+)</td>
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<tr>
<td>Eugenia elliptica</td>
<td>(+++)</td>
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<td>Tr</td>
</tr>
<tr>
<td>Eugenia orbiculata</td>
<td>(+)</td>
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<tr>
<td>Eugenia pollicina</td>
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<tr>
<td>Eugenia tinfoilia</td>
<td>(+)</td>
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<tr>
<td>Fernelia buxifolia</td>
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<tr>
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<tr>
<td>Myonima obovata</td>
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</tr>
<tr>
<td>Syzygium commersonii</td>
<td>(+++)</td>
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<td>(++)</td>
</tr>
<tr>
<td>Syzygium glomeratum</td>
<td>Tr</td>
<td>-</td>
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<tr>
<td>Syzygium mauritianum</td>
<td>(+++)</td>
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</tr>
<tr>
<td>Syzygium venosum</td>
<td>Tr</td>
<td>-</td>
<td>(++)</td>
</tr>
<tr>
<td>Trochetia boutoniana</td>
<td>(+++)</td>
<td>-</td>
<td>(++)</td>
</tr>
</tbody>
</table>

(++) = very prominent; (+) = prominent; (-) = present; tr = trace; (-) = not detected.
Estimated from the peak area of the HPLC profiles.
CONCLUSION

This review highlights the potentiality of the Mauritian endemic flora as an important source of naturally occurring bioactive compounds, hence providing an impetus to delineate their possible uses as prophylactic agents. The safety and efficacy of these products are of paramount importance and future perspective points to scientific research that will identify and/or confirm therapeutic benefits to these endemic plants in order to validate efficacy. In addition the potential toxicity resulting from the interaction of plants’ phytochemicals with conventional drugs also need to be addressed. Fallacies are often associated with use of traditional medicinal plant and include: (i) traditional plants, being natural are absolutely safe; (ii) traditional plants do not have side-effects; (iii) efficacy can be obtained over a wide range of doses. Considering these viewpoints, pharmacological and toxicological studies are vital to establish the true value of the traditional plants. Further investigations on phytochemical discovery and subsequent screening on the endemic flora may open new opportunities to exploit their properties as chemopreventive agents and to develop pharmaceuticals based on their constituents.

ACKNOWLEDGEMENTS

The authors wish to thank the Tertiary Education Commission and the Mauritius Research Council for financial support.
REFERENCES


A survey of the Mauritian endemic flora for potential prophylaxis. VS Neergheen et al.


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CHARACTERIZATION OF THE PHENOLIC PROFILE OF ENDEMIC MAURITIAN CHASSALIA SPECIES AND ASSESSMENT OF THEIR ANTIOXIDANT ACTIVITIES

M.A. Soobrattee¹, T. Bahorun¹ and P. Thaunoo²

¹Department of Biosciences, Faculty of Science, University of Mauritius
²Ministry of Environment, Ken Lee Tower, Port Louis

ABSTRACT

Accumulating biochemical, clinical and epidemiological evidence supports the chemopreventive effects of phenolic antioxidants against oxidative-mediated disorders. The current investigation examined the phenolic composition and antioxidant propensities of eight endemic Chassalia species of the Rubiaceae family, some of which being on the verge of extinction. The total phenolic content of the plant extract ranged from 662 to 3627 mg / 100g DW. The highest flavonoid level was observed in C lanceolata subsp. latifolia 1906 mg / 100g DW while the highest proanthocyanidin content was measured in C. capitata with 281 mg / 100g DW. TLC and HPLC analyses of the total extracts showed the presence of several flavonoid glycosides, namely rutin, hyperoside, isorhamnetin, kaempferol glucoside, quercitin, and chlorogenic acid. Based on the TEAC and FRAP assays the antioxidant potential of the extracts decreased in the following order: C. boryana > C. lanceolata subsp. latifolia > C. capitata > C. coriaceae var. coriaceae > C. coriaceae var. johnstonii > C. grandifolia > C. petrinensis > C. lanceolata subsp. lanceolata. For both antioxidant assays marked correlation was obtained with total phenolics (r > 0.8), flavonoid (r > 0.75) content, while weaker correlation was observed with proanthocyanidin content (r ≥ 0.5). The present study suggests that some of the endemic Chassalia species can be potential sources of natural antioxidants, thus warranting urgent conservation program.

Keywords: Chassalia, Mauritian endemic plants, conservation program, phenolic, flavonoid, antioxidant activities, TEAC, FRAP

INTRODUCTION

Cellular redox state is recognised as a critical component of stress-induced cellular responses and disease. Although the level of reactive oxygen species (ROS) is usually kept in check by the body’s antioxidant system, these entities when produced in excess are capable of damaging cellular components and accumulating evidence suggests that they may contribute to various diseases (Southorn and Powis, 1988). ROS are not only associated with lipid peroxidation leading to pathological conditions, but are also involved in the deterioration of food products resulting in the alteration of organoleptic properties (Kocchar, 1993).

Like animals, plants also have developed a strategy to preserve their cellular integrity or to overcome the deleterious effects of ROS in situation of oxidative stress. They react by enhancing the expression of multiple, synergistic, antioxidant mechanisms, which include the synthesis of primary and secondary metabolites. Among these natural substances, polyphenolics are a particularly attractive class ubiquitously distributed in the plant kingdom (Haslam, 1998). Interest in plant phenolics has increased greatly because of their roles as antioxidants and scavengers of free radicals and their potential effects on human health (Bors et al., 1990; Salah et al., 1995). Recent studies have revealed that phenolics can exert modulatory actions in cell by interacting with a wide spectrum of molecular targets central to the cell signaling machinery (Chung et al., 2003; Levites et al., 2002).

The Mascarene Islands originally covered by dense tropical forests have experienced the rapid transformation of native ecosystems and extinction of endemic species since their colonisation by man around the early sixteenth century (MacDonald et al., 1991). In Mauritius, the indigenous vegetation consists of only small relict patches in addition to the ten nature reserves covering some 585 ha. (Lorence and Sussman, 1986). With 45% (corresponding to about 311 species) of endemic plants, our small volcanic island is rightly considered as being the hotspot of endemism among the Mascarene
Characterization of the phenolic profile of endemic mauritian chassalia species and assessment of their antioxidant activities.
M A Soobrattee et al.

Islands. Among all the species recorded, 153 are classified as endangered, of which, 113 (73.5%) are endemic. 54 species are currently known with 10 individuals or less, of which 40 are endemic (Page 1995). Some of these critically endangered species include Hyophorbe amaricaulis (1 specimen), Diospyros angulata (1 specimen) and Badula reticulata (1 specimen) amongst others (Page 1995).

Members of the Chassalia genus, noted for their beautiful coral-shaped flowers, have unreservedly rarefied with species like Chassalia boryana being reduced to only one individual in the wild (D. Florens & G. d’Argent, 2001,Personal Communication; Page, 1995). These plants, that once were common under-storey shrubs, form part of those remnants that are now degraded and over-run by numerous exotic plants, like Lignum robustum, and Psidium cattleianum Sabine, and introduced animals (Baker, 1877; Lorence and Sussman, 1986). Like for most other Mauritian endemic species, very little data are available on the phytochemical and prophylactic potential of the Chassalia species. In line with our research interest on Mauritian endemic species and with the looming danger of these plants to become extinct, the present study was aimed towards the screening of their phytochemical profile and the assessment of their antioxidant potentiality for prophylaxis with the view to providing further justification to ongoing conservation program.

MATERIALS AND METHODS

Plant material

Mature leaves were collected from Le Pétrin Conservation management area (Table 1), Mauritius. They were weighed, washed with distilled water and stored at -20 °C within 2 hours following collection. Voucher specimens were deposited at the Mauritian Herbarium, Mauritius Sugar Industry research Institute, Réduit and at the Department of Biosciences, University of Mauritius.

Table1 Details of Mauritian endemic Chassalia species studied

<table>
<thead>
<tr>
<th>Specimen Collection</th>
<th>Nature Reserves</th>
<th>Harvest Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chassalia boryana</td>
<td>Mondrain</td>
<td>June 11 2001</td>
</tr>
<tr>
<td>Chassalia coriacea var coriacea</td>
<td>Perrier</td>
<td>October 4 2001</td>
</tr>
<tr>
<td>Chassalia coriacea var johnstonii</td>
<td>Pétrin</td>
<td>November 16 2001</td>
</tr>
<tr>
<td>Chassalia capitata</td>
<td>Pétrin</td>
<td>November 16 2001</td>
</tr>
<tr>
<td>Chassalia petrinensis</td>
<td>Pétrin</td>
<td>November 16 2001</td>
</tr>
<tr>
<td>Chassalia grandifolia</td>
<td>Brise Fer (Old Plot)</td>
<td>November 2 2001</td>
</tr>
<tr>
<td>Chassalia lanceolata subsp lanceolata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chassalia lanceolata subsp latifolia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Extraction

Extraction of plant material was carried out according to Bahorun et al., (1994).

Analysis of Total Phenolic Content

The total phenolic content of the extract was estimated using the Folin-Ciocalteu method adapted from Singleton and Rossi (1965).

Total Flavonoid Content

The AlCl3 method adapted from Lamaison and Carnet (1990) was used for the determination of the total flavonoid content of the extracts.
Characterization of the phenolic profile of endemic mauritian *chassalia* species and assessment of their antioxidant activities.

*M A Soobrattee* et al.

**Total Proanthocyanidin Content**

The modified acid / butanol assay of Porter et al., (1986) was used to quantify the total proanthocyanidin content.

**Thin Layer Chromatography (TLC)**

Flavonoids and phenolic acids from the total extracts as well as standards were separated by TLC using ethyl acetate: formic acid: water (8:1:1 v/v) solvent system adapted from Wagner et al. (1984) quoted from Lamaison et al., (1990).

**High-performance liquid chromatography (HPLC)**

Chromatographic analyses of total and hydrolysed plant extracts were carried out using a HP1100 series HPLC as described by Bahorun et al., (1994). The identification and quantification of the compounds were carried out from

(i) the retention time and 
(ii) peak area in comparison with authentic standards.

**Quantitative analysis of phenolic acid and flavan-3-ols in the plant extracts**

Gallic acid, (+) catechin, (-) epicatechin, (-) epicatechin gallate, (-) epigallocatechin, (-) epigallocatechin gallate, procyanidins B1 and B2 were identified and quantified by comparison with authentic standard in duplicates. Absorption wavelength was selected at 280 nm with a 65 mins running time.

**Hydrolysis of flavonoid conjugates and quantitative analysis of aglycones released**

Hydrolysis of flavonoid conjugates was carried out essentially as described by Crozier et al., (1997).

**Trolox Equivalent Antioxidant Capacity (TEAC) assay**

The total antioxidant activity of the extracts was assessed by the TEAC assay according to the method of Campos and Lissi (1996).

**Ferric Reducing Antioxidant Power (FRAP) assay**

The method of Benzie and Strain (1996) was employed to assess the reducing power of the extracts.

**STATISTICAL ANALYSIS**

Results are expressed as mean value ± standard deviation (n = 3). Simple regression analysis was performed to calculate the dose-response relationship of standard solutions used for calibration as well as for the test samples. Linear regression analysis was performed, quoting the correlation coefficient $r_{xy}$. 

RESULTS AND DISCUSSION

In recent years, there has been a surge towards the use of phytochemicals with antioxidant activity. Natural antioxidants have applications not only in the modern phytotherapy but also in the food and cosmetic industries. The present study evaluates the phenolic profile and antioxidant capacity of eight Chassalia species endemic to Mauritius. Chassalia boryana and Chassalia lanceolata subsp latifolia contained the highest level of phenolics with 3 627 mg and 3 077 mg GAE / 100 g dry material respectively (Figure 1). It is noteworthy that the two variants, Chassalia coriacea var coriacea and Chassalia coriacea var johnstonii, along with Chassalia capitata had comparatively the same amount of total phenols with 2 888 mg, 2 657 mg and 2 759 mg GAE / 100 g DW respectively. On the other hand, Chassalia grandifolia, Chassalia petrinensis and Chassalia lanceolata subsp lanceolata contained the lowest levels with only 1901 mg, 954 mg and 662 mg GAE / 100 g DW respectively. In situating the phytochemical content of Chassalia species in the context of literature data, the present study shows that the Chassalia species contain moderate to high level of phenolic compounds. Using similar methodology, Kähkönen et al., (1999) determined the phenolic content of 92 edible and non-edible plant materials (berries, fruits, vegetables, herbs, cereals, tree materials, plant sprouts and seeds) and reported levels of (0.2 – 1.3) mg GAE / g DW in cereals, (0.4 – 6.6) mg GAE / g DW in vegetables, and (12.4 – 50.8) mg GAE / g DW in berries. Herbal extracts contained (9.1 – 23.1) mg GAE / g DW whereas for the non-edible tree materials examined (excluding pine cork and birch bark) relatively high contents of phenolics (17.5 – 155.3 mg GAE / g DW) were reported (Kähkönen et al., 1999). Katsube et al., (2004) indicated that the phenolic content of 25 Japanese medicinal plants screened varied between 4.4 µmol and 188.5 µmol EGCG equivalent / g of sample.

Assessment of the flavonoid subclass show that Chassalia lanceolata subsp latifolia, Chassalia capitata and Chassalia boryana had maximum flavonoids amounting to 1906 mg, 1853 mg and 1453 mg / 100 g DW respectively. The other species had comparatively lower flavonoid content (< 1000
mg / g DW). The flavonoid levels of the two variants differed considerably; the amount quantified in *Chassalia coriacea var coriacea* (845 mg / 100 g DW) being nearly twice as high as that in *Chassalia coriacea var johnstonii* (486 mg / 100 g DW). The lowest flavonoid concentration was measured in *Chassalia lanceolata subsp latifolia* (120 mg / 100 g DW).

Analysis of proanthocyanidins (Figure 1) revealed that these compounds had very limited distribution in all the eight Chassalia species and these were found to occur in the range of 120 mg to 281 mg / 100 g DW. The highest levels were noted in *Chassalia capitata* (281 mg / 100 g DW) and the lowest in *Chassalia lanceolata subsp lanceolata* (120 mg / 100 g DW).

TLC is a rapid and useful technique for the identification of individual compounds from complex mixtures. Using this methodology, our data show that hyperoside was present in all species except *C. grandifolia*, while only trace amounts of isoquercitrin were observed in the *C. boryana* (Table 2). Although a high concentration of kaempferol-3-glucoside was detected in *C. boryana*, lower levels were observed in *C. capitata, C. lanceolata subsp latifolia* and *C. lanceolata subsp lanceolata*. Isoquercitrin was present only in the two variants. Furthermore, an unidentified polar phenolic with an Rf of 0.07 was prominent in all the species.

### Table 2 Thin Layer Chromatography data showing the distribution of leaf flavonoids in Mauritian endemic *Chassalia* species

<table>
<thead>
<tr>
<th>Species / Rf</th>
<th>Phenolic Acid and Flavonoid Compounds Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rutin</td>
</tr>
<tr>
<td>L1 C. grandifolia</td>
<td>++</td>
</tr>
<tr>
<td>L2 C. boryana</td>
<td>+++</td>
</tr>
<tr>
<td>L3 C. capitata</td>
<td>+++</td>
</tr>
<tr>
<td>L4 C. petrinensis</td>
<td>++</td>
</tr>
<tr>
<td>L5 C. coriacea var coriacea</td>
<td>+++</td>
</tr>
<tr>
<td>L6 C. coriacea var johnstonii</td>
<td>+++</td>
</tr>
<tr>
<td>L7 C. lanceolata subsp lanceolata</td>
<td>++</td>
</tr>
<tr>
<td>L8 C. lanceolata subsp latifolia</td>
<td>+++</td>
</tr>
</tbody>
</table>

Note: (+++): Very prominent; (++): Prominent; (+): Present; tr: Trace; (-): not detected

HPLC data confirmed the TLC results obtained (Table 3). In addition flavan-3-ol derivatives, namely (+) catechin, procyanidin dimers B1 and B2, were also identified in the extracts. In a previous study on the Mauritian endemic *Trochetia* genus several flavan-3-ols were also detected (Lai Fang et al., 2000) with however their level being much higher in the *Chassalia* species. It is noteworthy that the high antioxidant activities of teas, red wine and cacao products are ascribed mainly to the presence of flavan-3ol derivatives (Miura et al., 2000). Other studies have also shown that flavanols have a protective effect in neurodegeneration (Choi et al., 2001; Levites et al., 2003; Mandela and Youdim 2004). Pretreatment with (-) EC attenuated neurotoxicity induced by oxidized low-density lipoprotein in mouse-derived striatal neurons, as evidenced by apoptotic DNA fragmentation and caspase-3 activation (Schroeter et al., 2001). (-) EGCG protects against 6-OHDA and MPP+ induced human neuroblastoma cell damage (Levites et al., 2002). The *Chassalia* species therefore represent a potential source of prophylactic flavan-3ol derivatives.
Characterization of the phenolic profile of endemic Mauritian Chassalia species and assessment of their antioxidant activities.

M A Soobrattee et al.

**Table 3** HPLC data showing the distribution of some leaf flavonoids in the *Chassalia* species

<table>
<thead>
<tr>
<th>R_T / SPECIES</th>
<th>C. grandifolia</th>
<th>C. boryana</th>
<th>C. capitata</th>
<th>C. petrinensis</th>
<th>C. coriacea var. coriacea</th>
<th>C. coriacea var. johnstonii</th>
<th>C. lanceolata subsp. lanceolata</th>
<th>C. lanceolata subsp. latifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procyanidin B1</td>
<td>(15.210)</td>
<td>+</td>
<td>Tr</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>(+) Catechin</td>
<td>(18.951)</td>
<td>+</td>
<td>-</td>
<td>tr</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic Acid</td>
<td>(21.428)</td>
<td>tr++</td>
<td>++</td>
<td>+</td>
<td>tr</td>
<td>tr</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Procyanidin B2</td>
<td>(25.244)</td>
<td>-</td>
<td>-</td>
<td>tr</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Isoquercitrin</td>
<td>(67.573)</td>
<td>-</td>
<td>Tr</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hyperoside</td>
<td>(66.462)</td>
<td>++</td>
<td>Tr</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>Tr</td>
</tr>
</tbody>
</table>

Note: (+++): Very prominent; (++): Prominent; (+): Present; tr: Trace; (-): not detected

The TEAC values of the extracts varied from 41 μmol trolox / g DW (*C. lanceolata* subsp. *lanceolata*) to 379 μmol trolox / g DW (*C. boryana*) while the FRAP values ranged from 497 mmol Fe²⁺ / g DW (*C. lanceolata* subsp. *lanceolata*) to 3 743 mmol Fe²⁺ / g DW (*C. boryana*) (Table 4). Based on the TEAC and FRAP assays, the antioxidant activities of the extracts was in the following order: *C. boryana* > *C. lanceolata* subsp. *latifolia* > *C. capitata* > *C. coriacea var. coriacea* > *C. coriacea var. johnstonii* > *C. grandifolia* > *C. petrinensis* > *C. lanceolata* subsp. *lanceolata*. Furthermore, the two variants – *C. coriacea var. coriacea* and *C. coriacea var. johnstonii* had approximately the same redox capacity. Comparing the antioxidant activity of the *Chassalia* genus with other plants in the literature, the present study clearly shows that some of the endemic *Chassalia* species are relatively good antioxidants. Cook et al., (1998) reported that the antioxidant activities of 61 West African indigenous food plants were in the range of 3.9 to 26.3 μmol trolox equivalent / g DW. Ivanova et al., (2005) screened the phenolic content and antioxidant activity of 21 plants used in the Bulgarian phytotherapy for the treatment of respiratory, gastrointestinal and other inflammatory disorders; the TEAC value of the plant extracts ranged from 0.09 mM to 7.05 mM trolox equivalent. Linear regression analysis showed a very strong correlation between the antioxidant activities and the total phenolic (TEAC: r = 0.90; FRAP: r = 0.84) and flavonoid (TEAC: r = 0.85; FRAP: r = 0.76) content, while a lower correlation was observed with the proanthocyanidin content (TEAC: r = 0.66; FRAP: r = 0.50). Using the TEAC and FRAP assays, Luximon et al. (2002) reported that antioxidant activity of the *Cassia fistula* (Indian Laburnum) was strongly related to its total phenolics (TEAC: r = 0.99; FRAP: r = 0.95) and proanthocyanidin content (TEAC: r = 0.98; FRAP: r = 0.90). Using different lipid peroxidation model, Hsieh and Yen (2000) observed a direct relationship between the polyphenolic content and antioxidant activities of *Eucommia ulmoides* (Du Zhong) leaf extracts.

**Table 4** Antioxidant activities of Mauritian endemic *Chassalia* species

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>TEAC</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. grandifolia</em></td>
<td>157.40 ± 1.0</td>
<td>1 053.28 ± 2.2</td>
</tr>
<tr>
<td><em>C. boryana</em></td>
<td>379.52 ± 5.4</td>
<td>3 743.01 ± 12.2</td>
</tr>
<tr>
<td><em>C. capitata</em></td>
<td>268.27 ± 2.8</td>
<td>1 762.24 ± 5.7</td>
</tr>
<tr>
<td><em>C. petrinensis</em></td>
<td>80.94 ± 0.4</td>
<td>563.72 ± 6.1</td>
</tr>
<tr>
<td><em>C. coriacea var. coriacea</em></td>
<td>185.29 ± 2.4</td>
<td>1 407.24 ± 32.1</td>
</tr>
<tr>
<td><em>C. coriacea var. johnstonii</em></td>
<td>174.06 ± 2.8</td>
<td>1 285.55 ± 53.2</td>
</tr>
<tr>
<td><em>C. lanceolata</em> subsp. lanceolata</td>
<td>41.85</td>
<td>497.24 ± 1.0</td>
</tr>
<tr>
<td><em>C. lanceolata</em> subsp. latifolia</td>
<td>378.27 ± 2.0</td>
<td>3 314.69 ± 1.9</td>
</tr>
</tbody>
</table>

All analyses are mean of triplicate measurements ± standard deviations.

* Results expressed in μmol trolox equivalent / g DW.

** Values are expressed in units of mmol Fe (II) / g DW.

Characterization of the phenolic profile of endemic mauritian chassalia species and assessment of their antioxidant activities.

M A Soobrattee et al.

Similar positive correlation between phenolic content and antioxidant activities has also been reported in medicinal plants (Katsube et al., 2004), fruits (Luximon-Ramma et al., 2003), tea (Luximon-Ramma et al., 2005), vegetables (Velioglu et al., 1998; Bahorun et al., 2004) and cell cultures (Bahorun et al. 2003).

There is general consensus that a number of assays should be used when assessing the antioxidant activity as no one method can give a comprehensive prediction of antioxidant efficiency. A combination of rapid, sensitive and reproducible methods should be used whenever an antioxidant activity screening is designed. This ‘screening’ approach can be used to rule out direct antioxidant activity in vivo: a compound that is poorly effective in vitro will not be any better in vivo. Although several screening approach have been proposed, the trolox equivalent antioxidant capacity (TEAC) and the ferric reducing antioxidant power (FRAP) have been used in the present study to evaluate the antioxidant potential of the plant extracts. Their simplicity, reliability and reproducibility make these assays very popular as evidenced by literature data.

CONCLUSION

The accelerating rate at which the world’s botanical resources are being depleted threatens many plant species with extinction at a magnitude unparalleled in human history. Likewise, several Mauritian endemic plants are facing a great challenge for survival due to prolific exotic plant and animal competitors. Their disappearance will deprive us the golden opportunity to exploit biologically active compounds which they might harbour; this notion is clearly illustrated with the endemic Chassalia genus. The present study shows that some of the Chassalia species, particularly C. boryana and C. lanceolata subsp latifolia, are characterised by high phenolic content and potent antioxidant efficacy; thus making them potential candidate to be considered as functional food, nutraceuticals and nutriceuticals. Although the in vitro studies carried out facilitate the primary evaluation of the plant extracts, in vivo settings are essential to delineate their prophylactic potential; hence further research is needed in this regard.

ACKNOWLEDGEMENTS

We are grateful to the Mauritius Research Council and the Tertiary Education Commission for financial assistance.

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Characterization of the phenolic profile of endemic mauritian *chassalia* species and assessment of their antioxidant activities. 

*MA Soobrattee et al.*


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*MA Soobrattee et al.*


MAURITIAN EXOTIC FRUITS AND VEGETABLES: ANTIOXIDANT AND PRO-OXIDANT PROPENSITIES OF THEIR PROPHYLACTIC AGENTS

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ABSTRACT

The beneficial role of fruits and vegetables in the diet has convincingly been evidenced as regard to the maintenance of health and prevention of several diseases. The protective effects of fruits and vegetables are attributed largely to the antioxidant effects of their bioactive components. Our previous studies have clearly revealed high levels of antioxidants in Mauritian exotic fruits and vegetables namely Chinese goyava, goyava, starfruits, hogplum, Passion fruits, Chinese cabbage, onion, mugwort and broccoli. The antioxidant capacities as evaluated by two independent methods, the TEAC and FRAP assays, correlated strongly with total phenols and flavonoids while a much weaker influence was attributed to their vitamin C contents. The present work focuses on the assessment of the antioxidant and pro-oxidant actions of 10 Mauritian exotic fruits and vegetables using three newly established methods with biologically relevant reactive oxygen species at physiological pH. The results show that Chinese goyava “red type” exhibited highest scavenging capacities towards both the OCl⁻ and OH · radicals (IC₅₀ = 4.11 g FWL⁻¹ and 4.74 g FWL⁻¹ respectively) while the lowest potency was observed in litchi. Similarly among the vegetables studied the highest protection against radical attack was from broccoli (IC₅₀ = 43.79 g FWL⁻¹ and 144.76 g FWL⁻¹ respectively). Lettuce exhibited the poorest antioxidant activities in both assays. Both activities correlated positively with total phenol contents (OCl⁻ radical:  r = 0.68 and OH · radical:  r = 0.48). No pro-oxidant activities were observed for the vegetables studied within the range of 25-100 g FWL⁻¹ as compared to the positive control ascorbate (240 µM). However, pawpaw and litchi demonstrated some degree of pro-oxidant activities above 75 g FWL⁻¹ and 50 g FWL⁻¹ respectively. Thus, Mauritian exotic fruits and vegetables show important antioxidant activities that are highly relevant to maintenance of normal health and disease management.

Keywords: Mauritian exotic fruits, vegetables, total phenols, total flavonoids, antioxidant capacity, free radical scavenging capacity, pro-oxidant activity.

Abbreviations:
FRAP: Ferric Reducing Antioxidant Potential
FWL⁻¹: Fresh Weight / Litre
IC₅₀: Concentration for 50% inhibition
MDA: Malondialdehyde
OCl⁻: Hypoclorous radical
OH ·: Hydroxyl radical
TBA: Thiobarbituric acid
TBARS: Thiobarbituric acid reactive substance
TCA: Trichloroacetic acid
TEAC: Trolox Equivalent Antioxidant Capacity
INTRODUCTION

In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. ROS produced in vivo include superoxide radical (O²⁻), hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl) and the hydroxyl radical (OH·). The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease (Liao and Yin, 2000; Hensley and Floyd, 2002; Winterbourn 2002). Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS). In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Zang et al., 2003, Selloum et al., 2004; Horvathova et al., 2005). There is now a marked interest in antioxidant activity of phytochemicals present in the diet. Intake of dietary antioxidants is increasingly suggested to help maintain an adequate antioxidant status and, therefore, the normal physiological function of a living system.

Fruits and vegetables represent good sources of natural antioxidants for the human diet, containing various antioxidant components strongly proposed to be associated with reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer’s disease, cataracts and age-related functional decline (Ismail et al., 2004; Singh and Rajinia, 2004; Garcia-Alonso et al., 2004). The Mauritian diet comprises a wide array of exotic fruits, vegetables and beverages that have previously been characterised for their phenolic, vitamin C contents and antioxidant capacities (Luximon-Ramma et al. 2003; Bahorun et al., 2004; Luximon-Ramma et al. 2005). Chinese goyava, goyava, starfruits, hogplum and Passion fruits and vegetable cultivars of Chinese cabbage, onion, mugwort and broccoli have been found to be rich sources of flavonoid antioxidants and exhibited remarkably high antioxidant capacities when assessed using TEAC assay and FRAP assays. However, it is increasingly argued that due to the complex nature of the different classes of phytochemicals, no one method can comprehensively predict efficacy of food extract and the use of a multimethod approach in antioxidant activity assessment is strongly recommended (Frankel and Meyer, 2000; Aruoma, 2003).

The present work therefore focuses on the relationship between the in vitro antioxidant capacity of the Mauritian exotic fruits and vegetable extracts evaluated by two additional assays, the OCl-radical scavenging assay (HOCl method) and the hydroxyl scavenging assay (Deoxyribose method), and levels of total phenols, total flavonoids, total proanthocyanidins and vitamin C of the extracts. The pro-oxidant propensities of the extracts were also evaluated using the copper-phenanthroline assay (Aruoma 1993).

MATERIALS AND METHODS

Mauritian exotic fruit and vegetable cultivars

Fruit samples were collected during the year 2002 fruit-bearing season. Samples of red Psidium cattleianum fruits were obtained from Bigarra while Lettuce was collected at random from commercial gardens at Vacoas (Central region of Mauritius). All other mature ripe fruits were harvested at random from “La Compagnie Agricole de Labourdonnais” at Mapou, Mauritius while vegetables were purchased from the farms of the Ministry of Agriculture, Food Technology and Natural Resources (Mauritius). Table 1 lists the names of studied fruits and vegetables, their sample types, harvest sites and parts used for analysis. Voucher specimen of fruit and vegetable samples were deposited in the Department of Biosciences, Faculty of Science, University of Mauritius.

Extraction Procedure

100 g of the edible parts of fresh fruits or vegetables were homogenized in acetone/water (70/30 v/v) (2 x 300 ml) and left to macerate for 24 h at 4°C. After filtration the residue was homogenized in methanol 100% (2 x 300 ml) and left again to macerate for 24 h at 4°C. The combined filtrates were reduced to the aqueous phase in vacuo at 37°C before being washed with dichloromethane (3 x 150 ml) to remove fat-soluble substances. The aqueous extract was concentrated and divided into two equal
Mauritian exotic fruits and vegetables: antioxidant and pro-oxidant propensities of their prophylactic agents.

A Luximon-Ramma et al.

One was freeze-dried and redissolved in methanol at a final 1:5 fresh weight: volume ratio and was used for the quantitative analysis of phenolic compounds. The second aliquot was used to determine antioxidant activity.

**Table 1** Names of fruit species, their fruit types / subcultivars, harvest sites and parts used for analysis.

<table>
<thead>
<tr>
<th>Scientific Names</th>
<th>Common names</th>
<th>Sample Type / Variety</th>
<th>Collection sites</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psidium cattleianum</em> Sabine</td>
<td>Chinese goyava</td>
<td>Red Type</td>
<td>Bigarra</td>
<td>Whole</td>
</tr>
<tr>
<td><em>Psidium guajava</em> L.</td>
<td>Goyava</td>
<td>Pink Type</td>
<td>Mapou</td>
<td>Whole</td>
</tr>
<tr>
<td><em>Averrhoa carambola</em> L.</td>
<td>Starfruit</td>
<td>Sweet</td>
<td>Mapou</td>
<td>Whole</td>
</tr>
<tr>
<td><em>Carica papaya</em> L.</td>
<td>Pawpaw</td>
<td>Exotica</td>
<td>Mapou</td>
<td>Pulp</td>
</tr>
<tr>
<td><em>Litchi chinensis</em> Sonnerat</td>
<td>Litchi</td>
<td>-</td>
<td>Mapou</td>
<td>Pulp</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vegetables</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brassica oleracea</em> L. var. <em>botrytis</em> L. sub. var. <em>cymosa</em></td>
<td>Brocoli</td>
<td>Packman</td>
<td>Richelieu</td>
<td>Flower</td>
</tr>
<tr>
<td><em>Brassica oleracea</em> L. var. <em>capitata</em> L.</td>
<td>White cabbage</td>
<td>KKCross</td>
<td>Réduit</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Lactuca sativa</em> L.</td>
<td>Lettuce</td>
<td>Mignonette</td>
<td>Vacoas</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em> Mill.</td>
<td>Tomatoes</td>
<td>MST / 32</td>
<td>Quatre-Bornes</td>
<td>Whole</td>
</tr>
<tr>
<td><em>Capsicum annuum</em> L.</td>
<td>Chilly</td>
<td>Cypaye</td>
<td>Réduit</td>
<td>Whole</td>
</tr>
</tbody>
</table>

**Antioxidant Activity**

**Hypochlorous Acid Scavenging Assay**

This assay is adapted from Weiss et al. 1982 and the antioxidant capacity was based on the ability of the extract to scavenge hypochlorous acid. OCl⁻ was prepared by adjusting the pH of a 1% (v/v) solution of NaOCl to 6.2 with dilute sulphuric acid. The working concentration of the stock solution was determined spectrophotometrically by measuring its absorbance at 235 nm and applying a molar extinction coefficient of 100 M⁻¹ cm⁻¹. The reaction mixture contained taurine (10 mM), HOCl (1 mM), plant extract (variable concentrations), phosphate saline buffer (pH 7.4) in a final volume of 1 ml. The solution was mixed thoroughly and incubated for 10 minutes at ambient temperature followed by the addition of 10 µl of potassium iodide. A yellow coloration was developed and the absorbance was read at 350 nm. The results were expressed as IC₅₀ values (g FWL⁻¹).

**Hydroxyl Radical Assay**

The deoxyribose method for determining the scavenging effect of the extracts on hydroxyl radical was performed (Halliwell et al., 1987; Aruoma 1994). The reacting mixture contained in a final volume of 1 ml the following reagents: 200 µl KH₂PO₄-KOH (100 mM), 200 µl deoxyribose (15 mM), 200 µl FeCl₃ (500 µM), 100 µl EDTA (1 mM), 100 µl sample, 100 µl H₂O₂ (10 mM) and 100 µl ascorbic acid (1 mM). Reaction mixtures were incubated at 37 °C for 1 h. At the end of the incubation period, 1 ml 1% (w/v) TBA was added to each mixture followed by the addition of 1 ml 2.8% (w/v) TCA. The solutions were heated in a water bath at 80 °C for 20 min to develop the pink coloured MDA-(TBA)₂ adduct. As turbidity was encountered, the MDA-(TBA)₂ chromogen was extracted into 2 ml butan-1-ol and its absorbance measured at 532 nm. Results were expressed as IC₅₀ values (g FWL⁻¹).
Mauritian exotic fruits and vegetables: antioxidant and pro-oxidant propensities of their prophylactic agents.
A Luximon-Ramma et al.

Pro-oxidant Activity

_Copper-phenanthroline (Cu-phen) assay_

The Cu-phen assay (Gutteridge and Halliwell, 1982) was conducted essentially as described by Aruoma (1993). Reaction mixture contained in a final volume of 1.2 ml, the following reagents in order of addition indicated: 100 µl 1,10-phenanthroline (1.8 mM stock solution made up in water having initially dissolved the crystals in 50 µl ethanol), 480 µl copper (II) chloride (250 µM), 300 µl DNA (1.68 mg/ml) and 120 µl KH₂PO₄-KOH buffer at pH 7.4 (100 mM). Ascorbate (stock solution: 2.88 mM) or extracts (100 µl) were added to initiate the reaction. The tubes were incubated at 37°C for 1 hour. At the end of the incubation period 100 µl 0.1 M EDTA was added to stop the reaction. DNA damage was assessed by adding 1 ml of 1% (w/v) TBA in 0.05 M NaOH and 1 ml 25 % (v/v) HCl followed by 15 minutes incubation at 80°C. The pink chromogen formed was extracted into 2 ml of butan-1-ol and the absorbance measured at 532 nm. Results were expressed as the absorbance at 532 nm.

Statistical Analysis

Simple regression analysis was performed to calculate the dose-response relationship of standard solutions used for calibration as well as test samples. Linear regression analysis was performed, quoting the correlation coefficient rxy between antioxidant activities, phenolic classes and vitamin C. All results are expressed as mean value ± standard deviation (n = 3).

RESULTS

The OCl⁻ scavenging capacities of the Mauritian exotic fruit and vegetable samples studied are shown in Figure 1A. Chinese goyava showed the highest scavenging capacity as evidenced by the lowest IC₅₀ value (4.11 g FWL⁻¹) followed by starfruit (IC₅₀ =13.06 g FWL⁻¹). Litchi is less effective with an IC₅₀ value of 153.28 g FWL⁻¹. Among the vegetables, broccoli showed highest protection against the oxidation of KI by the OCl⁻ radical (IC₅₀ = 43.79 g FWL⁻¹). Tomato, chilly and cabbage demonstrated moderate potencies while lettuce showed the weakest radical scavenging capacity its high IC₅₀ value (Figure 1A) indicated

The fruit and vegetable extracts were also scavengers of the hydroxyl radical generated by a Fenton system in a concentration dependent manner (Figure 1B). A similar pattern of antioxidant capacities was observed using the in vitro deoxyribose damage protection assay. Chinese goyava afforded highest protection against degradation of the sugar moiety by the highly reactive OH. Radical (IC₅₀ = 4.74 g FWL⁻¹) while litchi extract gave a 50 % radical scavenging activity at 315 g FWL⁻¹. Similarly, among the vegetables, broccoli showed highest antihydroxyl radical activity (IC₅₀ = 144.76 g FWL⁻¹) while lettuce was characterized to be the poorest hydroxyl scavenger (IC₅₀ = 1160.09 g FWL⁻¹).

To determine a putative correlation between the observed antioxidant effects and the phenolic constituents, linear regression analysis was performed and the various correlation coefficients obtained are presented in Table 2. Based on the HOCl assay a relatively good correlation was observed with the total phenols (r = 0.68) and total proanthocyanidin contents (r = 0.61). Table 2 also indicates that flavonoid contents showed moderate influence while a poor correlation was observed between the ascorbate levels and the antioxidant capacity. Moderate correlations were also observed between the phenolic contents, vitamin C levels and the OH. Scavenging capacities as evaluated by the deoxyribose assay (Table 2).

Some compounds are capable of redox cycling metal ion required for hydroxyl generation, thus increasing radical production thereby exhibiting a pro-oxidant activity. In order to evaluate the pro-oxidant potential of the extract, the copper-phenanthroline assay was used. Data in Table 3 show that all vegetable extracts did not promote DNA damage above 100 g FWL⁻¹. The extent of DNA damage was lower than the basal value of ascorbate (TBARS value = 0.189). Similar observations were made on Chinese goyava, starfruit and goyava extracts. However, pawpaw and litchi showed prooxidant activities above 75 g FWL⁻¹ (TBARS = 0.180) and 50 g FWL⁻¹ (TBARS = 0.175) respectively.

Table 2 Correlation coefficients between OCl⁻ and OH scavenging capacities and total phenols, total flavonoids and total proanthocyanidin and vitamin C contents of Mauritian exotic fruits and vegetables evaluated by the linear regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>OCl⁻</th>
<th>OH⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Phenols</td>
<td>0.68</td>
<td>0.48</td>
</tr>
<tr>
<td>Total Flavonoids</td>
<td>0.44</td>
<td>0.34</td>
</tr>
<tr>
<td>Total Proanthocyanidins</td>
<td>0.61</td>
<td>0.45</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.19</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Figure 1 Concentration of extract used to obtain 50% inhibition (IC₅₀) of (A) OCl⁻ and (B) OH radicals
Mauritian exotic fruits and vegetables: antioxidant and pro-oxidant propensities of their prophylactic agents.

A Luximon-Rammat et al.

DISCUSSION

Epidemiological studies have shown strong and consistent protective effects of fruits and vegetable consumption against the risk of several age-related diseases such as cancer, cardiovascular disease, cataract and macular degeneration. A growing body of evidence suggests that compounds with antioxidant activity may play a major role to explain the benefits of plant-based diets. Antioxidant status assessment of commonly consumed food items is not only relevant to health care scientists and professionals but also to producers and consumers. In the present study, the in vitro free radical scavenging capacities of ten exotic fruits and vegetables commonly consumed in Mauritius were assayed using the HOCl, deoxyribose and copper-phenanthroline assays. These methodologies have increasingly been utilised in antioxidant and pro-oxidant status assessment of medicinal plants (Amarowicz et al., 2004; Zhao et al., 2004; Canadianovic-Brunet et al., 2005), fruits (Martynez et al., 2000; Franke et al., 2004), vegetables (Sandovala et al., 2002; Singh and Rajinia 2004), storage roots, (Kim et al. 2005; Houa et al. 2005), spice (Candan and Sokmen, 2004), seaweeds (Yan et al., 1998) and beverages (Arnous et al., 2002; Nagai and Yukimoto, 2003).

The involvement of non-specific oxidizing and chlorinating OCl\(^{\cdot}\) radical in a variety of chemical and biological processes including its reaction with various biomolecules such as proteins, carbohydrates, phospholipids and DNA (Pruzt, 1996; Schaur et al., 1998; Arnhold et al., 2001), has prompted several studies towards the assessment of potential compounds capable to inhibit its deleterious effects. The OCl\(^{\cdot}\) radical scavenging capacities of some Mauritian fruit and vegetable extracts have therefore been evaluated in this respect. Chinese goyava and broccoli possessed the highest OCl\(^{\cdot}\) radical scavenging capacity with IC\(_{50}\) values of 4.11 g FWL\(^{-1}\) and 43.79 g FWL\(^{-1}\) respectively. Starfruit, goyava, cabbage and tomato also exhibited important activities. However, pawpaw, litchi, chilly and lettuce were weak OCl scavengers as indicated by their high IC\(_{50}\) values (Table 2). These fruit and vegetable extracts had also low activities in the previously reported TEAC and FRAP assays (Luximon-Rammat et al., 2003, Bahoran et al., 2004)

HOCl is produced in vivo by the oxidation of Cl\(_{2}\) ions catalysed by neutrophil-derived myeloperoxidase in the presence of H\(_{2}\)O\(_{2}\), at sites of inflammation. One of the major extracellular targets of HOCl is \(\alpha\)-antiproteinase, the major inhibitor in human plasma of proteolytic enzymes, such as elastase (Arumoa, et al., 1989). The HOCl assay has been utilized by several authors to study the anti-inflammatory effects of plant extracts. Such extracts include Mangifera indica L. (Martynez et al., 2000), the brown algae Laminaria japonica (Zhao et al., 2004) and medicinal plants including Hypericum androsaerum and Lippia citriodora (Valentao et al., 2002a,b). Among these, Mangifera indica L. (Vimang) showed an IC\(_{50}\) of 0.04% thereby supporting its use in traditional medicine as an anti-inflammatory and cancer preventive agent where the protective effects seem to be mainly ascribed to its free radical scavenging capacities (Martynez et al., 2000). A similar observation was made in the bark extract of the medicinal plant, Uncaria tomentosa (Desmarchelier et al., 1997). Thus, it can be suggested that the Mauritian Chinese goyava and broccoli samples possessing important OCl\(^{\cdot}\) scavenging capacities may have potential protective effects against inflammation processes.

The fruit and vegetable extracts were scavengers of hydroxyl radical generated in the deoxyribose assay. Scavenging was dose dependant and the rank order was as follows for the fruits: Chinese goyava > starfruit > goyava > pawpaw > litchi and broccoli > cabbage > tomato > chilly > lettuce for the vegetables. The deoxyribose assay has been widely utilized in evaluating antihydroxyl efficacies of various plant extracts including ginseng (Kitts et al., 2000), mushrooms (Shon et al., 2003), potato (Singh and Rajinia, 2004), sea algae (Nagai and Yukimoto, 2003), green tea (Chen et al., 2005), wines (Arnous et al., 2002), orange juices (Franke et al. 2004) and medicinal plants (Schinella et al., 2002; Gynami and Aniya, 2002; Steenkampa et al., 2005). For instance, Chaminda et al. 2001 reported a 30 % hydroxyl scavenging capacity for the medicinal plant Cassia fistula at 125 \(\mu\)g/ml while Martynez et al., (2000) observed an IC\(_{50}\) value at 0.011 % w/v with Mangifera indica L.

Similar high antioxidant activities had been previously reported for the Mauritian Chinese goyava and broccoli cultivar (Luximon-Rammat et al., 2003, Bahoran et al. 2004). The TEAC value for Chinese goyava (45 \(\mu\)mol TEAC/g FW) was very close to that observed for blueberries (45.9 \(\mu\)mol TEAC/g FW) (Kaur and Kapoor, 2001), one of the richest source of antioxidant so far studied and higher than those reported for the Rubus species (0 to 25.3 \(\mu\)mol TEAC/g) which has been recommended for the improvement of nutritional value through germplasm programmes (Deighton et al., 2000). Extracts of broccoli have already been cited in the literature as good antioxidants in hydrophilic and lipophilic
antioxidant assay systems whereas chilly and lettuce have been reported to have weak radical scavenging capacities (Proteggente et al., 2002; Chu et al. 2002; Zhang and Hamauzu, 2004).

Data in Table 3 show that fruit and vegetable extracts did not promote DNA damage at concentrations ranging from 25-100 g FW L\(^{-1}\) except for pawpaw and litchi. The extent of damage was lower than the basal value observed for ascorbate (240 µM), which was taken as the positive control. Pawpaw and litchi extracts showed pro-oxidant activities above 75 g FW L\(^{-1}\) (TBARS = 0.180) and 50 g FW L\(^{-1}\) (0.175) respectively. This may be explained by the fact that some plant-derived compounds are capable of redox cycling metal ion required for hydroxyl generation. Pawpaw and litchi possess relatively important levels of vitamin C (929 ± 19 µg g\(^{-1}\) FW and 138 ± 15 µg g\(^{-1}\) FW respectively) (Luximon-Ramma et al., 2003) which at low concentration (4-240 µM) is now being considered as prooxidant due to their weak metal-chelating effects, their strong electron-donating capacity and their ability to stimulate oxidative effects (Yen et al. 2002). Ascorbate possesses the capacity to redox-recycle and maintain the supply of Fe\(^{2+}\) required for hydroxyl radical generation (Li and Xie, 2000; Schinella et al., 2002). However, Davey et al. 2000 argued that there is currently no clear evidence that the redox-recycle reactions of ascorbate are of significance in vivo. Thus, in spite of the observed pro-oxidant activities, the risk for human consuming these fruits and vegetables may be very low, due to enzymatic activities and pH changes in the digestive tract prior to their assimilation (Nemeth et al., 2003).

Table 3 Effect of fruit and vegetable extracts and ascorbic acid (240 µm) on copper 1-10 phenanthroline dependent DNA damage

<table>
<thead>
<tr>
<th>Fruit samples</th>
<th>Conc gL(^{-1})</th>
<th>TBARS (532 nm)</th>
<th>Vegetable samples</th>
<th>Conc gL(^{-1})</th>
<th>TBARS (532 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.189</td>
<td>Broccoli</td>
<td>25</td>
<td>0.033</td>
</tr>
<tr>
<td>Ascorbate (240 uM)</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>0.013</td>
</tr>
<tr>
<td>Chinese Goyava Red</td>
<td>25</td>
<td>0.033</td>
<td>Broccoli</td>
<td>25</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.065</td>
<td></td>
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<td>0.025</td>
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<td></td>
<td>75</td>
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<td>0.038</td>
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<tr>
<td></td>
<td>100</td>
<td>0.130</td>
<td></td>
<td>100</td>
<td>0.050</td>
</tr>
<tr>
<td>Goyava Pink</td>
<td>25</td>
<td>0.018</td>
<td>Cabbage</td>
<td>25</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.035</td>
<td></td>
<td>50</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.053</td>
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<td>75</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.070</td>
<td></td>
<td>100</td>
<td>0.080</td>
</tr>
<tr>
<td>Starfruit Sweet</td>
<td>25</td>
<td>0.028</td>
<td>Lettuce</td>
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<td>50</td>
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<td>75</td>
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<td>75</td>
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<td>100</td>
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<td>Chilly</td>
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<td>Tomato</td>
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<td>100</td>
<td>0.070</td>
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With the view to establishing correlations between phytophenolic constituents of the fruit and vegetable extracts and the antioxidant activities, linear regression analysis was performed. Total phenols and total proanthocyanidin contents strongly influenced the OCl\(^{-}\) scavenging capacities (Table 2). Similar observation was made by Neergheen et al., (2005) while characterising the phenolic contents and assessing antioxidant activities of some selected Mauritian endemic plants belonging to the Rubiaceae and Myrtaceae families. Valentao et al., (2002a) also observed the same type of relation
between phenolic levels and hypochlorous acid scavenging activities in the medicinal plant *Hypericum androsaemum*. Bahorun et al., 2004 highlighted the important contribution of these compounds to the antioxidant capacity in various Mauritian exotic fruits and vegetables. However, in this study these substances seem to moderately influence hydroxyl radical scavenging capacities of the extracts (r = 0.48 and 0.45 respectively). These observations therefore justify the use of a multimethod approach in antioxidant activity assessment as recommended in literature (Frankel and Meyer, 2000, Aruoma, 2003).

Bahorun et al., 2004 previously reported relatively important levels of quercetin derivatives prominently accounting for the antioxidant capacity of Mauritian vegetables. However, our data show that the flavonoid subclass had a moderate influence on both the OCl- and OH- scavenging capacity of fruit and vegetable extracts. This seems consistent with observations made by García-Alonso et al., 2004, who showed that high levels of flavonoids in fruits poorly correlated with antioxidant activities when assayed by the TBARS and TEAC methods. The antioxidant activities were mainly explained by the synergistic (or antagonistic?) action of the various phytochemicals present in the extracts.

Vitamin C levels poorly correlated with the OCl- radical (r = 0.19) and moderately with OH- Radical scavenging capacity of the fruit and vegetable extracts. Comparable data were previously reported by Bahorun et al., 2004 in exotic fruits and vegetables with the TEAC and FRAP assays. These observations are also supported by available literature data on fruits, vegetables and fruits juices (Kalt et al., 1999; Gardner et al., 2000; Szeto et al., 2002).

**CONCLUSION**

This study highlights the potential antioxidant propensities of some Mauritian exotic fruits and vegetables especially Chinese goyava, starfruits and broccoli that could be used as supplements in a balanced diet within nutritional programmes. The identification of the major bioactive components in these foods and the characterization of their biological activities may prove useful for their utilization against the incidence of a number of diseases, particularly cardiovascular diseases, diabetes and cancer which accounted for 51%, 6% and 11% of total deaths (CSO, 2004) respectively in the Mauritian population.

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SPECTROSCOPIC QUANTITATIVE ANALYSIS OF FOOD: CHEMOMETRICS IS A VITAL TOOL

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ABSTRACT

Spectroscopy is widely used in food science for quantitative analysis. Spectroscopic techniques use many data points and they are fast, accurate and non-destructive. Quantification using spectroscopic techniques involves analysis of standard samples leading to a calibration set and then the latter is used for samples to be investigated. The crux for the analysis is to have a good model from the calibration set so that errors for the prediction are within acceptable limits. Chemometrics is commonly used for analysing chemical data. Computers have given rise for a new age for acquisition, processing and interpretation of chemical data. However, although commercial software is available for the processing of data, there is a necessity for a deeper understanding of the methods used by the software. Understanding the concepts used for analysis helps to develop methods, which are more efficient and appropriate for treatment of experimental data and to validate the results obtained. This work reviews the applications of the Beer-Lambert Law and chemometrics methods, such as least squares regression model, classical least squares model, inverse least squares model, principal component regression and partial least squares regression, in quantitative food analysis.

Keywords: spectroscopy, quantitative analysis, statistical, chemometrics

INTRODUCTION

Spectroscopy (http://www.spectroscopynow.com/) is a technique that uses the interaction of energy with a sample to carry out analysis, which can be qualitative and quantitative (Duckworth, 1998). There are different spectroscopic methods including infrared spectroscopy, atomic absorption spectroscopy, and nuclear magnetic resonance spectroscopy. These spectroscopic methods differ in the source of energy used by the spectrometer to investigate the sample. A spectrum is the data that is generated from spectroscopy and it is generally a plot of the intensity of energy against the wavelength of the energy.

Spectroscopy has emerged into an increasingly important tool for the quantitative analysis of food materials (Christy et al., 2004). What has brought this importance? The most important advantage is that a spectrum of a sample contains many data points unlike other quantitative methods, for example single element absorption and pH measurement, where single point measurements are involved. Every data in a spectrum is related to the components made up the sample under investigation and thus many more measurements per sample are available compared to single point methods. Spectroscopic techniques are also fast, as they require little or no sample preparations, accurate and non-destructive. Further spectroscopic techniques have high acquisition speed with high spectral sensitivity due to advances in optical, mechanical, and microelectronic technologies.

Quantitative analysis using spectroscopic techniques assumed that the concentrations of the components of a sample are related to the spectral data generated from the measurement method. The crux for the analysis is to have a calibration set leading to a model, which can be applied to unknown sample. Also the model should have predictions within acceptable limits. Software packages using chemometrics algorithms are commonly used for the analysis of spectral data.

The term chemometrics was introduced by Wold and Kowalski in the early 1970s. Chemometrics is the chemical discipline that uses mathematics and statistical methods to design or select optimal measurements procedures and experiments. Chemometrics also provides relevant information on systems by analysing chemical data. The availability of personal computers has given rise for a new age for acquisition, processing and interpretation of chemical data. Quantitative analysis cannot go without chemometrics, 90% of quantitative analysis involves the use of hardware to obtain spectra and...
Spectroscopic Quantitative Analyses of Food: Chemometrics is a Vital Tool. P. Ramasami and S. Jhaumer-Laulloo.

the remaining 10% requires chemometrics for rigid statistical treatment (Bertrand et al., 1984, Cowe and McNicol, 1985, Cadet and Guardia, 2000, McClure, 2003). However, although commercial software is available for the processing of data, the methods used by the software have to be understood. This is because the procedure for analysis may be adapted for more efficient and appropriate treatment of experimental data. The results obtained can also be validated easily. Further, the definition of chemometrics shows that it has wide applications in chemical measurement sciences. It is not just limited to the analysis of experimental data but also, more important, considers what happens after analysis.

Quantitative analysis uses relationships between the spectral absorbances taken to be independent data and concentrations considered to be dependent data. Calibration equations are obtained relating these dependent and independent data using methods, which are classified as univariate and multivariate. Methods, which are univariate, attempt to solve one equation obtained from one measured value and one calibration parameter for the sample under investigation. However multivariate methods (Robert et al., 1987) attempt to solve a series of equations obtained from many measurements for one sample at a given calibration parameter. The advantage with multivariate methods is that more data in terms of spectral absorbance can be considered for analysis. This gives better results and can be understood due to averaging. Chemometrics is very helpful to achieve these results as it requires a change in one’s approach from univariate to multivariate thinking (Martens and Naes, 1989, Stordrange et al., 2003) and this requires a paradigm shift and a model is devised closer to reality.

This paper aims to review the fundamentals for spectroscopic quantitative analysis including the Beer-Lambert Law and chemometrics methods commonly used by commercial software. The different chemometrics methods reviewed include least squares regression model (LSR) (Lawson and Hanson 1974), classical least squares model (CLS), inverse least squares model (ILS), principal component regression (PCR) (Vigneau et al., 1997) and partial least squares regression (PLS) (Geladi and Kowalski, 1986). PCR and PLS have the largest number of applications of chemometrics methods. They are widely used techniques in quantitative analysis of complex mixture by spectroscopic methods, instead of time and chemicals consuming wet chemistry methods.

BEER-LAMBERT LAW

Spectroscopic methods for quantitative analysis use the Beer-Lambert Law and the latter defines a relationship between the spectrum and the component of a sample. The Beer-Lambert law is the basis of all chemometrics methods. Mathematically the law is expresses as Equation (1):

$$A_\lambda = \varepsilon_\lambda cL$$  

Where $A_\lambda$ is the sample absorbance value at specific wavelength $\lambda$, $\varepsilon_\lambda$ is the molar absorption coefficient at specific wavelength $\lambda$, $L$ is the cell thickness and $c$ is the molar concentration.

The molar absorption coefficient for every substance is different but it is a constant for a given compound and a given wavelength. The challenge for quantitative analysis using Equation (1) is to obtain the absolute value of the constant.

LEAST SQUARES REGRESSION

In this univariate model the height or area of a specific peak in the spectrum is assumed to be related to the concentration of the components as shown in Equations (2) and (3):

$$c = \sum_{i=1}^{n} \text{(coefficient)}_i \times \text{(area)}$$  

$$c = \sum_{i=1}^{n} \text{(coefficient)}_i \times \text{(height)}$$
Before the recent technological advances of computer, the measurement of peak heights or peak areas was the common technique for quantitative analysis. This model is applicable only if an isolated peak is identified in the spectrum with no inference due to the other components and the peak related to the component being investigated. It is helpful to have the spectrum of the pure component so that the peak to be considered may be decided and measured for all the standard samples and the unknown samples being investigated.

The LSR calculates the coefficients of a given equation such that the differences between the known spectral responses in terms of areas or heights and the predicted values are minimized. Since there can be more than one coefficient, there must be at least the same number of standard samples as the number of unknown coefficients. Once the coefficients have been obtained, the equation can be used for predictions. However the least regression model is limited to pure samples or mixtures where there are no interferences between peaks due to other constituents. Further although polynomials are usually considered, other functions can be more applicable and hence the equation to be used becomes another variable.

CLASSICAL LEAST SQUARES REGRESSION

If the cell thickness is kept constant, Equation (1) can be written as shown in Equation (4):

\[
A_i = K_i C
\]

Where \( K_i \) is a constant including the molar absorption coefficient. The constant at a given wavelength can be calculated from the absorbance and concentration of a standard sample. More reliable results are obtained if absorbances are measured for different concentrations and then applying least square regression. In cases where more than one component is present, it is required to have as many equations as constants. However it is possible to standardize only if resolved bands are obtained for the components in the sample. In case there is overlapping of bands, Beer-Lambert’s Law comes to the rescue and absorbances of different components are additive at the same wavelength. Hence for \( m \) components system, one of the equations is as shown in Equation (5) including residual errors \( E \):

\[
A_{i1} = \sum_{i=1}^{m} K_{j,i} C_i + E_{i1}
\]

There are \( p \) equations as Equation (5) for the calibration of \( m \) components sample. These \( p \) equations can be written in matrix form as shown in Equation (6):

\[
[A] = [K][C] + [E]
\]

Where \([A]\) is the matrix of absorbance with order \((p \times 1)\) at the \( p \) selected wavelengths, \([K]\) is the matrix of molar absorptivity constants with order \((p \times m)\), \([C]\) is the matrix of concentrations of the \( m \) components with order \((m \times 1)\) and \([E]\) is the matrix of absorbance error with order \((p \times 1)\). The \([E]\) matrix can be related to the offset or “bias” of the model.

Calibration using the CLS model is not limited to wavelengths but uses the entire spectrum. If the entire spectrum is used, Equation (6) can be extended to as shown in Equation (7):

\[
\begin{bmatrix}
A_{i,1} & \cdots & A_{i,p} \\
\vdots & \ddots & \vdots \\
A_{n,1} & \cdots & A_{n,p}
\end{bmatrix} = \begin{bmatrix}
c_{1,1} & \cdots & c_{1,m} \\
\vdots & \ddots & \vdots \\
c_{n,1} & \cdots & c_{n,m}
\end{bmatrix} \begin{bmatrix}
K_{1,1} & \cdots & K_{1,p} \\
\vdots & \ddots & \vdots \\
K_{n,1} & \cdots & K_{n,p}
\end{bmatrix} + \begin{bmatrix}
E_{1,1} & \cdots & E_{1,p} \\
\vdots & \ddots & \vdots \\
E_{n,1} & \cdots & E_{n,p}
\end{bmatrix}
\]

Where \( n \) is the number of samples, \( m \) is the components, \( p \) is the number of wavelengths used for calibration and the subscripts correspond to the order of the matrix. Equation (7) can be solved to obtain the \( K \)-matrix (Brown et al. 1982), Equation (8), and then it can be used to predict concentration of unknown samples.

\[
[K] = \left[ c^T \times c \right]^{-1} \times \left[ c^T \times \left[ A - [E] \right] \right] \quad (8)
\]

The major advantage of classical least squares is that the entire spectrum may be considered for quantification and thus the elements of the \( K \)-matrix represent genuine absorptivities with reference to the spectra of the individual components. However, the major disadvantage is that the equations must be calibrated for all the components of the sample and the unknowns must be of the same compositions as the standards. This method is also not applicable in case the components in a sample interact with each other.

**INVERSE LEAST SQUARES**

The composition of a mixture is difficult to know and sometimes only the concentrations of certain components are of interest. For these mixtures, it is not possible to use the classical least squares model but the quantification can still be achieved by ILS which is multivariate method. It is possible to rewrite Equation (4) to obtain Equation (9):

\[
c = \frac{A_i}{E_A} \quad (9)
\]

Equation (9) can be expressed as shown by Equation (10):

\[
c = A_i P + E \quad (10)
\]

Where \( c \) is the component concentration, \( P \) is the calibration coefficient, \( A_i \) is the absorbance at the wavelength \( \lambda \), and \( E \) is the concentration error. Equation (10) can be interpreted as the concentration to be a function of the absorbances for a series of given wavelengths. Equation (10) for the different standards and for the whole spectrum can be written in matrix form as shown in Equation (11):

\[
\begin{bmatrix}
  c_{i,1} - & - & c_{i,m} \\
  - & - & - \\
  - & - & - \\
  - & - & - \\
  c_{n,1} - & - & c_{n,n}
\end{bmatrix} =
\begin{bmatrix}
  A_{i,1} - & - & A_{i,p} \\
  - & - & - \\
  - & - & - \\
  - & - & - \\
  A_{n,1} - & - & A_{n,p}
\end{bmatrix} \times
\begin{bmatrix}
  P_{i,1} - & - & P_{i,m} \\
  - & - & - \\
  - & - & - \\
  - & - & - \\
  P_{p,1} - & - & P_{p,n}
\end{bmatrix} +
\begin{bmatrix}
  E_{i,1} - & - & E_{i,m} \\
  - & - & - \\
  - & - & - \\
  - & - & - \\
  E_{n,1} - & - & E_{n,n}
\end{bmatrix} \quad (11)
\]

Equation (11) is similar to Equation (7), it can be solved to obtain the \( P \)-matrix (Maris and Brown 1983, Brown 1984) and then the latter can be used to predict concentration of unknown samples.

The advantage of the ILS squares is unlike CLS, the coefficients of the \( P \)-matrix can still be evaluated even if all the concentrations of the components in a mixture are not known. The disadvantage of this method is that the elements of the \( P \)-matrix do not have physical meaning, since they do not reflect the spectra of the individual components. Further, due to the dimensionality of the matrix equations, the number of selected wavelengths cannot exceed the number of standard samples and thus wavelength selection is very important to achieve good calibration. In fact, the averaging effect due to selecting many wavelengths is lost and additional wavelengths lead to collinearity and overfitting.
PRINCIPAL COMPONENT REGRESSION

PCR is a two-step procedure combining principal component analysis (PCA) (Lefebvre 1983, Joliffe 1986) and inverse least squares regression to obtain the calibration model. PCA is a variable reduction process. If data for large number of variables is available and it is known that there is some redundancy in these variables, it is possible to reduce the number of variables into a smaller number of principal components that will account for most of the variance in the observed variables. The variables in a spectrum are the components in the sample, interactions between the components, instrument variations such as detector noise, variations in baseline and absorbance and differences in samples handling. PCA (Figure 1) reduces all these possible variations in original spectral data into a few new variables called scores. These new variables are linearly weighted combinations of the original spectral variables. The factors contain the weights used for each spectrum variable and thus reveal the influence of individual spectrum variables.

Thus PCA algorithm eliminates each independent variation from the standard spectra leading to two matrices namely the scores \((n \times f)\) matrix, \([S]\) and eigenvectors or spectral \((f \times p)\) orthonormal matrix, \([F]\). The calibration is as shown in Equation (12):

\[
[A] = [S][F] + [E] \tag{12}
\]

Where \([A]\) is the \((n \times p)\) matrix of spectral absorbances, \([E]\) is the matrix of residual spectra, \(f\) is the number of eigenvectors and other symbols are as defined before. It is to be noted that Equation (12) model only the spectral data and the concentrations matrix is not involved and thus PCA alone cannot be used for quantification and is rescued by inverse LSR.

**Figure 1:** Scheme for principal component analysis

![Diagram of principal component analysis](image)

The ILS regression carries out regression between the concentration matrix and the spectral absorbance matrix. It is possible to regress the concentration matrix with the scores matrix and the calibration Equation (12) can be written as shown in Equation (13):

\[
[c] = [S][R] + [E] \tag{13}
\]

Where \([c]\) is the components concentrations \((n \times m)\) matrix, \([R]\) is the \((f \times m)\) matrix of regression coefficients. Equation (13) can be solved as described before to obtain the matrix of regression coefficients.
Once the matrix of regression coefficients is obtained, Equations (12) and (13) can be combined to obtain Equation (14) which is the principal component regression model for quantification of unknowns.

\[ [c] = [A(F^T)]R + [E] \]  

(14)

The PCR model has the advantages of using the whole spectrum and complex mixtures can be analysed. However to be accurate large number of standards should be considered and prior knowledge of the model is required for the results to be interpreted efficiently.

**PARTIAL LEAST SQUARES REGRESSION**

PLS (Martens and Jensen, 1983) decomposes spectra using concentration in a single step unlike PCA, which consists of two steps to decompose the spectral matrix into a set of eigenvectors and scores, and then regressing them against the concentrations. Therefore PLS gives more weighting to spectra having higher components concentrations. PLS regression carries out decomposition both on the spectral and concentration data. Each time a new factor is calculated for the model, the scores are interchanged before the contribution is removed from the raw data. The reduced matrices obtained are then used to calculate the next factor and the process is iterated to be terminated when the desired number of factors has been calculated.

There are two forms of the PLS method and these are PLS-1 and PLS-2. PLS-1 calibrates one component, which is of interest at a time, but PLS-2 does calibration for all the components simultaneously. In case of PLS-1, the sets of eigenvectors and scores are specific for each component but if PLS-2 is used, the calculated vectors are not optimised for each individual component. Thus it is expected that PLS-1 should be more accurate for predictions that PCR and PLS-2. The major advantage of the PLS method is that it can be used for complex mixtures even if these mixtures are contaminated. The disadvantage of the PLS method is that large number of samples are required for the model to be accurate and for very large number of samples, calculations may be slow.

**CONCLUSION**

Automatic equipment has brought a situation, where routinely large quantities of data are collected and have to be processed. This causes a high degree of redundancy, where the important information first has to be extracted. Further significant information first has to be separated from unwanted data at high speeds. Chemometrics come to the rescue and it is widely used for the treatment of spectral data for quantitative analysis of food and quantification is becoming more versatile with the availability of software (website accessed Jan 2005) such as:

- MATLAB (http://www.mathworks.com/)
- Scilab (http://www-rocq.inria.fr/scilab/)
- Mathematica (http://www.wri.com/)
- Maple (http://www.maplesoft.com/)
- MathCAD (http://www.mathsoft.com/)
- MuPAD (http://www.mupad.de/)
- LastWave (http://www.cmap.polytechnique.fr/~bacry/LastWave/)
- Minitab (http://www.minitab.com/)
- Extract (http://www.extractinformation.com/)
- Sirius and Xtricator (http://www.prs.no/)
- The Unscrambler (http://www.camo.com/)
- Thermogalactic (http://www.galactic.com/)

Chemometrics is therefore a technique which can be used to model analytical instrument data. The model obtained can then be applied for quantification and quality assessment. It can be used to make routine the use of statistical models for data analysis.
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ABSTRACT

Infrared spectroscopy (IR) is a type of absorption spectroscopy, which is used to measure the ability of a sample to absorb different wavelengths of infrared radiation. The infrared energy band is defined for convenience as the near infrared (NIR) (14 000-4 000 cm⁻¹), the infrared or mid infrared (MIR) (4 000-400 cm⁻¹) and the far infrared (400-50 cm⁻¹). The MIR range consists of fundamental molecular vibrations while the NIR is composed of overtones and combination molecular vibrations. Therefore the absorption coefficients of the NIR bands are weaker than those of MIR. The NIR spectral region carries information related to CH, OH, CO and NH functional groups and this allows quantitative measurement of chemical concentrations in a matrix. However due to the strong absorption of water in the MIR and complex spectra obtained, the use of MIR was restricted for the analysis of food products. Both MIR and NIR have been developed into a powerful tool for food analysis with the advent of Fourier Transform and the use of powerful data analysis techniques. The field of application of IR in food industry is very wide; it covers the quantification of major constituents such as water, proteins, lipids and sugars. Additionally NIR has an advantage over MIR because NIR spectra can be obtained with no sample preparation and it can be used as a continuous and real time processing monitoring tool.

Key words: infrared spectroscopy, food products, analysis

INTRODUCTION

In recent years, infrared spectroscopy has been developed into an important and extremely useful method of analysis. In fact in food industry it has become an indispensable analytical tool because this fast and cost effective type of spectroscopy provides qualitative and quantitative information not available from other techniques. The most appealing feature of infrared spectroscopy is that many diverse parameters can be quickly assessed by a single scan of the ingredient. This stems from industrial developments, extensive use of computers and the development of appropriate chemometrics techniques. Applications of infrared spectroscopy in the fields of chemistry, drugs, the agro-food sector, life sciences and environmental analysis have been reported. This paper presents the characteristics, advantages, limitations and potentials of MIR and NIR. Special emphasis is placed on the application of these techniques in the food industry.

INFRARED SPECTROSCOPY

MIR and NIR are based on absorption techniques. The absorption bands in both MIR and NIR are due to molecular vibrations. While the bands in MIR are associated with fundamentals vibrations those associated with NIR are overtones and combination molecular vibrations. The MIR spectral region (4 000-400 cm⁻¹) gives distinctive patterns for many spectra and this permits the identification of different functional groups and compounds.

The absorption bands of NIR (14 000-4 000 cm⁻¹) radiation by organic molecules are due to overtone and combination bands primarily of OH, CH, NH and CO groups whose fundamental molecular stretching and bending absorb in the MIR region. These overtones are anharmonic making NIR spectra complex and not directly interpretable as MIR. Moreover, due to the lower transition probabilities in NIR, the absorption coefficient is lower by a factor of 10-100 for each step from the fundamental to the next overtone.
Infrared spectroscopy is based on the Beer-Lambert Law, which relates the absorbance of an observed band to the path length of the sample that the infrared energy passes through to its concentration and absorption coefficient. It is expressed as:

\[ A = εcL \]

Where \( A \) is the absorbance and \( ε \) is the absorption coefficient, \( c \) is the concentration and \( L \) is the path length (or thickness of the sample).

As absorption coefficient decreases, the path length must increase to measure the absorbance of the material. On the contrary if the absorption coefficient is large the path length must be small, otherwise the measured value for the absorbance will saturate the detector of the infrared spectrometer. The weaknesses of these absorption bands proved to be benefit as samples can be analysed directly without dilution.

**Instrument**

The field of instrument in infrared is constantly evolving. The first generation of MIR instruments used a high-resolution diffraction monochromator and this has been mainly used for qualitative analysis for the identification of chemical compound. With the advent of Fourier Transform spectroscopy, MIR has developed considerably with the use of powerful microcomputers and the advent of new techniques such as ATR (attenuated total reflectance) cells (Crocombe et al., 1987, Van de Voort and Ismail, 1991, Cadet et al., 1991).

In NIR spectroscopy, the most common type of instrument used in the analysis of food is the sequential instrument, where absorbances are collected sequentially in time.

However, the new trend is that analysis is moving closer to sampling point allowing real time analysis. Therefore the future generation of IR instrument is evolving into Fourier Transform spectroscopy, IR imaging spectrometry and hand held IR spectrometry.

**Infrared Calibration**

MIR spectra normally contain well-defined peaks, which are associated to different functional groups. However, very often the peaks overlap in the fingerprint region of the spectra. NIR spectra show overlapping bands, which are the result of the first and second overtones as well as of combination bands. As a result NIR spectra cannot be used to determine analytes concentrations directly because of the way in which near infrared radiation passes through and is reflected from the sample.

Meaningful information can be extracted from both NIR and MIR spectra with the help of sophisticated chemometrics techniques (Bertrand et al. 1984, Cowe and McNicol (1985). Robust prediction equations are normally based on calibration data sets. The calibration procedure includes:

- Selecting the calibration set
- Determine standard concentrations using classical chemistry and biochemical tests
- Collecting spectral data for the samples
- Developing the calibration model
- Validating the calibration model using separate set of samples

**Analytical characteristics**

The analytical characteristics of NIR and MIR display certain advantages that make these techniques attractive alternatives to classical analysis. First they are rapid, do not require chemicals and are non-destructive. The best illustration is the determination of protein content. The AOAC (Association of the Official Analytical Chemists) method for the determination of protein is the Kjeldhal method which takes 3 hours and use chemicals such as sulphuric acid, which are pollutants. The growing concern of scientists is to have environmentally safe by-products. In fact with NIR and MIR, it takes less than a minute to get the protein content. The other advantage of spectroscopic technique is that it requires no to minimal sample preparation. A similar illustration can be provided with regard to quantification of fat. The time required for a single determination using classical method (i.e. soxhlet

extraction) is 6-8 hours. In infrared spectroscopic method all these parameters can be detected simultaneously within minutes. Sample size or physical state (solid, liquid, gas) is also not a problem. The main disadvantage of spectroscopic techniques is that it is based on correlations derived from calibration set.

APPLICATIONS

Near Infrared spectroscopy

NIR is the most commonly used spectroscopic method in the food industry for the quantification of major biochemical constituents (Cadet et al., 2000, Pasquini et al., 2003, Bakeev, 2003). Its success is primarily due to its simplicity and its rapidity. NIR spectra are normally collected either as transmittance (light passing through translucent media) or reflectance (light diffusely reflected from opaque media) mode.

Norris developed the first NIR apparatus for the quantification of water in foodstuffs (Norris, 1962 and Hart, 1965). The measurement of the components such as proteins, lipids and carbohydrates was hindered by the presence of water. But with the advent of multiregression analysis to spectral data this problem was solved and it has also allowed the measurements different constituents. NIR has been used to measure moisture, sugars, protein and fat in food (Davies and Grant, 1987, Hong and Tsou, 1998 and Osborne, 1993).

Cereals and Cereal Products

NIR is currently being used as a quality testing of crossbred material from wheat breeding programmes. The application of NIR analysis gives the protein and moisture in the wheat, which in turn is an indication of the quality of the wheat and flour obtained. Breeders use this as an indication to verify the quality of wheat and the yield of flour to be obtained.

NIR has now been used over the world for example in Canada, Australia and Europe to monitor grower’s deliveries. The use of NIR to determine the protein and the moisture content has become a common practice in flourmills and it is replacing conventional chemical test. Since spectral analysis can be done on solid grains this has greatly reduced analysis time. Many bakeries also monitor the quality of flour using NIR spectroscopy.

NIR is applicable to the analysis of moisture, protein, fat, starch, sugars and fibre in intact cereal foods such as bread, cakes, mixes, breakfast cereals, pasta and snack foods (Osborne 1993).

Milk and Dairy Products

NIR has a key role in the analysis of dairy products (Ozaki, 2001). It offers flexibility in the analysis of protein, fat and lactose content in a wide variety of dairy products including:

- Liquid milk
- Dried whole milk, skim milk and whey powders
- Cream
- Traditional processed cheese

Many of these products are emulsions whose classical analysis is difficult. For example blending such samples changes their physical characteristics. NIR offers the possibility of on-line analysis.

Meat

NIR spectroscopy is widely used in the meat industry (Cozzolino and Murray, 2002). A special interactance fibre optic probe has been designed to spear carcasses and determine the fat content. The protein, fat and moisture contents of ground meat and meat products are available for processed meat. The meat samples are minced then blended in a food processor before being packed in an open sample cell.
The spectra of meat are dominated by water bands at 1450 nm (first overtone of the OH stretching mode) and 1934 nm (the combination band of OH vibrations). However, bands at 762, 960 and 1152 nm are well defined in the second derivative of the spectrum of water for the moisture content (McClure, 2002).

**Fish**

The analysis of fish flesh by NIR spectroscopy has been reported (Gjerde and Mertens, 1987; Mathias et al., 1987), where a good correlation has been obtained between laboratory and spectral data for fat and moisture contents on the lyophilized material. Non-destructive NIR analysis (760-200 nm) of fish has been reported by Rasco et al., 1991; Lee et al., 1992; Sollid and Solberg, 1992 Wold et al., 1996 and Downey, 2003.

Salmon fish is a high value food product. The salt and moisture contents are critical factors that influence and inhibit the growth of foodborne pathogens and spoilage bacteria (Euckland, 1995, Gram and Huss, 2000). Knowing the salt concentration in fish will help to control salt content of the final product. There is normally a large variation in the salt absorption among samples within the same production and also because of high value of fish material, only a rapid and non-destructive method for moisture and salt determination is useful. Huang et al. 2003 have determined the salt and moisture content in cured Atlantic salmon using short-wavelength near infrared spectroscopy (600-1100 nm).

**Fruits and vegetables**

In the past ripeness of fruits was sorted by optical spectroscope in the visible region. However appearance of fruits is not a reliable guide to sweetness.

NIR spectroscopy has been used as an automatic online method for evaluating qualities of foods. A number of researchers have used NIR spectroscopy coupled with regression analysis to determine compositions of different types of fruits and vegetables.

Athansia et al., 2003, used NIR to measure the moisture, sugar, acid protein and salt in a variety of tomato juice. These are important criteria in the determining the nutritious energetic value, which also influences the physical characteristics hence the quality of the food products. Moisture limits are often specified in the product regulations. But in addition to moisture, sugar, acid, protein and salt must be analysed routinely in order to achieve standardisation of the product according to the label specification.

In Japan it is a common practice to use NIR in the food industry for sorting fruits such as peaches, kiwi, apples and melons (Hasegawa et al., 2000, Tsuta et al., 2002), by visualizing the sugar content based on the absorption band in the NIR wavelength region. The second derivatives absorbances at 874 and 902 nm correlate with the sugar content. Muramatsu et al., 2003, coupled NIR with neural computing self-organisation map (SOM) have developed a non-destructive quality evaluation technique for apples.

**Fermentation**

Arnold et al. 2002 have reported that NIR spectroscopy can improve fermentation processes by incorporating rapid (analysis within seconds or minutes), non-destructive, multiconstituent analyses of fermentation broth media directly into monitoring and control strategies with minimal or no sample preparation or pretreatment. In fact NIR spectroscopy (Blanco et al. 2004) has been used for analysis and control of fermentation processes in both support laboratories and directly in the manufacturing environment.

During fermentation process biomass accumulates, the initially translucent media transforms opaque media that strongly scatters light. The important absorption bands in the NIR spectra of fermentation samples are at 1450 nm and 1940 nm corresponding to OH first overtone and the OH combination band of water. Because of the large absorption of water molecule it is a common practice to work with a second derivative data, where absorbance maxima are converted into minima flanked by positive lobes. In this way spectral data band-with is reduced and baselines differences are greatly reduced between spectra.
Because of the complexity of fermentation process, it remains difficult to relate spectral variations observed in the broth spectra to changes in the concentration levels of the individual broth constituents. Basic assignments can only be made if the fermentation broth is unmodified by comparing the NIR of pure component. Interfering absorption bands from other components, matrix variations from complex media and widely varying accumulation profiles affect spectroscopic measurements. Hence proper calibration set of samples must be included and the spectral data are treated using mathematical model.

**Mid-infrared spectroscopy**

Mid-infrared spectroscopy is perhaps one of the most widely used vibrational spectroscopic techniques. However its use has been restricted in the food industry because:

- Water being a major component in biological samples is also a strong infrared absorber
- Sample preparation
- Complex spectra
- Weak penetration of incident rays

In MIR, because of the strong absorption coefficients, samples have to be diluted before making transmission measurement. Unlike NIR, MIR has been more currently used in off-line analyses.

**Sugars**

MIR spectroscopy has been used since 1950’s for the study of carbohydrates. The combination of mid-infrared spectroscopy and multivariate statistics for determining glucose, fructose, and sucrose in aqueous mixtures has been investigated (Ramasami et al., 2004). In contrast with other classical methods the different sugars present can be determined in one run.

Cadet et al., 1991 has used MIR spectroscopy for the study of sucrose in raw sugarcane juice. The absorption band at 997cm\(^{-1}\) was used to quantify the sucrose content. This is an important criterion since the level of sucrose determines the price of sugar.

**Fat**

The rapid control of the quality of lipids is a major preoccupation in the food industry. Wheeler (1954) has intensively investigated the structural analysis through their MIR absorption spectra. MIR is now currently used for the determination of cis and trans fatty acids in oils and fats (Belton et al., 1988, Gobhurdhun et al., 2000) and has been adopted as an official routine method by the American Oil Society (AOCS) and the International Union of Pure and Applied Chemistry (IUPAC). The bands at 3 015 and 967 cm\(^{-1}\) are associated to the cis and trans of fatty acid (Stuart, 2004).

MIR has been used to analyze the fat content of milk and cream (Tay, 2001). The attenuated total reflectance (ATR) sampling technique was found to be fast and easy to use as compared to the transmittance sampling technique. The absorption band in the region 1,730 - 1,760 cm\(^{-1}\) (characteristic of ester group) is used to quantify the amount of lipids. This study showed that the ATR technique is fast, easy to use and can be implemented for on line monitoring of fat composition.

**Protein**

Etzion et al. 2004 have investigated the use of ATR MIR for the determination of protein concentration in raw milk. The determination of protein concentration is based on two absorbance bands in the 1 500-1,700 cm\(^{-1}\) range, known as amide band I and amide bands II, and 1,060 – 1,100 cm\(^{-1}\) range which is associated with phosphate groups covalently bind to casein proteins. The absorption bands due to water spectra were subtracted to reduce the effect of water, which overlaps with the amide bands (1,640 cm\(^{-1}\)).
CONCLUSION

Undoubtedly, NIR and MIR spectroscopy will play an important role in the food sector. However they present some limitations. Some of them are related to their nature as a secondary method. This means that a conventional, well-accepted supporting (reference) method must be available to supply the analytical results required for the modeling step of IR spectral data. Furthermore, the models need to be frequently updated to accommodate changes in the sample matrix, even for the same type of sample and analyte. Robust models may require hundreds or even thousands of samples preanalysed by the reference method.

On the other hand, the universal nature of the information that IR spectroscopy generates, the non-invasive and non-destructive use allowed by the technique, its expeditiousness, and the robustness of the IR spectrophotometers commercially available today may overcome the disadvantages indicated herein. The number of scientific papers and the successes of international congresses on the IR are evidence of this fact.

REFERENCES


BIOSENSOR AS A TOOL FOR MONITORING THE STATUS OF FRUITS AND VEGETABLES

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ABSTRACT

Biosensors offer the possibility of carrying out reliable, cost-effective and rapid field-based monitoring of a produce. Components of fruits and vegetables, indicators of quality, and/or maturity can thus be measured to determine the status of the produce accurately. We report here the development of an electrochemical biosensor array device to monitor the quality and stage of maturity of fruits of economic importance to Mauritius. Such a multi-analyte sensor has the ability to simultaneously monitor a range of fruit quality indicator analytes offering a robust analytical output. Initially, Ananas comosus (pineapple) has been chosen. Fruit components, namely β-D-glucose, total D-glucose, sucrose and ascorbic acid, all indicators of fruit quality and maturity, have been selected as target analytes. Preliminary results on real sampling of the fruit indicate that the biological markers β-D-glucose, total D-glucose and sucrose can successfully discriminate between the different fruit physiological stages. For simple end-user data interpretation from the sensor array, principal component analysis was used to allow measured chemical parameters to be related directly to a simple “score” of fruit ripeness. The biological markers β-D-glucose, total D-glucose and sucrose were found to successfully discriminate between the different fruit physiological stages.

Keywords: biosensor, array sensors, enzymes, fruit and vegetable quality

INTRODUCTION

With the difficulties currently encountered within the sugar sector, the Mauritian non-sugar sector is being called upon to assume a more important role in the agricultural economy (Ministry of Agriculture, Food Technology and Natural Resources 2003). In order to be competitive on the world market, it is important to ensure that the fruits and vegetables produced meet the required standards. The final quality of these produce depends on the physiological processes occurring during their development, maturation and/or ripening as well as on post-harvest practices. Fruits and vegetables must therefore be monitored from their early stage of development in the fields through to their destination market. This requires rapid and portable methods for the assessment of the produce’s status. Biosensors have the potential to meet these demands.

What is a biosensor? A biosensor may be defined as a device incorporating a biological recognition entity (e.g. an enzyme or antibody) either intimately connected to or integrated within a transducer (Jaffari and Turner, 1995; Hall, 1990). The latter translates the chemical/biochemical recognition by the biological component into a viable signal, which is proportional to the concentration of a specific chemical or set of chemicals (Jaffari and Turner, 1995; Higson, 1994). Biosensors, in principle, can offer many advantages to suit the requirements of the agro industry.

• They can be mass producible, economic and disposable
• They can measure a wide range of substances
• They can have a high degree of specificity for the analytes
• With modern techniques of sensor fabrication and the processing power of modern microelectronics, a degree of miniaturisation can be inferred to the sensors. Thus an array of sensors can be integrated into a small portable device for multiple parameter determination
• They can enable rapid in situ real-time monitoring of analytes
• They can operate in complex matrices that do not necessitate much sample preparation


51
Biosensor as a tool for monitoring the status of fruits and vegetables. S. Jawaheer et al.

- They can be user friendly and not require technical skills for operation
- Their measuring range can be tailored to the analytical problem. (Hall, 1990; Davis et al., 1995; Wollenberger, 1995; Turner and Newman, 1998).

In view of the requirements of the agro-industry and advantages offered by biosensors, a multi-analyte enzyme sensor based on amperometric measurements is being developed to monitor fruits and vegetables of economic importance to Mauritius. The sensor has the ability to simultaneously monitor a range of fruit quality indicator analytes thus offering a robust analytical output for quality determination. Fruit components, namely β-D-glucose, total D-glucose, sucrose and ascorbic acid, all indicators of fruit quality and maturity, were selected as analytes (Jawaheer et al., 2003). To address the problem of enzyme stabilisation, pectin was used as a novel immobilisation / entrapment matrix for the enzymes (Jawaheer et al., 2002).

This paper reports the preliminary testing of the array sensor using pineapple, Ananas comosus, as initial target fruit. The fruit was selected as it forms part of the main exports in the fruit sector in Mauritius. Previously, the array sensor performance was assessed with its pure substrates (Jawaheer et al., 2003). The study showed that the different individual sensors demonstrated good stability and the initial non-Faradaic current took only twenty seconds to fall to the background level. No cross-talk were noted across the sensors and the array biosensors gave appreciable signal to noise ratio with their respective substrates with a detection range of 0-30 mM analyte. The current signals produced by the individual sensors were appreciable and their relative signals with respect to each other were as expected. In contrast to studies using pure substrates, fruit sap is a complex system made up of several components including sugars, organic acids, and enzymes, all of which are interconnected biosynthetically and could interact (e.g. enzymatic breakdown of the components) or even react with the immobilising matrix or membrane. It was therefore important to assess the response of the array sensors with real samples, so as to ensure that their performance is not affected.

MATERIALS AND METHODS

Chemicals and biochemicals

Glucose oxidase (EC 1.1.3.4) from Aspergillus niger (product code GO4F, 274 U mg⁻¹), mutarotase (EC 5.1.3.3) from pig kidney (product code MUR1F, 1866 U mg⁻¹) and ascorbate oxidase (EC 1.10.3.3) from Cucurbita sp. (product code AO2, 416 U mg⁻¹) were purchased from Biozyme Laboratories Ltd (Blaenavon, U.K.). Invertase (EC 3.2.1.26) from bakers yeast (product code I9274, 400 U mg⁻¹) was obtained from Sigma-Aldrich Company Ltd (Poole, U.K.). Pectin from citrus fruits (9000-69-5, lot no. 77469) was purchased from ICN (Aurora, U.K.) and cellulose acetate with 40 % acetyl content and average Mr ca. 6 100 from Fluka Biochemica (Poole, U.K.). All other reagents were of analytical grade and purchased from Sigma-Aldrich-Fluka (Poole, U.K.).

Plant material

U.K. Tesco supermarket branded pineapple fruits and Queen Victoria variety obtained from the University of Mauritius farm were tested. The fruits were classified into three categories based on their skin colour: fully mature fruit corresponding to the skin colour starting to turn yellow, early ripe fruit where the skin colour was partially yellow, and ripe fruit corresponding to the skin colour being completely yellow. Two fruits as homogeneous as possible by visual assessment were examined per group.

Sample preparation and analyte concentration establishment

Fruit sap was manually extracted by squeezing sectioned fruits. The dilution factor for the samples was determined by first establishing calibration graphs for the pure substrates based on the two methods of analysis, glucose analysis test kits and array sensors. Samples of the different stages of ripeness were then diluted, such that the data collected fitted within the calibration graphs. The analyte concentration was calculated from the equation of the trend line of the established calibration curves. All sample dilutions were carried out using phosphate buffer. The following buffer was used: 0.1M sodium phosphate containing 0.1 M potassium chloride at pH 6.3.
Biosensor as a tool for monitoring the status of fruits and vegetables. S. Jawaheer et al.

**Biosensor fabrication**

Array sensors made up of eight individual sensors (Figure 1), each with a working area 4 mm$^2$ and consisting of a three-electrode system, were designed and fabricated using thick-film screen-printing. Melinex polyester films (ICI, Middlesborough, U.K.) were used as support substrates. To print the electrodes, graphite based P.T.F. ink (Acheson Colloids, Plymouth, U.K) was used for the conducting tracks, Ag / AgCl ink (MCA Services, Cambridgeshire, U.K.) for the reference electrode, metalised carbon paste, MCA4a (MCA Services) for the working electrode and blue 242-SB screen printable coating (ESL Europe, Reading, U.K.) for the insulating layer. The screen-printing paste for the working electrode was prepared by mixing thoroughly 15 g of MCA4a with 50 g of 3 % w / v pectin in buffer. The electrodes were screen-printed using a fully automated screen-printing machine (model DEK 248 from DEK Printing Machines Limited, Weymouth, U.K.).

**Figure 1** Screen-printed single array sensor. (E= electrode number)

**Enzyme immobilisation**

The enzymes were immobilised in a pectin matrix over the individual electrodes and consisted of 10 U of each of the different enzymes involved in the respective enzyme systems being dissolved in 1 µl of 3 % w / v pectin in buffer solution and dispensed over the working electrode using a positive displacement pipette. The sensors were allowed to dry for at least three hours. Before use, the sensors were spin coated with 1.5% w / v cellulose acetate in acetone solution. Spin coating was carried out by first depositing 1 ml of membrane solution over the array sensor and spinning at 2000 rpm for 20 seconds using a programmable precision spin coater from Specialty Coating Systems (INC, Indianapolis, USA). The sensors were allowed to dry for at least 24 hours at ambient temperature.

**Electrochemical measurement**

Each assay was carried out using two single use array sensors corresponding to a total of 16 electrodes. 75 µl of sample was dispensed over strips of absorbant paper (medical wipes, Kimberley-Clark) 30 x 9 mm in size, placed over a row of 4 sensors. All electrochemical measurements were carried out in a quiescent medium using an Autolab PGSTAT 10. A working potential of $+350$ mV vs. Ag / AgCl was used. To eliminate the major interferent, ascorbic acid, when carrying out real sampling, 1000U of ascorbate oxidase dissolved in 75µl pectin solution was dispensed over the absorbent paper used for sample deposition. The sample was deposited eight minutes prior to the electrochemical measurement (Jawaheer et al., 2003).

**Food analysis test kits**

D-glucose enzyme based food analysis kits were purchased from Digen Limited (Oxford, U.K). A Camspec M350 double beam U.V. visible spectrophotometer (Cambridge, U.K) was used for light absorbance measurements.
Principal component analysis (PCA)

The data matrix was scaled such that the sum of the measurements for each fruit was one. A PCA was then performed using the Matlab version 6.0 (Mathworks Inc., USA) to reduce the original six variables to two, so that each sample can be represented on a 2D graph.

RESULTS AND DISCUSSION

Array sensor performance with pineapple samples

Figure 2 shows the levels of analytes measured in the Tesco pineapple fruits of different ripening stage using the array sensor. The amount of analytes measured in the fruits belonging to the same stage of ripeness was found to vary considerably. It is possible that the fruits could have been harvested from different fields resulting in biochemical differences due to agronomic and environmental factors. Also, the classification of the pineapple fruits was based on the fruit skin colour, which depends on the level of chlorophyll, which in turn is greatly influenced by the amount of sunlight available to the fruit especially during maturation and the early stage of ripeness. These results clearly indicate that, for pineapples, skin colour is not a very reliable indicator of the levels of simple metabolites, which contribute to fruit quality. Thus, methods based on fruit metabolite component analysis, such as biosensors, may give a more reliable indication of the produce status in terms of parameters that directly relate to the management of fruit production and storage.

As for the trend of the different constituents measured, the levels of α-D-glucose, sucrose and ascorbic acid were found to decrease with increasing fruit ripeness while β-D-glucose increased. The background signal was also considered as an indicator of fruit status since electrochemically active compounds such as malic acid, that are potential fruit status indicators, contribute to the signal. The latter was found to decrease with increasing ripening stage.

Figure 2 Trend of selected analytes measured in Tesco pineapple of different ripening stages using array sensors
In order to assess the reliability of the sensors as analytical tools, the results obtained with the net D-glucose sensors were compared to that of a colourimetric enzyme-based D-glucose test kit having a detection range of 0.8-5.5 mM. The tests were therefore carried out on the same samples and on the same day to minimise any biotransformation, within the extracted juice, that might have interfered with the results. The amount of D-glucose detected using the electrochemical sensor measurement was almost four times that obtained using the D-glucose test kit. The trends measured during ripening also diverged slightly. It must however be noted that the food test kit method is not very accurate as one of the enzymes involved, hexokinase, has a low specificity towards its monosaccharide substrate; being able to catalyse the phosphorylation of D-glucose, D-fructose, D-mannose and D-glucosamine (Goodwin and Mercer, 1990). Nevertheless, hexoses other than glucose and fructose are rarely found in fruits and when found; they are usually in trace amounts (Whiting, 1971). The levels of total D-glucose transformed therefore depend on the amount of hexokinase present, the affinity of the enzyme for α-D-glucose, β-D-glucose and D-fructose as well as the levels and relative proportions of these substrates in the fruit sample. The method used to determine the dilution factor for this test was based on the instructions provided with the analytical kits and did not take into consideration the levels of fructose. These results are therefore not conclusive. The test was therefore repeated with Queen Victoria cultivars using a greater dilution factor for the test kit samples though this implied a higher margin of error. The level of D-glucose measured with the sensors was this time almost twice that obtained with the test kit though the trend obtained with the fruits of different ripeness was somewhat similar. Since biochemical changes are dependent on plant genotypes and influenced by environmental factors, different trends can be obtained depending on the cultivar, origin, seasonal and climatic factors (Kermasha et al., 1987; Bartolomé et al., 1995). It was therefore difficult to compare the results obtained with the Queen Victoria cultivar to previous work reported on other pineapple cultivars.

In general, the standard deviation across the analyte sensors during real sampling was found to be larger than that obtained with their pure substrate. For real sampling, contrary to assays with pure analytes, the samples were deposited over tissue paper impregnated with dried ascorbate oxidase in pectin. Uneven distribution and rehydration of the ascorbate oxidase in pectin could have been a contributing factor to the decreased reproducibility. To improve the sample distribution, the ascorbate oxidase could in future be deposited onto the lower ply of the double ply tissue paper, and the pristine upper ply used for sample deposition. The tissue paper free of pectin and enzyme, due to its good absorption and diffusion properties, would thus ensure an even distribution of the sample.

**PCA analysis of real sampling data**

In this work, the biochemical markers β-D-glucose, total D-glucose, sucrose, ascorbic acid and the background signal were chosen to discriminate fruits belonging to different physiological stages. Figure 2 clearly indicates that the levels of these fruit components change with fruit ripening. It was therefore important to confirm whether these markers could effectively be used for this classification. Consequently, Principal Component Analysis was performed to reduce these numerous variables (markers of maturity), to two composite indices PC1 and PC2 so as to facilitate the comparison between the different fruit classes.

Table 1 and 2 represent the scaled data matrix of the real sampling array output and the derived loadings of the variables respectively, while Figure 3 illustrates the score plot. From the loadings in Table 2, it can be deduced that the two principal components PC1 and PC2 place little emphasis on ascorbic acid and the background signal implying that these markers play a less significant role in the fruit classification as compared to the sugars. The score plot shows that the fruits of three different physiological stages group in different sections of the graph. This suggests that the variables β-D-Glucose, total D-Glucose and sucrose can effectively be used to distinguish between the different pineapple fruit physiological stages. The big difference within the different groups can be accounted to the fact that the fruits were categorised by their skin colour, which is not a reliable indicator of fruit ripeness / status.
Table 1  Scaled data matrix such that the sum of the measurements for each fruit is one

<table>
<thead>
<tr>
<th>Pineapple Fruit</th>
<th>β-D-Glucose</th>
<th>α-D-Glucose</th>
<th>Sucrose</th>
<th>Total D-Glucose</th>
<th>Ascorbic acid</th>
<th>Background signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0.15463</td>
<td>0.23170</td>
<td>0.22549</td>
<td>0.38662</td>
<td>0.00132</td>
<td>0.00025</td>
</tr>
<tr>
<td>1b</td>
<td>0.13037</td>
<td>0.18415</td>
<td>0.37039</td>
<td>0.31452</td>
<td>0.00044</td>
<td>0.00012</td>
</tr>
<tr>
<td>2a</td>
<td>0.27415</td>
<td>0.10100</td>
<td>0.24905</td>
<td>0.37515</td>
<td>0.00053</td>
<td>0.00013</td>
</tr>
<tr>
<td>2b</td>
<td>0.31664</td>
<td>0.06739</td>
<td>0.23217</td>
<td>0.38328</td>
<td>0.00039</td>
<td>0.00013</td>
</tr>
<tr>
<td>3a</td>
<td>0.29420</td>
<td>0.15282</td>
<td>0.10587</td>
<td>0.44701</td>
<td>0.00002</td>
<td>0.00009</td>
</tr>
<tr>
<td>3b</td>
<td>0.35795</td>
<td>0.11550</td>
<td>0.05290</td>
<td>0.47345</td>
<td>0.00013</td>
<td>0.00007</td>
</tr>
</tbody>
</table>

Table 2  Loadings for the different variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>β-D-Glucose</th>
<th>α-D-Glucose</th>
<th>Sucrose</th>
<th>Total D-Glucose</th>
<th>Ascorbic acid</th>
<th>Background signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC 1</td>
<td>0.48419</td>
<td>0.26195</td>
<td>0.37475</td>
<td>0.74599</td>
<td>0.00085</td>
<td>0.00024</td>
</tr>
<tr>
<td>PC 2</td>
<td>-0.46780</td>
<td>0.26638</td>
<td>0.81841</td>
<td>-0.20104</td>
<td>0.00194</td>
<td>0.00029</td>
</tr>
</tbody>
</table>

Figure 3  PCA scores plot for the scaled data matrix. PC1 and PC2 represent the first and second principal components respectively.

CONCLUSION

The trends observed during real sampling of pineapple fruits suggest that α-D-Glucose, β-D-Glucose, sucrose, ascorbic acid and the background signal can potentially be used as indicators for fruit status. The preliminary results obtained with the sensors look very promising though the large standard deviations obtained with the biosensors calls for further optimisation. Screen-printing the enzymes in the MCA4a paste and using a more reproducible membrane coating method will certainly improve the reproducibility of the sensors as will designing more appropriate sensor formats and sizes that allow direct insertion into fruits and the subsequent controlled, reproducible and passive application of sap to the active sensor areas.
Chemometrics analysis suggests that the data collected for β-D-Glucose, total D-Glucose and sucrose were sufficient to effectively discriminate between the different fruit physiological stages. Additionally, the use of PCA may allow simple communication of the sensor output to unskilled use as a simple level of maturity based upon the clustering within the PCA data. Once the sensors are fully optimised, these results need to be confirmed using larger number of samples. Since environmental and agronomic factors affect fruit composition, accurate models for determination of the status of selected fruits should be built with fruits grown in different regions and seasons.

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QUALITY SYSTEMS IN THE FOOD SECTOR IN MAURITIUS

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ABSTRACT

Measures taken to ensure the quality and safety of imported and exported food have been strengthened with the globalisation of food trade. The application of the HACCP (Hazard Analysis Critical Control Point) system in many countries is becoming mandatory for domestically produced and imported food products. Food quality assurance systems are also necessary to develop effective controls to ensure food of acceptable quality. Food organizations have the responsibility to implement systems which will ensure the safety and quality of food. The objective of this paper was to find out the extent to which systems are implemented in food organizations in Mauritius. A survey was conducted for this purpose. The questionnaire used, was structured in several sections and in each section questions were asked on one system which can be implemented in the food sector. Proportionate stratified random sampling method was used. Questionnaires were administered both personally and partly mailed to respondents. A 100% response rate has been obtained.

Keywords: Food organizations, survey, implementation, systems, HACCP, GHP, SQF, EFSIS

INTRODUCTION

Globalisation of food trade and the recognition that ‘emerging hazards’ are a significant issue has increased the risk of cross-border contamination of food. The potential for significant transfer of contaminated food between countries and/or regions is therefore much increased with potentially catastrophic consequences for public health. The standards, guidelines and recommendations adopted by Codex for food safety are recognised in the World Trade Organisation Sanitary and Phytosanitary agreement (WTO, 1995 a,b) as the basis for harmonisation of sanitary measures and HACCP is a central food safety system of choice promulgated by Codex. It is inevitable therefore that HACCP and ISO management systems will play an increasingly important role, not only as the systems of choice within individual countries, but also on the world-wide stage as an important contributor to the facilitation of world trade in foodstuffs. The current survey aims to find out the extent to which systems are implemented in the food organizations, in both the manufacturing and catering sector, in Mauritius.

METHOD

A questionnaire was designed to be answered by quality practitioners at management level. Both open and closed ended types of questions were used in the questionnaire. Where opinions were sought, open-ended questions were asked.

The questionnaire consisted of several sections, each dealing with the different systems (e.g. Food Safety Management System and quality management system) that can be implemented in food organisations. Questions related to the different systems implemented and the reasons they were implemented.

Sampling method used was proportionate stratified random sampling. This method involves a process of stratification or segregation, followed by random selection of subjects from each stratum. In stratified random sampling the population is first divided into mutually exclusive groups that are relevant, appropriate and meaningful in the context of the study. The subjects are drawn in proportion to their original numbers in the population.
In the case of this survey, the population was identified as all organisations in the food sector, which consisted of manufacturing, distribution, wholesalers, retailers and catering. The Food manufacturing and catering sectors made up the larger part of the sector, and therefore for the purpose of the survey, only these two subgroups were sampled. A sampling size of 40 was chosen.

For the catering sector, a list of hotels was compiled based on information obtained from ARHIM (Association des Hoteliers et Restaurateurs de l’Ile Maurice) and from the MEDIA (Mauritius Export Development Investment Authority). The list consisted of 66 hotels in all and these were classified according to the number of rooms, namely 10-50 rooms for small hotels, 51-80 rooms medium and > 80 rooms large hotels. Locally, there were 30 large hotels, 9 medium and 27 small hotels. For the survey, 9 large hotels, 3 medium and 8 small hotels were sampled.

Manufacturing organizations were classified according to the Central Statistics Office (CSO) that is based on the number of employees. Small enterprises are those with less than 10 employees and large enterprises have more than 10 employees. A list of all manufacturing organisations was compiled based on information collected at the Mauritius Chamber of Agriculture, the Ministry of Industry and International Trade and the MEDIA. There were 70 organisations in all, of which 55 are large scale and 15 are small-scale organisations. 16 large scale and 4 small-scale organisations were randomly sampled for this survey.

Data was collected by both personal interview and by mail. Questionnaires were sent by mail only when it was not possible to go on site. Data was then analysed.

RESULTS

Good Hygiene Practices (GHP) Based on Codex International Code of Hygiene

Companies were questioned on the food hygiene practices i.e. whether they had the following criteria in place on a yes / no scale viz:- design and layout of premises, cleaning and disinfection programme, effective pest control, transportation under appropriate conditions, good waste disposal system, quality and sanitisation programme, high degree of personnel hygiene, a good health of personnel system, training plan, product identification, product traceability and product recall; they were also asked for how long they had the above in place.

Twenty-one organisations claimed to be fully conforming to all the requirements of GHP. The remaining organizations have reported non-conformances related to some clauses of the standard, as shown in Figure 1.

It was further observed that there were three major non-conformances in organisations not fully complying to GHP namely: improper design and layout of premises, product traceability and training. Eight catering organizations did not have a proper design and layout of their kitchen, with no separation between dirty and clean areas and no proper waste disposal system. The length of time that they have had a food hygiene system in place varied with companies. Some had it since they were operational and for others it dated back to two to three years.

Safe Quality Food and HACCP

Questions were asked if companies had SQF 2000 and HACCP in place, if they were certified, and for how long and if they found SQF and HACCP effective.

Only 3 catering organizations claimed to have Safe Quality Food (SQF) 2000 system and they reported the system as being very effective as it helped them to work to maintain high level of food standard within their organisation. Respondents who do not have SQF 2000 already had HACCP which is a more common standard for the food industry or were unaware of the system itself. A few however reported that it was a matter for management decision or it is not a customer requirement.

The study revealed that 18 out of 40 food organizations had HACCP in place and only 14 were certified. Only the large food businesses had HACCP in place. The most cited reason why HACCP was not in place was that it was a management decision. Some manufacturing businesses stated that
HACCP was not a priority given the nature of their product. A number of respondents, especially those from small and medium enterprises, were not very aware of what HACCP is and one respondent did not seem to have heard of it before.

**Figure 1** The number of food organizations which are non-conforming to Codex Good Hygienic practices criteria.

<table>
<thead>
<tr>
<th>Design and layout of premise</th>
<th>A</th>
<th>Personal hygiene</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product traceability</td>
<td>B</td>
<td>Product identification</td>
<td>H</td>
</tr>
<tr>
<td>Training</td>
<td>C</td>
<td>Effective pest Control</td>
<td>I</td>
</tr>
<tr>
<td>Waste disposal system</td>
<td>D</td>
<td>Hygienic design of equipment</td>
<td>J</td>
</tr>
<tr>
<td>Product recall</td>
<td>E</td>
<td>Transport of food under appropriate conditions</td>
<td>K</td>
</tr>
<tr>
<td>Cleaning and disinfection</td>
<td>F</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Quality Management System**

Companies were asked if they have a quality management system, to specify which systems and the length of time since they were registered.

**Figure 2** shows that only 12 organizations have a quality management system in place, 11 are ISO 9000 registered and the remaining organizations having implemented their own company’s quality management system e.g. Coca Cola has a Coca Cola Quality System in place. From **Figure 3**, it can be seen that both in the catering and manufacturing sector, only large organizations have been registered to a quality management system. All, except for one manufacturing organization, also have HACCP in place.

For some the reasons for them for not having a quality management system would be because it is too expensive to implement or that it is a question of management decision. A few respondents reported that they preferred to focus on their food safety management system first.
Figure 2 The number of organizations having HACCP, a Quality Management System and an Environmental System in place.

Figure 3 The number of organizations in the catering and manufacturing sector which has a Quality Management System and HACCP in place.
Other systems

Only one food manufacturing organization reported to have been registered to European Food Safety Inspection Service certificate (EFSA). Six organizations have an Environmental Management system, 2 have ISO 14001 and the remaining 4 have Green Globe as shown in Figure 2.

DISCUSSION

Despite difficulties in carrying out the survey, a 100% response rate was finally obtained. Some organizations were very willing to help and allowed on site verifications of facilities available. Good Hygiene Practices (GHP) are prerequisite programmes which provide general level of control applicable across all areas of the food operation (Mayes and Mortimore, 2001). The programmes screen out the general hazards. The Codex International Code of Practice on General Principles of Food Hygiene states that the design and layout of food establishments should permit good food hygiene practices including protection against cross contamination between and during operations by food stuff (CAC, 1997). However, the study revealed that locally the infrastructure in a number of organizations was inadequate with no one-way flow process and no separation between clean and dirty areas. The infrastructure in these organizations is not according to GHP. The code of practice requires that food products be permanently marked to identify the producer and the lot (CAC, 1997). The documentation should be such that any batch of food produced, can be correlated with deliveries of the respective raw materials used in its manufacture and with the corresponding laboratory records (IFST, 1998). This would ensure product traceability which is important in case there is a problem with a batch of product and a recall needs to be done. However, the Good Catering Practices of the IFST (1998) does not require catering organizations to ensure product traceability which may explain why a number of organizations do not ensure that their food is traceable back to the supplier.

Training has two main roles. It first helps to develop awareness and motivation in the workforce which lead to change in attitudes of people. It also aims at enhancing competency by providing technical and practical knowledge. Furthermore, training has to be an ongoing programme in the workplace so as to be a catalyst for lasting change (Mayes and Mortimore, 2001). However, the study revealed that locally the organizations did not realize the importance of proper training and that adequate training is not provided to the employees. This may lead to problems later on when the organization may decide to implement other systems which need a proactive culture throughout the organization, from senior management to line operators.

The Safe Quality Food 2000 Code was developed in Australia in 1995 and is an HACCP-based food safety and quality risk management system. The Code requires that SQF system address food safety hazards relating to potential biological, chemical and physical contamination and customer identified product quality issues (SQF, 2002). Organizations with HACCP and quality management systems in place did not feel that this system will bring much to their organization. Also, SQF Code is not recognized worldwide and thus the need to implement such a system is not felt.

The number of large organizations with HACCP in place shows that they are conscious of the need for an internationally recognized food safety management system, although HACCP is not mandatory locally. However, this does not seem to be the case among the small and medium organizations, where none of the organizations surveyed had the system in place. It was noted that a number of respondents did not know what HACCP was. This is reflected in a recent study in UK which have found that small and medium organizations are less likely to invest in food hygiene and food safety than large organizations and are less likely to have HACCP in place (Mortlock et al., 1999).

Arguments against ISO 9000 in the food organizations are that it can add more work, constraints, paper and is expensive to implement. However, arguments identified locally by the respondents were cost associated, management decision and priority to HACCP. ISO 9000 and HACCP are complimentary and a food organization can not have food quality without food safety. ISO 9000 and HACCP both focuses on preventing, rather on detecting or correcting a program during final inspection. The only difference is that ISO 9000 focuses on the system and HACCP on the product safety (Newslow, 2001). Locally all organizations except for one, have both HACCP and a quality management system in place. The eight organizations which have an environmental management system in place are those which are committed to quality in all aspects of their business. One manufacturing organization which had an
EFSIS certificate, is the only organization which manufactured products mainly for export towards European countries. The other organizations do not export to Europe and the need for such a system in place is not justified.

CONCLUSION

Mostly large organizations have implemented and are committed to systems like Food safety and ISO 9000, ISO 14000, Green Globe management systems in place. However, most of the small and medium organizations do not conform to a number of criteria for the Good Hygiene Practices and do not have any systems in place. The situation is the same as in UK where a recent study found that small and medium organizations were less likely to have a food safety management system (Mortlock et al., 1999)

There is thus a need to provide information regarding the importance of having prerequisites programmes and a food safety management system. Adequate technical support like availability of appropriate, current and scientific support and low cost analytical services will also be of valuable help to these organizations (World Health Organisation, 1999). High quality training will also have to be provided to all food handler’s and to managers for a better understanding of food hygiene and of hazards associated with a particular food.

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Ms. Esha Amjaud, Lecturer in Food Science, University of Mauritius.

REFERENCES


INNOVATION EN MATIERE DE SALAISON DE VENAISON EN MILIEU TROPICAL

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RESUME

De façon générale, les procédés traditionnels de transformation des produits carnés sont basés sur l’utilisation des opérations de salage, séchage, cuisson, fumage, fermentation / maturation seul ou en combinaison pour aboutir à une large gamme de produits. Dans les pays du Sud, les procédés de transformation utilisés sont de préférence rapides et combinent souvent le salage, séchage / cuisson et le fumage. Quant à la fermentation / maturation, elle est essentiellement pratiquée dans les pays tempérés où les conditions climatiques et économiques permettent aisément leur mise en œuvre.

L’objectif de ce travail est d’élaborer un procédé innovant de salaison de la viande de venaison adapté au milieu tropical en s’inspirant des procédés utilisés en pays tempérés. Le procédé retenu combine la déshydratation-imprégnation par immersion (DII) pour saler et présécher la viande suivie d’une deuxième opération de fermentation pour améliorer la stabilité et les propriétés sensorielles du produit fini. Les résultats montrent que le traitement d’une pièce de viande par immersion en solution eau / sel / sirop de glucose permet une bonne déshydratation et un salage satisfaisant de la viande. Une étude menée avec un soluté modèle, le polyéthylène glycol (PEG), a permis de mieux comprendre la performance du sirop de glucose en terme de perte en eau et du contrôle du salage. Les résultats préliminaires sur le couplage DII / fermentation sont prometteurs. Néanmoins, les conditions expérimentales demandent à être revisitées pour mieux orienter le métabolisme des ferments vers une fermentation lactique.

Mots-clés: viande, déshydratation, salage, fermentation

INTRODUCTION

Dans les pays du Nord et du Sud, les produits d’origine animale, quand ils ne sont pas consommés à l’état frais, sont généralement transformés par une large gamme de techniques traditionnelles, notamment le salage, le séchage, la cuisson, le fumage, la fermentation / maturation, seules ou en combinaison aboutissant à un large éventail de produits. Quelques exemples de viandes traditionnelles transformées par ces techniques sont la poitrine salée (Europe), le kilishi (Sahel), le biltong (Afrique du Sud), le charque et le carne do sol (Brazil) et le boucané (La Réunion) (Collignan et al., 2001).

Dans les pays du Sud, les procédés de transformation utilisés sont de préférence rapides et combinent souvent le salage, séchage / cuisson et le fumage. La plupart de ces procédés bénéficient d’un savoir-faire ancestrale mais reste empirique. La maîtrise de ces procédés est presque entièrement dépendante de l’expérience des artisans et la qualité du produit fini n’est pas toujours garantie.

Quant à la fermentation / maturation, elle est essentiellement pratiquée dans les pays tempérés où les conditions climatiques et économiques permettent aisément sa mise en oeuvre. En effet, la fermentation est un procédé long, réalisé de préférence à température basse et nécessitant des cellules à atmosphères contrôlées. Pour le jambon sec ou cru, exemple type d’un produit salé / séché / fermenté, les étapes de fabrication sont les suivantes: salage pendant une période d’environ 20 jours à 1-4°C; repos déshydratant à 2-5°C pendant 12 semaines au total; étuvage à 20-30°C et une hygrométrie de 65
– 85% pendant une semaine, et enfin un séchage / maturation à 13-16°C avec une hygrométrie de 75-85% en condition de ventilation intermittente pendant 2 à 7 mois ou plus (Girard, 1988; Durand, 1999). Ces conditions expliquent que l’on retrouve peu de produits de salaison crus en milieu tropical.

La venaison, viande de gibier d’élevage ou de chasse, ne se prête pas bien au procédé classique de salaison. Étant une viande particulièrement maigre (<5% lipides), elle tend d’une part à s’imprégner trop en sel et d’autre part, les arômes subtils de gibier qui la caractérisent ont tendance à disparaître au cours du procédé (Deumier et al., 1996, Collignan et al., 2003). Aussi, le travail en cours vise à proposer un procédé simple à mettre en œuvre et adapté aux conditions tropicales, combinant la déshydratation-imprégnation par immersion (DII) et la fermentation / maturation.

Le traitement de la viande par immersion dans des solutions concentrées (DII) présente l’avantage de sécher et de saler la viande en une seule opération, évitant ainsi le séquençage des opérations successives de salage et de séchage. De plus, cette technique s’applique particulièrement bien aux aliments de petite taille, caractéristique des muscles du cerf rusa. Des travaux antérieurs ont montré qu’en utilisant des solutions ternaires eau-sel-sirop de glucose, on pouvait atteindre des niveaux de séchage importants tout en limitant l’imprégnation en solutés, en particulier en sel (Collignan et Raoult-Wack, 1994). Dans le cadre de ce travail, une première étude est menée pour voir si la venaison se prête bien à cette technique, l’objectif étant de favoriser la déshydratation et limiter le salage de la viande. Dans un deuxième temps, le rôle que joue le sirop de glucose sur les transferts de matières, notamment la perte en eau et les gains en solutés, est évalué en utilisant un soluté modèle, le polyéthylène glycol. Une fois les performances du sirop de glucose mieux cernées, d’autres solutés moins coûteux et plus disponibles en milieu tropical seront testés.

Cependant, le traitement par immersion conduit à un produit relativement brut par rapport aux propriétés sensorielles et peu stable à température ambiante (Raoult-Wack, 1994). Une deuxième opération s’avère donc nécessaire pour mieux stabiliser le produit. Notre choix a porté sur la fermentation / maturation dont les mécanismes aboutissent généralement à une amélioration de la texture, la couleur, la flaveur, et la stabilité du produit. Il s’agit alors de tester si le couplage DII / fermentation est possible et, dans l’affirmatif, d’identifier les conditions optimales pour un procédé adapté au milieu tropical (température, petite échelle, faible coût).

MATERIELS ET METHODES

Matières premières

Les pièces de viande de bœuf utilisées, (rond de gîte, partie maigre, équivalent à la viande de cerf rusa) proviennent d’une boucherie locale et sont découpées en parallélépipèdes rectangulaires de 70 mm x 50 mm x 10 mm. Les solutions d’immersion sont préparées en mélangeant à chaud les solutés (sel et sirop de glucose DE 21 (Roquette, France) ou PEG (ACROS Organics, Belgium)) avec de l’eau distillée jusqu’à dissolution complète (Tableau 1). La molalité en sel des solutions est fixée à 3,0 mole kg⁻¹ d’eau.

Traitement de la viande par immersion dans une solution eau-sel-sirop de glucose ou PEG

L’expérimentation est conduite dans des récipients en polypropylène de 2,5 L, fermés hermétiquement, sous agitation (1,7Hz, amplitude 2 cm, agitation horizontale) dans une étuve à 25°C. Les morceaux de viande sont placés entre des grilles horizontales maintenues en position par un système de tiges en acier inoxydable avant d’être immérghés dans la solution concentrée. Celle-ci est utilisée en large excès, avec un rapport massique solution / produit de 10:1, ce qui permet de considérer que les variations de la concentration moyenne de la solution durant la manipulation sont négligeables.

Fermentation de la viande traitée par immersion

Les filets de viande après traitement DII sont ensemencés avec les ferment lactiques Lactobacillus sakei (Rhodia / Texel) à raison de 105 cfu g⁻¹. Les filets sont ensuite suspendus dans une étuve à 20°C et une hygrométrie moyenne de 80%.
Techniques analytiques

La masse des échantillons est pesée sur une balance analytique avec une précision de ± 0,1 mg. L’activité en eau est mesurée à l’aide d’un Awmètre (FA-st / 1, GBX-France). La teneur en eau est déterminée par gravimétrie selon la norme AFNOR (1968) et la teneur en sel à l’aide d’un chloruremètre (Corning 926) après extraction dans de l’acide nitrique 0,3N. La viscosité dynamique est mesurée par un viscosimètre (Brookfield digital viscosimètre Model LV1 DVII). L’acide lactique est dosé à l’aide d’un kit enzymatique (Enzytec, L+D-Lactic acid, ID-No.1 002 889). Les sucres et les PEGs sont dosés par chromatographie liquide haute performance. La flore totale de la viande est dénombrée sur milieu PCA (Plate count agar) et la flore lactique sur milieu MRS (Mann- Rogosa – Sharpe).

RESULTATS

Transferts de matière lors de l’immersion de la viande dans une solution concentrée complexe

Les résultats relatifs au traitement par immersion de la viande de bœuf en solution eau / sel / sirop de glucose (Figure 1) ont montré que la perte en eau en 24h est nettement plus importante (de l’ordre de 40%), que les gains en sel (<5%) et en sucres (<3%). L’analyse de la composition du sirop de glucose a révélé la présence de mono-, di-, oligo- et poly- mêres de glucose en proportion variable (Tableau 1). Les essais réalisés avec un soluté modèle, le polyéthylène glycol (PEG) (Tableau 1), ont montré qu’en augmentant la masse molaire et la molalité en PEG de la solution eau / sel / PEG, la perte en eau augmente et les gains en solutés diminuent. Par contre, l’influence de la viscosité sur ces transferts n’est pas effective dans nos conditions expérimentales.

Tableau 1 Nature et masse molaire des solutés utilisés

<table>
<thead>
<tr>
<th>Soluté</th>
<th>Masse molaire</th>
<th>Masse molaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirop de glucose DE 21</td>
<td>1,4*</td>
<td>180</td>
</tr>
<tr>
<td>Glucose (G1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose (G2)</td>
<td>6,0*</td>
<td>342</td>
</tr>
<tr>
<td>Maltotriose (G3)</td>
<td>8,2*</td>
<td>504</td>
</tr>
<tr>
<td>Maltotetraose (G4)</td>
<td>7,5*</td>
<td>666</td>
</tr>
<tr>
<td>Maltopentaose (G5)</td>
<td>6,5*</td>
<td>828</td>
</tr>
<tr>
<td>Maltohexaose (G6)</td>
<td>10,0*</td>
<td>990</td>
</tr>
<tr>
<td>Maltoheptaose (G7)</td>
<td>6,5*</td>
<td>1,152</td>
</tr>
<tr>
<td>Maltoctaose (G8)</td>
<td>3,5**</td>
<td>1,314</td>
</tr>
<tr>
<td>Maltononaose (G9)</td>
<td>3,2**</td>
<td>1,476</td>
</tr>
<tr>
<td>Saccharides de degré de polymérisation 10</td>
<td>47,2</td>
<td>&gt;1638</td>
</tr>
<tr>
<td>PEG 200</td>
<td>190-210</td>
<td></td>
</tr>
<tr>
<td>PEG 300 (» G1)</td>
<td>285-315</td>
<td></td>
</tr>
<tr>
<td>PEG 400 (» G3)</td>
<td>380-420</td>
<td></td>
</tr>
<tr>
<td>PEG 600 (» G4)</td>
<td>570-630</td>
<td></td>
</tr>
<tr>
<td>PEG 1,000 (» G5-G7)</td>
<td>950-1,050</td>
<td></td>
</tr>
<tr>
<td>PEG 1,500 (» G8-G9)</td>
<td>1,400-1,600</td>
<td></td>
</tr>
<tr>
<td>PEG 20,000 (» G123)</td>
<td>20,000</td>
<td></td>
</tr>
</tbody>
</table>

* déterminé par CLHP-CI
** fourni par Roquette, France

Dans une tentative de reproduire les performances du sirop de glucose, un mélange de PEGs de masse molaire variant de 200 à 20 000 a été utilisé en substitution au sirop de glucose dans la solution concentrée. L’évolution des transferts d’eau, de sel et de PEGs totaux est illustrée sur la Figure 2. La combinaison de PEGs reproduit de façon satisfaisante la performance du sirop de glucose par rapport au gain en solutés. Par contre, en ce qui concerne les transferts d’eau, des écarts significatifs sont notés. Ceci pourrait être dû au fait que le mélange de PEGs ne respectent pas de façon identique la composition en sucres du sirop de glucose. En effet, la combinaison de PEGs reproduit bien la distribution de masse molaire des sucres du sirop de glucose allant du glucose au maltononaose (G9), mais au delà on a complété le mélange avec du PEG 20 000. Une autre explication pourrait être la nature des molécules de PEG qui est différente de celle des sucres.

Figure 1 Evolution de (a) la perte en eau (PE), du gain en sel (GS), du gain en sucres totaux (GSu), lors d’un traitement d’immersion de la viande de bœuf dans une solution composée de sel (NaCl, 3,0 mole kg\(^{-1}\) d’eau) et de sirop de glucose (DE21, 950 g kg\(^{-1}\) d’eau)

Figure 2 Comparaison des performances du sirop de glucose et d’une combinaison de PEGs de masse molaire variant de 200 à 20 000. (PE perte en eau, GS gain en sel, GSu gain en sucres, GPEG gain en PEGs)
Des essais préliminaires de fermentation ont été réalisés sur la viande traitée au préalable par DII pendant 3h, 7h et 24h. Il en ressort que le traitement de DII abaisse nettement la flore totale de surface de la viande d’une à deux unités log de $10^4$ à $10^2$ cfu/g. L’effet est d’autant plus important que le temps d’immersion est long. Par contre, la flore lactique initiale de la viande ($10^3$ - $10^4$ cfu/g) n’est pas affectée par le traitement. Il apparait ainsi que la DII permet de réduire la flore banale de la viande au profit de la flore lactique utile pour la fermentation. Au cours de la fermentation, la flore totale ainsi que la flore lactique présentent une croissance significative de 2 unités log. Par contre, le pH et la teneur en acide lactique ne montrent aucun changement significatif. On constate aussi une perte en eau importante pouvant atteindre 30% en 72 h.

**DISCUSSION**

**Transferts de matières pendant la DII dans une solution eau / sel / PEG**

Sur la base des résultats obtenus, un schéma des mécanismes de transferts de matière est proposé (Figure 3). Cette hypothèse suppose la formation d'une couche concentrée en PEGs à la périphérie de la viande, qui va régir les transferts d'eau et de sel. Cette couche se maintient du fait de la diffusion lente des PEGs vers l'intérieur de la viande et de la rétraction de la matrice suite à la sortie d'eau. Par effet d'encombrement stérique et de tortuosité accrue, les mouvements de sel vers l'aliment sont freinés. Par contre, un gradient favorable à la diffusion de l'eau du centre du produit vers la surface est maintenu. Ces effets sont d’autant plus importants que la masse molaire du PEG est élevée.

**Figure 3** Schéma des hypothèses des mécanismes régissant les transferts d'eau et de sel lors de l'immersion de la viande dans une solution ternaire eau / sel / PEG

![Schéma des hypothèses des mécanismes régissant les transferts d'eau et de sel](image)

- Les molécules de PEGs se retrouvent à la surface, formant une couche barrière concentrée et d’une certaine épaisseur
- Diffusion lente des PEGs vers l'intérieur
- Couche barrière freine l'entrée de sel (encombrement, tortuosité)
- Maintien d'un gradient favorable entre l'intérieur et l'interface du produit, favorisant le départ d'eau
- Couche barrière d' épaisseur plus faible et plus concentrée en PEGs
- Diffusion très faible et très lente des PEGs vers l'intérieur
- Encombrement plus important freinant davantage l'imprégnation en sel
- Maintien d’un gradient plus important, augmentant la perte en eau

**Couplage DII / fermentation**

La flore lactique naturelle de la viande résiste au traitement de DII, malgré les conditions généralement défavorables aux microorganismes (solution très salée, pression osmotique élevée). De plus, la viande traitée par DII s’imprégne légèrement en sucre, ce qui constitue un apport supplémentaire en nutriments pour les ferment. Ces résultats tendent à soutenir la possibilité d’une étape de fermentation après traitement de DII. Par ailleurs, après ensemencement par les ferment lactic, une croissance des bactéries lactiques est observée. Cependant, cette croissance n’est pas...
accompagnée de formation d’acide lactique et d’abaissement du pH, comme c’est le cas normalement lors d’une fermentation lactique de la viande. Ceci pourrait être due aux conditions expérimentales qui n’ont pas été favorables à une fermentation lactique (haute teneur en sel de la viande, faible taux d’ensemencement en ferments lactiques, durée de fermentation courte, séchage top important pendant la fermentation).

Cependant, l’ensemencement par les ferments lactiques de la viande prétraitée par DII permet d’élever de manière significative la stabilité du produit à température ambiante. Ainsi, même si la fermentation n’est pas déclenchée de manière satisfaisante, il est intéressant de noter que la multiplication des bactéries lactiques empêche la prolifération des microorganismes d’altération.

CONCLUSION

Cette étude a permis de mettre en évidence l’importance de la taille des molécules des solutés et de leur concentration sur les transferts de matière lors d’un traitement de la viande par immersion dans une solution complexe. Ces résultats devraient faciliter l’identification de solutés moins coûteux et plus disponibles en milieu tropical que le sirop de glucose comme la mélasse, les amidons et les gommes végétales.

Le couplage DII / fermentation est envisageable. Cependant, les conditions expérimentales devront être améliorées pour favoriser le développement des ferments lactiques et orienter leur métabolisme vers une fermentation.

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DETERMINATION OF GENETIC VARIATION AMONG SOME ANTHURIUM CUT-FLOWER CULTIVARS

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ABSTRACT

Knowledge of the genetic relationships existing among Anthurium andraeanum cultivars, the most economically important horticultural crop of Mauritius, is primordial in assisting future collection strategies for cultivar improvement and breeding. The genetic variation among 15 cut-flower anthurium cultivars was evaluated using randomly amplified polymorphic DNA (RAPD). High molecular weight DNA was initially extracted from young leaves. A subset of five distinctive anthurium cultivars was chosen at random and screened with 52 decamer primers in optimized PCR reactions to detect polymorphic primers. The four most polymorphic primers were chosen and used to amplify all the anthurium cultivars. RAPD fingerprint profiles generated a total of 59 scorable repeatable fragments which were used to compute the Nei and Li’s genetic distance coefficients for all pair-wise cultivar combinations. A significantly low average genetic distance of 0.08 (representing a mean genomic similarity of 91.7%) indicated that cultivars were closely related to each other. The dendrogram generated by unweighted pair-group method of arithmetic averages (UPGMA) clustering algorithm indicated that ‘Midori’ was the most genetically distinct cultivar. The remaining anthurium cultivars were distributed into 2 separate clusters which were found to be independent of spathe colour, shape and cultivar provenance. Our results demonstrated that RAPD markers could successfully assess the extent of genetic variation among Anthurium andraeanum cultivars.

Keywords: Anthurium andraeanum, RAPD, dendrogram, UPGMA.

INTRODUCTION

Anthurium is grown throughout the world for trade as cut-flowers and blooming potted plants. Cut flower anthuriums, referred to as Anthurium andraeanum Hort., are believed to be hybrids between Anthurium andraeanum Linden and several closely related species of the Calomystrium section (Kamemoto and Kuehne, 1996). Currently, numerous cultivars with different flower colours, sizes, shapes and some with delicate fragrances are available for commercial exploitation.

Introduced to Mauritius from Hawaii in 1956 and from Brazil in 1965 (Reetoo et al., 1989), anthurium is presently cultivated on some 200 acres of land and is the pre-eminent flower of the island. With some 10 million blooms exported annually (representing about one fifth of the world’s export supply), Mauritius is the third largest anthurium producer in the world after Holland and Hawaii. Japan, Italy, Hong Kong, France and USA are among the principal importers of Mauritian anthurium.

The success of the local anthurium industry essentially depends on its ability to provide a portfolio of flowers that will please the final consumer as demands for various spathe colours change constantly. New cultivars, developed principally in Holland and Hawaii, are constantly being imported, mass propagated using tissue culture and introduced for commercial cultivation. However, the competitiveness and growth of the Mauritian cut flower anthurium industry depend on its ability to develop novel cultivars to exploit the growing global flower market. Breeding for new anthurium cultivars is traditionally performed through sexual hybridization followed by progeny evaluation and selection. Efforts to select parental cultivars for breeding require an assessment of the genetic relationship among the various cultivars. Knowledge of the level of genetic variability present in the gene pool is essential for elaboration of a successful breeding strategy for varietal improvement and can be helpful in the efficient management and utilisation of anthurium germplasm. Determination of relationship between anthurium cultivars based on morphological characters is expected to be difficult as the majority of cultivars are known to be phenotypically similar (except for their spathe colour and shape). Cultivars have so far been identified mainly by morphological measurements of flowers.
Determination of genetic variation among some anthurium cut-flower cultivars. P. Nowbuth et al.

(Kobayashi et al., 1987). Clear scope exists for the assessment of anthurium genetic diversity using molecular markers which are known to be abundant in number and independent of environmental influences and plant maturity. RAPD markers, developed by Williams et al., (1990), have been successfully used for identifying cultivars and assessing level of genetic diversity in pot plant anthurium species (Ranamukhaarachchi et al., 2001).

The genetic variability accessible in the gene pool is the major resource available to breeders. The objectives of this study were to fingerprint cultivars using RAPD markers so as to assess the level of genetic variation among some commercially grown anthurium cultivars.

MATERIALS AND METHODS

Plant material

Fifteen *Anthurium andraeanum* Hort. cut-flower cultivars namely ‘Ozaki’, ‘Paradiso’, ‘Mickey mouse’, ‘Marcovie’, ‘UH’, ‘Jose’, ‘AC10’, ‘KF1’, ‘Midori’, ‘Chloe’, ‘Fleur des iles’, ‘Bianca’, ‘La Coquille’, ‘Rose’ and ‘Mauna kea’ were sampled at Exotic Exports Ltd situated at Hermitage. The youngest leaves were collected and quickly brought to the lab. *Spathiphyllum* sp, also belonging to the Araceae family, was collected to serve as out-group in the study.

DNA extraction

Genomic DNA was extracted from the leaves using the DNeasy® Plant Mini kit (QIAGEN, Germany) according to the manufacturer’s protocol. DNA quality was verified by electrophoresis on a 1% agarose gel in 1xTAE buffer (Sambrook et al., 1998).

RAPD analysis

A total of 52 decamer primers (Operon Technologies, Alameda, Calif.) were initially used to amplify a subset of 5 cultivars chosen at random. The RAPD protocol described by Ranamukhaarachchi et al. (2001) was carried out with minor modifications. Each 25µL PCR reaction mixture contained 2.5 mM Mg2+, 200µM of each dNTP , 2.5 pmol decamer primer (Operon Technologies, Alameda, California, USA), 0.5 units HotstarTaq™ DNA polymerase (QIAGEN GmbH, Germany), 0.5 µl template DNA, 1x PCR buffer, 250 ng BSA (Life Technologies, UK) and 1x Q solution. The thermal cycling program used was: 94°C / 15 mins ; 3 cycles of 94°C / 25 sec, 35°C / 25 sec and 72°C / 2 mins followed by 40 cycles of 94°C / 25 sec, 37°C / 25 sec and 72°C / 2 mins. Following a final extension of 72°C / 7 mins, reactions were ended with an indefinite hold at 4°C. Negative controls namely ‘no template’ and ‘no primer’ were included to check the fidelity of the PCR reaction. The four most polymorphic primers were used to amplify all the cultivars. Following amplification, RAPD products were separated on 1.5% agarose gels, stained with ethidium bromide and photographed. Sizes of all the amplification products were estimated using the 100-bp ladder marker (DNA molecular weight marker XIV, Roche Diagnostics, Germany). Experiment was repeated twice to get reproducible RAPD patterns.

Data analysis

The presence and absence of bands was coded in binary (1,0) form. Relationships among individuals were determined by calculating the Nei and Li (1979) indices of genetic distance (GD<sub>NL</sub>) using PAUP* software version 4.0 (Swofford, 2000). GD<sub>NL</sub> values were subjected to clustering analyses using the unweighted pair group method with arithmetic averages using PAUP* software to produce a dendrogram showing the relationship among the cultivars. Percentage genetic similarity was calculated by the formula: [1- GD<sub>NL</sub>] x100% (Nei and Li (1979).
RESULTS

The number of bands for each primer varied from 1 to 7, with an average of 4 bands per primer. Band sizes ranged from 200 to 1500 bp, mostly concentrated from 200 to 800 bp. Primer B13 produced the highest level of polymorphism (shown in Figure 1).

**Figure 1.** RAPD profiles obtained by amplifying 24 cultivars of *Anthurium andraeanum* cultivars and *Spathiphyllum* species with primer B13. Lanes 1 to 29 are as follows: 100bp DNA weight ladder (lane 1), Paradiso (lane 2), Midori (lane 3), Mauna Kea (lane 4), AC 10 (lane 5), Fleur des Iles (lane 6), Bianca (lane 7), José (lane 8), Chloé (lane 9), KFI (lane 10), Mickey Mouse (lane 11), Rose (lane 12), UH (lane 13), Ozaki (lane 14), La Coquille (lane 15), Marcovie (lane 16), Breton (lane 17), Michelle (lane 18), Fontasia (lane 19), *Spathiphyllum* (lane 20), Salsa (lane 21), Marian Seefurth (lane 22), Tango (lane 23), Antartica (lane 24), Nita (lane 25), Bourgogne (lane 26), negative control with no DNA template (lane 27), negative control with no primer (lane 28) and 100bp DNA weight ladder (lane 29).

Most pairs involving the out-group had higher GD_{NL} values indicating that *Spathiphyllum* was distantly related to the anthurium cut flower hybrids. With a GD_{NL} value of 0.03, ‘Mauna kea’ was found to be 97% genetically similar to ‘Chloé’ thus making the closest cultivar pair. On the other hand, ‘Mauna kea’ and ‘Midori’ constituted the most distant pair with a GD_{NL} value of 0.21 (representing a genetic similarity of 79%). Average genetic distance among cultivars was 0.08 representing a mean genomic percentage similarity of 91.7%.

The dendrogram obtained upon subjecting all pair-wise GD_{NL} values to UPGMA clustering analysis was represented in Figure 2. Dendrogram based on 59 reproducible RAPD bands indicated that 14 cultivars were grouped into 2 clusters. ‘Midori’ was found to be genetically distinct from the remaining anthurium cultivars.

DISCUSSION

Polymorphism detected by RAPD analyses allowed distinction of cultivars. UPGMA cluster analyses using Nei and Li’s genetic distance coefficient revealed little variation among the 15 anthurium cut flower cultivars. Our results were consistent with numerous other studies showing anthurium hybrids and species to be closely related. For instance, Sheffer and Croat (1983) and Marutanni et al., (1993) found that cytological diversity among cut flower anthurium cultivars was significantly low. Cross compatibility testing indicated that species within the *Calomystrium* section were taxonomically closely related (Marutanni et al., 1988). Ranamukhaarachchi et al., (2001) showed that pot plant anthurium species were closely related to each other using RAPD markers polymorphism.
Table 1  \( GD_{NL} \) coefficients for 15 anthurium cultivars using PAUP 4.0b

Highest \( GD_{NL} \) value of 0.21 was between Mauna kea and Midori.
Lowest \( GD_{NL} \) value of 0.03 was between Mauna kea and Chloë.

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Clusters were not associated with flower colour, spathe type (standard or obake) and cultivar provenance. Spathes of different colours grouped together in the dendrogram. Similarly, no pattern was discernable on the basis of cultivar provenance since cultivars from Hawaii were likely to cluster with cultivars from Mauritius. A wide genome variance was not observed despite the fact that cultivars were of diverse provenances. Moreover, finding correlations between specific morphological traits and cluster groupings were expected to be difficult since most of the amplified RAPD products originated from non-coding regions of DNA which constitute the bulk of the chromosome. As a result, bands obtained did not control phenotype. Additionally, distance estimates based on RAPD markers could not be correlated with expectations based on known pedigrees as breeding history of many local and imported cultivars were not well documented.

Relating genetic distance indices to genetic diversity, the genetic base of the local anthurium germplasm was found to be narrow. Various reasons have been put forward to explain this narrow genetic base. The massive replacement of numerous older cultivars (considered to be obsolete) with fewer commercially successful ones has definitely resulted in genetic erosion of the germplasm leading to a decrease in genetic diversity. Due consideration should be given to old hybrids which no doubt comprise important alleles. Moreover, the close relationship among anthurium cultivars demands the use of a fairly large number of molecular markers to allow efficient discrimination. With a much higher multiplex ratio than RAPD markers, AFLP markers (Vos et al., 1995) can prove to be extremely useful in identifying anthurium cut flower cultivars and are actually under progress in our laboratory. Furthermore, a more intensive drive towards the protection of new plant varieties has been noted over the years and consequently breeders need methods to efficiently and quickly trace infringements of Plant Breeders’ Rights. AFLP has the potential of being an integral part of plant variety registration (Jan De Riek, 2001).

Assessment of level of variation is clearly feasible using RAPD markers and scope exists for application of other more powerful molecular markers in future anthurium diversity evaluation studies. Selection of promising parental anthurium genotypes in a breeding program should be based on morpho-agronomic traits complemented with molecular data which provide additional useful information. The successful improvement of cultivars will require the broadening of the narrow genetic base of the Mauritian anthurium gene pool.
ACKNOWLEDGEMENTS

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REFERENCES


APPLICATION OF MICROSATELLITE MARKERS TO THE SUGAR CANE BREEDING PROGRAMME

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ABSTRACT

Microsatellite markers or simple sequence repeats (SSRs) consist of short and repeated DNA sequences (1-6 nucleotides) densely interspersed throughout the eukaryotic genomes. They are regarded as the marker of choice for a number of plant species because they are highly polymorphic and species specific. Here we report the application of microsatellite markers in the sugar cane breeding programme. Six SSR primer pairs were selected based on their high level of polymorphisms. A non-radioactive fingerprinting method using fluorescently labelled SSR primers on an ABI 310 Genetic Analyzer was optimised. The primers proved useful for the identification of cultivars and in the verification of true to type clones regenerated from callus culture. The identification of male parents in polycrosses was also possible using these primer pairs. The hybrid nature of clones derived from intergeneric crosses (Saccharum officinarum x Erianthus arundinaceus) was also investigated using microsatellite markers and further confirmed by PCR using specific primers to the 5s ribosomal DNA spacer region.

Keywords: Microsatellites, SSR, fingerprinting, intergeneric hybrids, Saccharum, Erianthus, 5s rRNA.

INTRODUCTION

Molecular markers are powerful tools for studying plant genetic diversity and for fingerprinting of varieties. Several types of molecular markers have been proposed and include restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and microsatellites or simple sequence repeats (SSRs). However, lately, microsatellites have been established as the marker of choice in plant genetic studies.

SSRs are a small array of tandemly arranged nucleotides repeats (2-6 bases), which are widespread throughout eucaryotic genomes. They are highly polymorphic, reproducible, and are inherited in a codominant manner. In addition, they can be analysed using PCR-based methods.

DNA profiling is of considerable interest to modern plant breeders for measuring genetic diversity, identification and registration of genotypes, and assessment of genomic composition of hybrids. Microsatellites have been successfully utilised to assess genetic diversity in barley (Saghai-Maroof et al., 1994), wheat (Plaschke et al., 1995), rice (Xiao et al., 1996) and sugar cane (Corderio et al., 1999, 2000). Microsatellite markers are frequently used in DNA fingerprinting and variety identification in many species including peach (Downey and Jezzoni, 2000; Testonlin et al., 2000), apricot (Hormaza, 2002), grapes (Sanchez-Eschribano et al., 1999) and numerous others.

SSR fingerprinting is a valuable tool in assessing genetic integrity. Genetic changes can occur in micropropagated plants due to somaclonal variation during the process of cell division and differentiation (Shenoy and Vasil, 1992). At the Mauritius Sugar Industry Research Institute (MSIRI), callus culture is performed in view of eliminating Sugarcane yellow leaf virus (SCYLV) and sugarcane yellows phytoplasma (SCYP), two pathogens associated with yellow leaf syndrome of sugar cane (Parmessur et al., 2002). It is therefore necessary to maintain the true-to-type nature of micropropagated plants vis-à-vis the explant source. Morphological analysis is useful only when genotypic changes are expressed. This is a time consuming process especially for sugar cane and is not always straightforward. Microsatellite markers have shown their usefulness in confirmation of genetic fidelity in potato (Perazzo et al., 2000). These markers were also used to detect changes resulting from long-term in-vitro storage (Angel et al., 1996; Borner et al., 2000).
In plant breeding, there is concern about the reduction of the gene pool available for improvement of varieties. In such instances, breeders often resort to related genera for introgression of novel genes (e.g. disease and pest resistance, tolerance to floods, drought, and vigour). Monitoring the extent of introgression is essential but not readily feasible through morphological means and other conventional methods. Oliveira et al. (2002) reported the use of a combination of leaf apex morphology and SSR markers in the identification of citrus hybrids. Similarly, *Festulolium* hybrids (*Lolium x Festuca*) were assessed through species difference alleles revealed by microsatellite markers (Momotaz et al., 2004).

Modern hybrid sugar cane varieties descend from interspecific hybridisation mainly between *Saccharum officinarum* and *S. spontaneum* (Bull and Glasziou, 1979). The genetic base of modern varieties is very narrow since very few parental clones were utilised in the initial crosses (Arceneaux, 1965; Price, 1965). Thus, breeders want to introgress new traits from related genera of the 'Saccharum complex' comprising of the following species: *Saccharum*, *Erianthus* (sect. *Ripidium*), *Miscanthus* (sect. *Diandra*), *Sclerostachya* and *Narenga*. There is however a limited number of publications relating to the introgression of these species. One serious drawback has been the difficulty in identifying genuine intergeneric hybrids. With the help of PCR primers based on the highly variable 5s rRNA spacer region, genuine hybrids were successfully identified by D’hont et al., (1995).

In this study, we report the application of a non-radioactive, fluorescent-based, SSR technique in the sugar cane breeding programme at the MSIRI. The objectives were:

1. to identify unknown cultivars in the field,
2. to verify true to type clones issued from tissue culture,
3. to confirm pollen progenitor in polycross and
4. to identify true F1 hybrids from *Saccharum x Erianthus* intergeneric crosses.

## MATERIALS AND METHODS

### Preparation of genomic DNA

DNA was extracted from fresh meristematic sugar cane tissue using the CTAB method as described by Aljanabi et al., (1999). DNA was resuspended in 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA) and the quality and concentration were checked by agarose gel electrophoresis. Appropriate dilutions were made and the solutions stored at -20°C until further used.

### Microsatellite PCR amplification

The PCR amplification for the SSR loci were performed in a PTC-200 thermal cycler (MJ Research, Inc, USA) in a 25 µl total volume containing 2.5 µl of 10 X PCR buffer (Roche Diagnostics, USA), 1 U of Taq polymerase (Roche Diagnostics), 200 µM of each dNTPs, 0.2 µM of forward primer (fluorescently labelled with either TET-Yellow or HEX-green coloured dye), 0.2 µM reverse primer, 25 ng template DNA and sterile distilled water to 25 µl. Cycling conditions included an initial denaturation at 94°C for 3 min; followed by 30 cycles of 94°C for 45 s, appropriate annealing temperature (50-56°C) for 45 s, 72°C for 30 s; and a final step of 72°C for 5 min. The following primer pairs were selected based on their high level of polymorphisms; mSSCIR12, mSSCIR19, mSSCIR32, SMC119CG, SMC278CS, and SMC749BS. These primers were developed by the International Consortium for Sugarcane Biotechnology (ICSB).

The PCR products were loaded on an ABI Prism 310 Genetic Analyzer (Applied Biosystems Inc, USA) for capillary electrophoresis to determine the size of alleles. The ABI Genescan Analysis V. 3.1 and ABI Genotyper V. 2.5 softwares (Applied Biosystems Inc, USA) were used for analysis of results and sizing of alleles.
Fingerprinting of sugar cane varieties

Identification of an unknown variety

An unknown variety in the field was suspected to belong to either variety M 1334/84 or R 570 or R 575 based on morphological characteristics. Microsatellite analysis of these four varieties was performed using primers SMC119CG and mSSCIR32.

Verification of true to type clones regenerated from callus culture

Twenty sugar cane varieties were regenerated through callus culture in order to produce SCYLV-free and SCYP-free plants. These included M 1030/71, M 1042/86, M 1156/66, M 1176/77, M 1186/86, M 1315/86, M 1334/84, M 1394/86, M 1400/86, M 1551/80, M 1557/70, M 1565/87, M 1906/87, M 2024/88, M 2119/88, M 2256/88, M 292/70, M 3014/87, M 96/82, and M 1246/84. Using SSR analysis, in-vitro plantlets of each variety were compared with the original clones maintained in the field to check if they were true-to-type. SSR primers SMC119CG and mSSCIR32 were used for this purpose.

Identification of male parents in Polycrosses

SSR markers being co-dominantly inherited, it is expected that alleles in the progeny are provided by the parents. Twenty eight percent of crosses made at the MSIRI are derived from polycrosses involving several female and male varieties (MSIRI, 2003). The identification of the male parent involved in five polycrosses with known female parent (Table 1) was attempted based on presence of 'male-specific alleles' in the progeny (but which were absent in the female parent). Where the male parent was not identified using one set of primers, other primer pairs were evaluated.

Table 1 Clones involved in each polycross

<table>
<thead>
<tr>
<th>Polycross</th>
<th>Progeny</th>
<th>Female parent</th>
<th>Male parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>POL S 638/91</td>
<td>M 747/93</td>
<td>M 563/79</td>
<td>N 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na 6390</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SP 703370</td>
</tr>
<tr>
<td>POL S 1588/89</td>
<td>M 2238/89</td>
<td>M 555/60</td>
<td>CP 44101</td>
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<td></td>
<td></td>
<td></td>
<td>CP 5243</td>
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<td></td>
<td></td>
<td></td>
<td>CP 5268</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 2343/77</td>
</tr>
<tr>
<td>POL S 1490/95</td>
<td>M 683/85</td>
<td>W 681049</td>
<td>CP 66346</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>CP 811302</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F 802147</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RB 739953</td>
</tr>
<tr>
<td>POL S 859/90</td>
<td>M 1672/90</td>
<td>M 134/75</td>
<td>M 1729/80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 2229/80</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M 523/81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R 570</td>
</tr>
<tr>
<td>POL X 1889/94</td>
<td>M 1112/94</td>
<td>M 220/80</td>
<td>M 1371/78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 292/70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 220/80</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>M 515/79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 596/78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 860/70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 937/77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M 1107/81(not available)</td>
</tr>
</tbody>
</table>
Local modern hybrid cultivars (M 1176/77 and M 387/85) were used as female parents in crosses with *E. arundinaceus* (IK 7647) as summarised in table 2. Putative F1 hybrids were analysed to confirm their true identity.

### Table 2 Intergeneric *Saccharum x Erianthus* crosses performed

<table>
<thead>
<tr>
<th>Female parent Saccharum hybrid</th>
<th>Male parent Erianthus sp.</th>
<th>Putative F1 hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 1176/77</td>
<td>IK 7647</td>
<td>M 1156/00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1157/00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1158/00</td>
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<tr>
<td></td>
<td></td>
<td>M 1159/00</td>
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<tr>
<td></td>
<td></td>
<td>M 1160/00</td>
</tr>
<tr>
<td>M 387/85</td>
<td></td>
<td>M 1161/00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1162/00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 1163/00</td>
</tr>
</tbody>
</table>

**Amplification of the 5s ribosomal DNA spacer region by PCR**

To further confirm the identity of the *Saccharum x Erianthus* hybrids, they were analysed by PCR using primers based on the 5s rRNA spacer region. The sequence of primer pair P1/P2, (Cox et al., 1992) were 5'-TGGGAAGTCCT(C/T)GTGTTGCA-3' and 5' (T/G)T(A/C)G(T/C)GCTGGGTATGATCGCA respectively. Each PCR reaction consisted of 5 ng of genomic DNA, 2.5 µl of 10X PCR buffer (Roche Diagnostics, USA), 200 µM of each dNTP (Roche Diagnostics, USA), 0.2 µM of each primer, 1 U of Taq polymerase (Roche Diagnostics, USA) and sterile distilled water to 25 µl. The reactions were performed in a PTC-200 thermal cycler (MJ Research, Inc, USA) with the following thermal regime: an initial denaturation step of 3 min at 94°C, followed by 35 cycles of denaturation for 55 s at 94°C, annealing for 30 s at 55°C and extension step for 30 s at 72°C, and a final extension of 6 min at 72°C. After agarose gel electrophoresis and ethidium bromide staining, the PCR products were visualised under UV light.

### RESULTS

**Fingerprinting of sugar cane varieties**

**Identification of unknown variety**

The fingerprint profile of the different varieties obtained using primer SMC119CG are displayed in Figure 1. The profile of the unknown variety was compared with those of clones R 570, R 575 and M 1334/84 and found to match with the latter. These results were also confirmed using primer mSSCIR32. Thus, the unknown clone was established as being variety M 1334/84.

**Verification of true to type clones regenerated from callus culture**

The fingerprints for 19 out of 20 varieties in the field were identical to those derived from callus culture in-vitro. Variety M 1246/84 produced different profiles for the in-vitro and the original plant in the field (Figure 2). This variation may be due to mislabelling of the original clone or somaclonal variation and is being further investigated.
Figure 1 The unknown variety produced a similar fingerprint as variety M 1334/84 using primer SMC119CG.

Identification of male parents in Polycross

The identity of four out of five male parents in the respective polycross was successfully established. Table 3 summarises the results of the microsatellite analysis of each polycross. Due to the large number of male parents involved, three pairs of primers were used (SMC119CG, mSSCIR32 and mSSCIR 12). However for POL X 1889/94, no conclusive results could be drawn with the sets of markers used. Variety M 220/80 was used both as a male parent and pollen donor in this cross and also variety M 1107/81 was not available for analysis rendering the task difficult.

Table 3 Identity of the male parent in the polycrosses

<table>
<thead>
<tr>
<th>Polycrosses</th>
<th>Progeny</th>
<th>Female parent</th>
<th>Male parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>POL S 638/91</td>
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<td>M 2343/77</td>
</tr>
<tr>
<td>POL S 1490/95</td>
<td>M 683/85</td>
<td>W 681049</td>
<td>RB 739953</td>
</tr>
<tr>
<td>POL S 859/90</td>
<td>M 1672/90</td>
<td>M 134/75</td>
<td>R 570</td>
</tr>
<tr>
<td>POL X 1889/94</td>
<td>M 1112/94</td>
<td>M 220/80</td>
<td>Inconclusive results</td>
</tr>
</tbody>
</table>
Figure 2: Electropherograms from a 310 ABI Genetic Analyzer showing size polymorphisms detected using primer pair SMC119CG in tissue culture derived plantlets (T) and field material (F) of variety M 1246/84. The other two varieties, M 1186/86 and M 2119/88 produced similar profiles from tissue culture and field derived materials.

Identification of true intergeneric Saccharum x Erianthus hybrids with SSR markers

Hybrid clones should contain alleles derived from both Saccharum and Erianthus sp. Figure 3 represents the profiles for the putative hybrids, IK 7647 (E. arundinaceus) and M 1176/77 (Saccharum hybrid) using marker SMC119CG. Alleles 146, 157 and 194 bp were present in IK 7647 but were absent in the Saccharum parent M 1176/77. Among the putative hybrids, M 1156/00 and M 1159/00 contained all these three additional alleles while M 1157/00 and M 1158/00 contained the Erianthus alleles at 146 and 194 bp. This indicates the presence of Erianthus material in the chromosome of the putative hybrids since microsatellites are co-dominant markers. It was not possible to ascertain whether these clones also shared Saccharum characters as all alleles common in the putative hybrids and cultivar M 1176/77 were also present in the Erianthus sp, IK7647.
Figure 3 Profiles of putative hybrids M 1156/00, M 1157/00, M 1158/00 and M 1159/00 using primer pair SMC119CG are compared with M 1176/77 (Saccharum hybrid) and IK 7647 (E. arundinaceus). Alleles 146, 157 and 194 bp are specific to IK 7647.

Amplification of the 5s ribosomal DNA spacer region by PCR

To further confirm the nature of the clones, the 5s ribosomal DNA region was amplified by PCR. With the 5s rRNA primer pairs used, it is expected that a major band of approximately 370 bp is obtained from Erianthus sp., while Saccharum sp. is expected to produce a major product of about 230 bp. Figure 4 shows the various PCR products obtained with the DNA from the putative hybrids, IK 7647 and the Saccharum parents M 1176/77 and M 387/85. The expected band of 370 bp was amplified from IK 7647 (Lane 1). Similarly, the Saccharum specific 230 bp product was obtained with variety M 1176/77 and M 387/85. When DNA from IK 7647 and M 1176/77 were mixed, two major bands of 230 and 370 bp (lane 12) were obtained. All the putative hybrids exhibited exclusively the
Application of microsatellite markers to the sugarcane breeding programme. N. Joomun et al.

Erianthus specific product of 370 bp. Thus it is proposed that clones M 1156/00, M 1157/00, M 1158/00, M 1159/00, M 1160/00, M 1161/00, M 1162/00 and M 1163/00 are E. arundinaceus selfs and not hybrids of Erianthus x Saccharum.

Figure 4 Agarose gel electrophoresis of PCR products. Lane 1, IK 7647 (E. arundinaceus); Lanes 2 and 3, M 1176/77 and M 387/85 respectively (Saccharum hybrids). Lanes 4-11, putative F1 hybrids M 1156/00, M 1157/00, M 1158/00, M 1159/00, M 1160/00, M 1161/00, M 1162/00 and M 1163/00 respectively; Lane 12, control with a DNA mix of M 1176/77 and IK 7647; Lane 13, water control; lane 14, molecular weight marker IX (Roche Diagnostics, USA).

DISCUSSION

The semi-automated ABI fluorescent system is an easy and reproducible method for SSR analysis. It is now routinely used as an aid in the breeding programme at the MSIRI. The SSR markers used were highly polymorphic. It was possible to confirm the identity of the unknown clone in this study using two sets of primers (mSSCIR32 and SMC119CG). Morphological analysis could be coupled with SSR analysis in order to reduce the number of clones for testing. In future, it would be desirable to have a variety database to effectively manage local germplasm collection. With SSR markers, it is possible to have unique DNA profiles for different varieties. The genetic diversity of a collection of noble canes (Saccharum officinarum) was evaluated previously (Aljanabi et al., 2003). This marker system is being extended to local cultivars and as shown in this study, clones can be accurately fingerprinted. Characterisation of local cultivars will also be useful for registration purposes and protection of plant breeders’ rights.

Prior to the application of SSR markers, it was not possible to identify the pollen donor in a polycross. The ability of a cross to produce elite varieties (proven cross method) is useful in sugar cane plant breeding. Plant breeders can now use these information for directed crosses and thus transfer useful traits to the progeny.

The success rate of intergeneric Saccharum x E. arundinaceus crosses is usually low (D’Hont et al., 1995) due to the high number of seedlings produced as a result of self-pollination or pollen contamination. In Queensland, out of 96 intergeneric crosses producing 1000 seedlings, only 19 putative hybrids survived (Piperidis et al., 2000). Genuine hybrids were observed at very low frequency (2.8 %) and all were sterile and had poor vigour (Piperidis et al., 2000). Using 5s rRNA primers, none of the putative hybrids produced at MSIRI amplified the 230 bp marker in Saccharum and the 370 bp marker observed in Erianthus showing that they were not true hybrids. However, both fragments were amplified from the control mixture of both DNAs’. Hence, this technique can be applied to the breeding programme for rapid screening of putative hybrids.

SSR fingerprinting is also a valuable tool for analysis of putative hybrids. Momotaz et al., (2004) selected markers revealing species-difference alleles. For sugar cane, the study of hybrids can be improved by screening a larger number of primers, which would enable selection of markers specific for *Saccharum* and *Erianthus*.

ACKNOWLEDGEMENTS

The authors are thankful to the Plant Breeding Department, MSIRI, for providing relevant information regarding the crosses performed. Primers used in this study were developed by members of the International Consortium for Sugarcane Biotechnology (ICSB) to whom thanks are extended.

REFERENCES


IN-VITRO MUTATION STUDIES OF TARO
(Colocasia esculenta Var. esculenta) IN MAURITIUS.

S. Seetohul ¹ and D. Puchooa ²

¹AREU
²University of Mauritius

ABSTRACT

The combination of in-vitro culture and mutation breeding has been employed for many years to generate novel plant mutants and cell lines of agricultural and industrial interest. Taro (Colocasia esculenta var. esculenta) plants, being vegetatively propagated and hardly bearing seeds, induced mutation method offers the only way to induce variability and breeding. In Mauritius, Taro production was severely affected, since 1997 by Taro leaf blight disease (TLB), caused by Phytophthora colocasiae. Both types of Taro, Colocasia esculenta var. esculenta and Colocasia esculenta var. antiquorum are susceptible to the disease. In the in-vitro culture, shoot tips trimmed to only the apical dome with one or two primordial leaves were used as explants for the initiation of tardo. Optimum disinfection of axilliary buds of developing suckers that are sources of explants was obtained when treated with 2% sodium hypochlorite and 1 drop of tween 20 per 100 ml for 15 minutes. Shoot-tips were cultured on Murashige and Skoog media with different concentrations of IAA. Optimum initiation was obtained with IAA (10 mgL⁻¹). In the radiosensitivity analysis, shoot-tips were treated with irradiation doses of 0-60 grays of a ¹³⁵Co gamma irradiation source and cultured on MS Medium supplemented, with 10 mgL⁻¹ IAA. The effective mutation dose (LD₃₀), that causes 30% reduction in growth was found to be 7.65 grays. The in-vitro mutation technique is an efficient method to produce large numbers of mutant colocasia germplasm which will be selected for Taro Leaf blight resistance.

Keywords: taro, mutation breeding, in-vitro culture, taro leaf blight

INTRODUCTION

Taro (Colocasia esculenta) is a herbaceous plant with a swollen underground stem, the corm, belonging to the family Araceae. It is one of the most ancient crops and continues to be a key component of livelihoods in areas of Pacific, South East Asia, West Africa and the Caribbean, where it has special cultural, dietary and economic importance. Worldwide, taro ranks fourteenth among staple crops with 9 million tons produced globally on some 2 million hectares of land (S. Caillon et al., 2004). Both the corm, which can be baked, roasted or boiled, and the leaves are eaten. The latter is a significant source of vitamins, especially folic acid.

The cultivated species of tardo may be distinguished into two main groups, namely the Eddoe type, and Dasheen type. Eddoe type (Colocasia esculenta var. antiquorum), which is locally known as arouille carri, has one main tuber (corm) and several side tubers (cormels). On the other hand, the Dasheen type (Colocasia esculenta var. esculenta), which is locally known as arouille violette, has one main tuber.

In Mauritius, the average annual production of tardo is of about 192.3 tonnes (1993 – 2001). The production has dropped drastically from 480 tons in 1993 to 45 tonnes in 1997, with a subsequent increase in its retail price in the local market (Jugurnauth et al., 2001). This situation was mainly due to the epidemic outbreak of tardo leaf blight (TLB) disease, caused by Phytophthora Colocasiae. This disease was first recorded in Mauritius in the year 1995. In fact, Taro Leaf Blight is the most destructive fungal disease of tardo. It is considered to have originated in South East Asia (Trusillo, 1967; Zhang et al., 1994) and is widely distributed throughout the tropical regions of the World (CMI, 1997).

As locally available colocasia germplasm are susceptible to tardo leaf blight disease, a breeding programme using in vitro mutagenesis technique was initiated with a view to develop mutants of tardo.
(Colocasia esculenta var. esculenta) showing resistance to the disease. This method of improving taro was chosen because the introduction of new germplasm in Mauritius is prohibited due to the embargo laid by the local quarantine services on importation on any plant from the Araceae family. This measure is important to protect the Anthurium industry from bacterial infection. Even in vitro plantlets of taro are not allowed to be introduced. Moreover, taro being vegetatively propagated, it is difficult to obtain genetic variation.

Mutagenic agents such as radiation and certain chemicals have been used to induce mutations at a higher frequency and generate genetic variation from which desired mutants may be selected. Generally, radiation and especially gamma rays have most often been used to generate desired characters for crop breeding. Today, the FAO/IAEA mutant varieties database includes nearly 2,300 officially released varieties of 154 plant species (Jain 2004).

The combination of mutation breeding and in-vitro culture (also called in-vitro mutagenesis) has been found to make the induction and the selection of induced mutations more effective and it speeds up the production of mutants as a result of an increased propagation rate and a greater number of generations per unit time and space (Morpurgo et al., 1997).

The aim of this paper is to present part of the findings of this study namely, the in vitro initiation of taro and the radiosensitivity analysis to determine the effective mutation dose (LD30).

MATERIALS AND METHODS

Plant material and surface sterilization

Selected plants of taro of the dasheen type (Colocasia esculenta var. esculenta) were collected and grown in open field under sprinkler irrigation at Richelieu Crop Research Station. These plants were grown until new suckers and stolon were produced. Axillary buds of developing suckers (Figure 1) were used as source of explants for the in-vitro culture.

Figure 1 Axillary buds of developing suckers

The axillary buds were rinsed under running water for 1 hr, cleaned and then trimmed to approximately 2 cm³. They were then washed for 15 minutes in a solution of benlate at 0.06% on a shaker. The buds were rinsed under running water for 15 minutes. They were then transferred under laminar flow and washed in four different levels of sodium hypochlorite (NaOCl), namely 1.0%, 1.5%, 2% and 2.5% with one drop of Tween 20 per 100 ml in each treatment for 15 minutes. Finally, buds were rinsed three times with sterile distilled water and then trimmed until only the apical dome with one or two primordial leaves remained (2-3 mm). The shoot tips from each treatment were then transferred to culture jars containing Murashige and Skoog (MS) media supplemented with IAA (20 mg L⁻¹). Explants were cultured at 23 ± 2°C under 12 hr photoperiod. Treatments were laid in a randomized block design with 30 replicates.
In-vitro Mutation Studies of Taro (Colocasia Esculenta Var. Esculenta) in Mauritius. S Seetohul and D Puchooa.

Parameters measured were percentage contamination, percentage survival and percentage of buds that developed shoots.

**In-vitro initiation media test for taro**

This experiment was carried out to determine the optimum media for the *in-vitro* initiation of taro. MS media with different concentrations of IAA (0, 10, 15, 20 and 25 mg L\(^{-1}\)) were tested.

Developing buds from selected taro plants from germ plasm collection were collected for removing the shoot tips. The buds were cleaned and rinsed under running water for 1 hour. They were then washed in a solution of benlate of 0.06% for 15 minutes and then rinsed under running water for another 15 minutes. They were then treated with 2% sodium hypochlorite and 1 drop of tween 20 per 100ml for 15 minutes. Finally, the buds were rinsed three times with sterile distilled water under laminar flow.

Media were solidified with 0.18% Phytagel; pH was adjusted to 5.7 ± 0.1 before autoclaving for 15 minutes at 121°C. Explants were cultured at 23 ± 2°C under 12 hours photoperiod.

**Determination of effective mutation dose (LD\(_{30}\))**

This experiment was carried out to determine the appropriate mutation dose which induces a 30% reduction in the growth of treated explants. An irradiator with gamma rays as source of irradiation from a caesium-137 source was used.

30 shoot-tips of 3-4mm, excised from tissue culture plantlets, were irradiated with 0, 2, 4, 6, 8, 10, 12, 14, 16, 20, 40, 60 Gy. Treated explants were then cultured on MS media supplemented with IAA (10mg L\(^{-1}\)). Data on percentage survival, number of leaves and roots, length of leaves and roots and number of buds were recorded on a weekly basis for a duration of 8 weeks.

**RESULTS and DISCUSSION**

**Plant material and surface sterilization**

Different concentrations of NaOCl were evaluated in combination with Tween 20. Tween 20 was used to reduce the surface tension of the cutting explant of taro and to improve the surface contact of the bleach solution.

The results showed that NaOCl / 15 minutes and Tween 20 reduced, in all the cases, the presence of contaminants in the *in-vitro* culture media (Figure 2). NaOCl (2.5%) did eliminate all the surface contaminants but it was toxic to cellular tissue of taro. NaOCl (2%) did not eliminate all the contaminants as some bacteria and fungi were observed in the culture media. However, the survival rate as well as the percentage of green buds that developed shoots after five weeks were highest. The average length of shoots after five weeks is illustrated in Figure 3.

Contamination obtained were both fungal (mainly of *Aspergillus* and *Fusarium* species) and bacterial. Sodium hypochlorite is one of the common disinfectant used in tissue culture to promote surface sterilization and improve the establishment of aseptic cultures (Pierik, 1989). This is, because its mechanism of action causes biosynthetic alterations in cellular metabolism and phospholipid destruction, formation of chloramines that interfere in cellular metabolism, oxidative action with irreversible enzymatic inactivation in bacteria and lipid and fatty acid degradation (Estrela et al., 2002).

(Pécora and Souza 1993) reported that NaOCl exhibits a dynamic balance as is shown by the reaction.

\[
\text{NaOCl} + \text{H}_2\text{O} \leftrightarrow \text{NaOH} + \text{HOCl} \leftrightarrow \text{Na}^+ + \text{OH}^- + \text{H}^+ + \text{OCl}^-
\]

Hypochlorous acid, a substance present in NaOCl solution when in contact with organic tissue acts as a solvent, releases chlorine that, combined with the protein amino groups, forms chloramines. Chlorine (strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible
oxidation of SH groups (sulphydryl group) of essential bacterial enzymes. The antimicrobial effectiveness of NaOCl is also based on its high pH (hydroxyl ions action).

**Figure 2** Effect of 4 levels of NaOCl on disinfection of taro

**Figure 3** Average length of shoots, five weeks after treatment with NaOCl.

**In-vitro initiation media test for taro**

In the initiation media test with IAA at levels of 0, 10, 15, 20 and 25 mg L⁻¹, more healthy and vigorous growth in terms of highest average number of leaves and roots was obtained with IAA at 10 mg L⁻¹ (Figure 4).

**Figure 4** Effect of different concentrations of IAA on in-vitro initiation of taro @ 8 weeks
MS media supplemented with IAA at concentrations above 15mg L\(^{-1}\) did not support growth of taro explants as compared to Basal MS Media. Growth in basal MS Media may be attributed to the effect of endogenous growth regulators. Similar observations regarding the role of endogenous levels of growth regulators in determining the shoot forming-capacity of tomato leaf disks have been reported (Kartha et al., 1976, Frankenberger et al., 1981). Another study (Elliot et al., 1987) has also demonstrated that a critical endogenous level of growth regulators has to be attained before cell division and organogenesis can occur.

**Determination of effective mutation dose (LD\(_{30}\))**

Apart from the control where the explants were not irradiated, taro shoot tips were subjected to eleven doses of irradiation (2, 4, 6, 8, 10, 12, 14, 16, 20, 40, 60 Gy)

The optimal dose for mutation induction is dependent on the parameter studied. Among all the parameters studied, number of leaves was preferred because it resulted in less experimental error than other parameters recorded such as survival rate, number of roots, length of leaves and roots. The effect of different doses of gamma irradiation on growth of taro plantlets is presented in **Figures 5 and 6**.

**Figure 5** Response of *in-vitro* culture of shoot-tips of taro after irradiation.
Figure 6  Tissue culture plantlets from shoot-tips irradiated at 0, 2, 6, 8, 10, 20, 40, 60 grays after 8 weeks in culture

The average number of leaves of treated shoot-tips with 2 Gy was higher, even than that of the control after 18 days indicating the boosting effect of this dose. A similar response was obtained when Anthurium Andreanum *in-vitro* leaf explants were irradiated with 5 Gy. The calli and seeds also expressed better responses at the 5 Gy, but lethality at 15 Gy (Puchooa, 2005). In our study, irradiation doses above 20 Gy were lethal to taro explants. It should also be pointed out that the effective mutation dose is controlled by a number of parameters including the genotype, the type of explant, the orientation of explant on the culture medium, and the origin of the explant from the mother plant (Douglas, 1995).

Data recorded on the number of leaves showed that the effective mutation dose, which caused a 30\% reduction in growth, was 7.65 Gy as shown in Figure 7.

Figure 7  Average number of leaves 52 days after irradiation.
Future Research Activities

Having determined the optimum initiation and multiplication media for in-vitro culture of taro and the effective mutation dose, shoot-tips of taro will be irradiated with 7.65 Gy for induction of mutation. The treated explants will be grown and multiplied 4 times in-vitro.

Following mutagenic treatment and multiplication, the plants will be subjected to screening and selection for resistance against Phytophthora Colocasiae, pathogen responsible for the colocasia leaf blight disease. Screening and selection will be carried out using regenerated mutant plants under a polycarbonate house. Selected mutants of Colocasia esculenta var. esculenta will be characterized using molecular tools.

A limitation commonly associated to the technique of in-vitro mutation is that the probability of achieving success is somehow aleatory. However, this intrinsic characteristic of the technique will be compensated with the use of large treated populations, an efficient screening protocol to select mutant of disease resistance against Phytophthora Colocasiae among the other mutants and the non-mutated tissue.

Tissue-cultured based mutagenesis has been employed for many years to generate novel plant mutants and cell lines of agricultural and industrial interest (Collin & Dix, 1990). In this study, this technique is being used to generate mutants of Colocasia esculenta var. esculenta resistant to Phytophthora Colocasiae. Induced mutants reported in Arabidopsis in recent years have opened new possibilities for analyzing defense response in plants. These studies have been possible with the availability of well characterized induced mutants altering response to pathogens. Mutations affecting gene resistance, hypersensitive response and systemic acquired resistance (SAR), lesion mimic mutants, phytoalexin deficient mutants, enhanced susceptibility, enhanced resistance and signal transduction pathways have been identified (Bhatia, 2000).

CONCLUSION

This study has helped in the establishment of a protocol for the disinfection of taro explants and the in-vitro initiation of the crop. These can be successfully used for the rapid multiplication of taro.

The study has also led to the determination of the effective mutation dose (LD30) which will be used to irradiate shoot-tips of Colocasia esculenta var. esculenta with a view to create mutants showing resistance to the taro leaf blight. Such technique of in-vitro mutagenesis can be further extended to other vegetatively propagated crops of economic importance in Mauritius.

ACKNOWLEDGEMENTS

The author wishes to thank IAEA for partly funding the project, FARC for providing tissue-culture facilities for implementing the project, Assistant Research Scientists of the Agronomy Division and the Management of Agricultural Research and Extension Unit for support in this study.

REFERENCES


IN VITRO AND MOLECULAR STUDIES ON Asparagus officinalis

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ABSTRACT

Regeneration of asparagus plantlets through indirect organogenesis was achieved by using different plant growth regulators. Callus induction and shoot proliferation were attempted using various combinations and levels of NAA, KIN and BAP. Rooting efficiency was assessed on MS media enriched with IAA, NAA, 6% and 9% sucrose. The effect of nodal position of explants on shoot formation was also tested. Regenerated plantlets were transferred to field where their survival, growth rate, and spear production were evaluated. Highest shoot production was obtained on MS medium supplemented with 0.015 mgL⁻¹ NAA combined with 0.25 mgL⁻¹ BAP. When nodes from different positions on the seedlings were used, no significant difference in shoot formation was observed. Rooting was highest (89.7 %) on MS medium supplemented with 6 % sucrose and 1 mgL⁻¹ NAA. Plantlets transferred to field produced spears and flowers with a survival percentage of 58.6%. RAPD technique was used to screen for genotypic similarities and differences in two mother plants that were used to establish clonal lines. Out of the 23 primers tested, 21 primers giving scorable bands in the range of 300-1950 bp have been identified. Among these 9 were sensitive enough to generate polymorphic bands that could potentially be used to differentiate between the two mother plants.

Keywords: Asparagus officinalis, micropropagation, RAPD, somaclonal variation, field trial.

Abbreviations: BAP – benzylaminopurine; IAA – indole-3-acetic acid; KIN – kinetin ; MAS – marker assisted selection; MS – Murashige and Skoog (1962); NAA - α-naphthaleneacetic acid; PCR – polymerase chain reaction; PGR – plant growth regulator; RAPD – random amplified polymorphic DNA.

INTRODUCTION

Asparagus officinalis is a member of the Liliaceae family and is a very promising vegetable crop for Mauritius, the reasons being its adaptability to a wide range of climatic conditions (Reuther 1984), and its high price on the world market. At present, it is not a traditional crop in Mauritius and is still considered as a luxury vegetable item. It is mostly cultivated by sugar estates, and its production for the whole country is approximately 35 tonnes (data provided by Agricultural Research and Extension Unit – AREU / FARC). It generally propagates only at a slow rate by conventional method of crown division (Ellison, 1986; Yang and Clore, 1973). To boost up its cultivation and its commercial potential there is a need for fast production of plantlets.

In this regard, a study was initiated three years ago with the main objective of establishing a protocol for micropropagation of A. officinalis cv Mary Washington. Two pathways were targeted to be evaluated, namely: somatic embryogenesis and indirect organogenesis. A protocol for micropropagation of Asparagus through indirect organogenesis was established after one year (Bojnauth et al., 2004). It was found that MS medium should be supplemented with 0.015 mgL⁻¹ NAA and 0.5 mgL⁻¹ BAP to promote both callus, shoot and crown formation. A rooting media allowing a rooting success of 50 % was also devised.

However, one of the major concerns in micropropagation, is the development of somaclonal variants among regenerated plantlets. This warrants the establishment of reliable techniques to determine their genetic stability. RAPD technique (William et al., 1990) is a suitable candidate for screening genomic DNA for somaclonal variation as it is simple and quick to perform, requiring small quantities of DNA and no prior knowledge of DNA sequence.
The aims of the present study were to re-evaluate and optimise the established protocol of regeneration (Bojnauth et al., 2004); to use RAPD technique to generate scorable bands with asparagus DNA; and also to test the growth, production and survival of regenerated plantlets under field condition.

**METHODOLOGY**

**Plant material**

*In vitro* seedlings were grown on ½ MS media and kept for 1 week in darkness followed by 3 weeks in light. The nodes (starting from base of shoot) were used as explants for callus induction for ultimate regeneration of plantlets.

**Culture conditions**

The basal medium used was the Murashige and Skoog (1962) medium supplemented with 3% sucrose, and 0.6% agar (oxid number 3) and growth regulators as specified (unless otherwise stated). The pH was adjusted to 5.7±0.1 with either 0.1M NaOH or 0.1M HCl, and then autoclaved for 20 mins at 15 psi, 121°C. The light condition in the culture room was 16 hours light (3000-4000 Lux) and 8 hours darkness.

**Indirect organogenesis**

*Induction of callus from nodal sections*

Several media were tested for their ability to induce callus formation and to initiate shoot development. Two sets of media were used: M1 containing 5 different concentrations of NAA in combination with 5 concentrations of BAP; and M2 comprising 5 levels of NAA in combination with 5 concentrations of Kinetin (Table 1). In all there were 50 treatments each with 4 replicates.

<table>
<thead>
<tr>
<th>Media</th>
<th>PGR concentrations used mgL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAA</td>
</tr>
<tr>
<td>M1</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>0.020</td>
</tr>
<tr>
<td>M2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NAA</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
</tr>
</tbody>
</table>

The *in vitro* seedlings were used as explants source. Three nodes of size of about 2-3 mm were inoculated per petridish. After inoculation these were arranged in a completely randomised design and kept in light condition.

*Development of shoots from callus*

After 28 days, nodes were subcultured in 125 mL jars containing 20 mL of media (of similar composition as the petridishes). The jars were sealed with parafilm and kept in light condition in
culture room. A second subculture was performed after 28 days. After a further 28 day period, the number of shoots of size 1 cm or more was counted for the 5 best treatments for each of the 2 sets of media.

**Rooting of the regenerated shoots**

Four rooting media containing MS basal medium supplemented with varying concentrations of plant growth regulators and sucrose levels were used (Table 2). The effect of presence and absence of light was studied.

<table>
<thead>
<tr>
<th>Media tested</th>
<th>Culture condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 0.5 mgL⁻¹ IAA + 3% sucrose</td>
<td>Dark</td>
</tr>
<tr>
<td>MS + 1.0 mgL⁻¹ IAA + 3% sucrose</td>
<td>Dark</td>
</tr>
<tr>
<td>MS + 1 mgL⁻¹ NAA + 6% sucrose</td>
<td>Light</td>
</tr>
<tr>
<td>MS + 9% sucrose</td>
<td>Light</td>
</tr>
</tbody>
</table>

The regenerated shoots were separated into units containing at least 2 shoots and a portion of the crown, which were then placed at a frequency of 2 per jar (250 mL jar containing about 35mL of medium). The rooting percentage was recorded after 6 weeks.

**Induction of callus from nodal sections derived from different positions on shoot**

Nodes from 3 different positions, from shoot base upwards (1st, 2nd and 3rd), in 28 days old seedlings were inoculated on MS medium supplemented with 0.015 mgL⁻¹ NAA and 0.25 mgL⁻¹ BAP. This medium had been optimised for maximum callus and shoot development earlier in this study (induction of callus from nodal sections). The medium prepared was poured into petridishes and each of these was inoculated with 3 nodes of size of about 2-3 mm. In all there were 3 treatments (3 nodal positions), each with 6 replicates. After inoculation, the petridishes were arranged in a completely randomised design and kept in light condition. The nodes developed into shoots as stated earlier (development of shoots from callus). At the end of 3 months the average number of shoots produced per node for each nodal position was recorded.

**Field trial of tissue culture plantlets**

Twenty-nine fully rooted plantlets were transferred to field following hardening. They were planted in 2 rows separated by 75 cm and the closest distance between 2 plants in the same row was 50 cm. They were watered once or twice per week depending on climatic conditions. No fertiliser or pesticide was used.

After ten months, all mother stalks from the plants were measured (tallest stalk from each plant), then cut except for plant number 17 where stalks were left intact. Plant number 17 was left out to act as a control (to show that complete removal of mother stalks did have an influence on spear production). Spears were harvested from the 18 surviving plantlets and their head diameter, length and weight were noted. During harvest, the plants were treated on a fortnightly basis with the fungicide mancozeb at concentrations specified by the manufacturer.

**Molecular analysis**

**DNA extraction**

Plant DNA was extracted from 0.1 g of *in vitro* plants (2 mother plants and shoots from clonal lines developed from the two mother plants) by using the Qiagen Dneasy Plant Mini Kit. Its concentration was calculated from the optical density at a wavelength of 260 nm. Before use, all DNA samples were diluted to a working concentration of 12.5 ngµL⁻¹.
**RAPD analysis**

Twenty-three decamer arbitrary primers were screened for their ability to bind and amplify regions of the asparagus genome. PCR was performed in 25µL of 20 mM Tris-HCl; 50 mM KCl; 3 mM MgCl2; 100 µM dNTP; 0.6 µM primer; 25ng DNA; and 1 unit Taq DNA polymerase. DNA amplification was performed in MY cycler thermal cycler BIORAD. The PCR cycling conditions were as follows: 1 cycle of 5 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C and a final time delay cycle of 5 min at 72°C. The RAPD / PCR products were resolved on a 1.5 % agarose gel and electrophoresed at a constant voltage of 90 V. The DNA bands were stained with ethidium bromide and visualised under a UV transilluminator. Marker VI and Hyperladder VI were used as molecular standards.

**RESULTS**

**Indirect organogenesis**

**Callus induction and shoot proliferation**

MS medium supplemented with 0.015 mgL⁻¹ NAA and 0.25 mgL⁻¹ BAP gave the highest mean number of shoot per explant (12.84) and there was significant difference between this treatment and the 4 other conditions used (Table 3). These results clearly show the importance of both auxin and cytokinin for shoot formation.

**Table 3** Mean number of shoots produced per explant per treatment after 84 days of culture.

<table>
<thead>
<tr>
<th>Treatment (NAA / BAP)</th>
<th>Mean Number of shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015 / 0.25</td>
<td>12.84 a</td>
</tr>
<tr>
<td>0.02 / 1</td>
<td>7.75 b</td>
</tr>
<tr>
<td>0.015 / 1.0</td>
<td>6.67 b</td>
</tr>
<tr>
<td>0.01 / 1</td>
<td>4.42 b</td>
</tr>
<tr>
<td>0 / 0.5</td>
<td>4.17 b</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different (p<0.05) from each other based on L.S.D.

The very low mean number of shoots per explant produced when NAA was used in combination with KIN (Table 4), clearly indicated that shoot development is influenced by the type of cytokinin used. Our results therefore emphasise that MS medium supplemented with NAA and KIN seems inappropriate for use on *Asparagus officinalis*. Given the low shoot production, no statistical analysis was attempted.

**Table 4** Mean number of shoots produced per explant per treatment after 84 days of culture.

<table>
<thead>
<tr>
<th>Treatment (NAA / KIN)</th>
<th>Mean Number of shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 / 1.25</td>
<td>2.33</td>
</tr>
<tr>
<td>1.0 / 0.5</td>
<td>2.33</td>
</tr>
<tr>
<td>0.5 / 0.75</td>
<td>2.00</td>
</tr>
<tr>
<td>0.75 / 1.0</td>
<td>1.60</td>
</tr>
<tr>
<td>1.25 / 1.0</td>
<td>1.43</td>
</tr>
</tbody>
</table>

**Rooting of regenerated plantlets**

The roots produced on media enriched with 6% and 9 % sucrose were white, thick and of adventitious type, whereas those which developed on IAA enriched media were thinner and had one main root. MS medium supplemented with 1 mgL⁻¹ NAA and 6% sucrose gave the highest rooting percentage of 89.7% (Table 5).
Table 5 Percentage rooting on the 4 media studied.

<table>
<thead>
<tr>
<th>Media tested</th>
<th>Rooting %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 0.5 mgL⁻¹ IAA</td>
<td>46.0</td>
</tr>
<tr>
<td>MS + 1.0 mgL⁻¹ IAA</td>
<td>67.0</td>
</tr>
<tr>
<td>MS + 1 mgL⁻¹ NAA + 6% sucrose</td>
<td>89.7</td>
</tr>
<tr>
<td>MS + 9% sucrose</td>
<td>78.5</td>
</tr>
</tbody>
</table>

Effect of nodal position on shoot development

Nodal explants obtained from position 3 gave the highest number of shoots. However, statistical analysis showed that no significant difference in shoot number was observed when nodal position 2 and 3 were used as explant. However, the number of shoots formed when nodes from position 1 was used, was significantly lower than for the other 2 nodal positions (Table 6).

Table 6 Mean number of shoots produced per explant from each nodal position after 84 days of culture.

<table>
<thead>
<tr>
<th>Nodal position</th>
<th>Mean Number of shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd</td>
<td>10.39 a</td>
</tr>
<tr>
<td>2nd</td>
<td>8.13 a</td>
</tr>
<tr>
<td>1st</td>
<td>2.50 b</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different (p<0.05) from each other based on L.S.D

Field trial of successfully acclimatised tissue culture plantlets

Survival of acclimatised tissue culture plantlets in the field was 62.1 %. The plants that survived grew normally, giving flowers and fruits but the rate of growth differed giving rise to shoots of various height (ranging from 89 – 245 cm after 10 months). The diameter of the spears ranged from 0.4 – 1.8 cm and their mean weight ranged from 5.0 - 19.1 g (Table 7).

Table 7 Data on spears harvested from the 18 plants growing in the field over a two week period.

<table>
<thead>
<tr>
<th>Plant No</th>
<th>Diameter range cm</th>
<th>Spears harvested</th>
<th>Total weight g</th>
<th>Mean weight g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5-1.1</td>
<td>77.0</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.6-1.4</td>
<td>116.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.8-1.1</td>
<td>31.0</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Nil</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.8-0.9</td>
<td>10.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.8-1.8</td>
<td>153.0</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.4-1.2</td>
<td>90.5</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.9</td>
<td>7.0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.6-1.2</td>
<td>90.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.6-1.0</td>
<td>45.0</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.8-0.9</td>
<td>24.0</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.6-1.3</td>
<td>85.0</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
<td>20.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.5-1.0</td>
<td>64.0</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Nil</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.5-1.3</td>
<td>98.5</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.1-1.3</td>
<td>30.0</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.6-1</td>
<td>22.0</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>
During the 2 weeks period, the diameter of spears produced was quite consistent for the 5 plants. Plant number 8 gave spears of better quality with a spear diameter ranging from 0.8-1.8 cm (Figure 1).

**Figure 1** Variation in diameter of spears produced over 2 week by 5 plants with highest yield

![Graph showing variation in diameter of spears produced over 2 weeks by 5 plants with highest yield.](image)

**RAPD analysis**

Out of the 23 primers screened, 21 gave scorable RAPD markers, out of which 9 produced polymorphic bands. A total of 107 bands were generated, of which 19 (17.7%) were polymorphic between the 2 parent plants (Plates 1 and 2). The bands ranged from 300 to 1950 bp with a majority of the polymorphism being distributed between 450 and 1500 bp. On average each primer set produced 5 bands.

**DISCUSSION**

The number of shoots produced per explant per treatment when NAA and BAP are used as growth regulators is much higher than with NAA and KIN. This indicates that BAP is more efficient than KIN to induce shoot formation in asparagus when used in combination with NAA.

Data in **table 3** show that a number of combinations of NAA and BAP can produce a large number of shoots in 3 months. Statistical analysis confirmed that there is significant evidence (at 5% level) that treatment M1/0.015/0.25 is different from the four other treatments and that no significant difference exist among the treatments M1/0.02/1, M1/0.015/1, M1/0.01/1 and M1/0/0.5.

Indirect organogenesis pathway has been thoroughly studied by Reuther (1977, 1984) and Hunault (1973, 1974, 1975, 1981), but in both cases different media were used for callus induction and shoot production. The protocol established in this study has the advantage of being simpler to implement as only one medium is involved and moreover all regenerated shoots possess a crown. The latter organ has been found to be of prime importance for rooting and survival of regenerated plantlets in the field (Doré, 1988).
In vitro and molecular studies on *Asparagus officinalis*. G Bojnauth et al.

MS medium supplemented with 6% sucrose and 1 mgL⁻¹ NAA gave the highest rooting percentage (89.7%) compared to the other media. The use of 9% sucrose alone (without plant growth regulator) in the rooting media however, was less efficient than when 6% sucrose and 1 mgL⁻¹ NAA was used, implying that the presence of auxin in the medium is instrumental to rooting. In 1987 Desjardins et al. compared the effect of supplementing rooting media with 7% sucrose and iso-osmotic concentration of mannitol. Their data showed that rooting occurred only on sucrose enriched media, that rooting was not induced by increased osmotic potential. The actual mechanism of action of increased sucrose concentration on rooting has yet to be determined in our study.

**Plate 1** Polymorphic bands scored with 2 primers used on DNA from mother plants

1 2 3 4 5

Lane 1 and 2: Primer OPC 08
Lane 3 and 4: Primer OPC 01
Lane 5: Marker VI

**Plate 2** Similar bands scored with 2 primers used on DNA from the 2 mother plants.

1 2 3 4 5

Lane 1: Marker VI
Lane 2 and 3: Primer OPC 16
Lane 4 and 5: Primer DK02

The results obtained from our experiment on nodal position show that the further away the node is from the base, the greater the number of shoots produced. This could be due to higher internal auxin level in nodes of position 2 and 3 as they are close to the shoot tip where auxin is produced. This finding is in contradiction with observation made by Yang and Clore (1973). According to their data, plantlets regenerated from explants closer to the base are more vigorous and root more easily. One possible explanation is that, Yang and Clore (1973) used field explants, and these due to their different auxin distribution gave different response during *in vitro* culture.
The growth rate varied greatly among the 18 regenerated plantlets. This could be due to the fact that they were regenerated from explants derived from different seedlings. This emphasises the need to work with clonal lines (plants regenerated from one seedling only) where the generated plants would grow at the same rate and would be ready for harvest at the same period. In this field trial, harvest was quite early (18 months after inoculation of explant) as compared to the 2-4 years period required for seed generated plants. Growing clonal lines in the field would be highly beneficial to the planters as it will allow saving on time and cost of harvest.

Field observation shows that some plants (such as number 8) grow much faster than others and also produce spears of larger diameter (Table 7). These data can be used to isolate plants with appropriate phenotypic characteristics (high yield, large spear diameter, tightness of spear head), that can be used as explants to establish stable clonal lines to be propagated on a large scale. From the collection of 18 plants growing in the field, plant number 8 seems an appropriate candidate for cloning and mass propagation as its spear diameter is quite stable (Figure 1) and it gives highest yield. This leads to the need to establish another protocol whereby field spears can be used as explants for micropropagation.

The production pattern and field conditions for growing asparagus has not been studied in the Mauritian context. Most work in this area has been conducted in regions other than tropical countries, and applying their know-how on asparagus cultivation in Mauritius would most probably not give the best result.

The molecular work undertaken has shown that RAPD analysis is sensitive enough to detect variation between 2 seed generated plants belonging to the same cultivar. So far no polymorphism has been detected among the regenerated plantlets tested with 6 primers that scored marker bands with the parent plants. The banding patterns observed were totally conserved between the mother plants and the tissue culture shoots. Nevertheless, it cannot be concluded that no somaclonal variation has arisen, as mutations could have occurred in a section of the genome where none of the primers tested anneal. In order to reach a valid conclusion as to whether somaclonal variation has occurred, more regenerated plantlets and primers need to be screened.

RAPD markers have so far been used to screen for somaclonal variation among embryogenic calli (Hollingsworth et al., 1999). The technique was sensitive enough to detect changes in DNA structure induced during culture of embryogenic calli. This clearly emphasises the relevance of RAPD technique for detection of somaclonal variation.

An appropriate step would be to test for the presence of somaclonal variation only among tissue cultured plantlets that survive at field condition. There are great chances that mutations occurring in vitro make the plantlets weaker or inefficient, hence these get eliminated by the process of natural selection. The mutations occurring in vitro could also be beneficial, in which case plantlets transferred to field would show improved characteristic such as tighter head, thicker spear, better tolerance to drought. The improved phenotypic characteristics could then be linked to molecular markers that could ultimately be used in marker-assisted selection (MAS). This method would allow tissue culture plantlets to be screened for the desired characteristics before hardening, hence saving on space, time and labour.

Molecular work could also be extended to find sex-linked markers, which allow detection of sex of a plant before it actually reaches the developmental stage for setting flowers. Some work has been carried out along these lines (Ozaki et al., 1999, Caporali et al., 1996), but no RAPD markers tightly linked to the sex determining genes have been detected. Since male plants are earlier and give more and generally larger spears than female plants (Gonzalez Castañón, 1990), the sex-linked marker identified could allow early detection of male plants which could then be mass produced through tissue culture.
CONCLUSION

An optimised protocol has been established for regeneration of asparagus plantlets. Induction of callus and shoot development are optimally produced when 2nd or 3rd nodes from the base are cultured on medium supplemented with 0.015 mgL⁻¹ NAA and 0.25 mgL⁻¹ BAP. Regenerated shoots can then be rooted on MS medium supplemented with 6 % sucrose and 1 mgL⁻¹ NAA.

Research should be focused on the production pattern and field growing condition pertaining to asparagus, as these would be beneficial to growers in Mauritius. Once markers for specific characteristics and lethal somaclonal variations have been identified, these could be used to develop healthy clonal lines for mass propagation.

ACKNOWLEDGEMENTS

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MAPPING THE ACTION PROCESSES INVOLVED IN THE MANAGEMENT OF INFORMATION BY FARMERS – A CASE OF THE SMALL-SCALE CATTLE KEEPERS IN A VILLAGE IN MAURITIUS

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ABSTRACT

The information action processes involved in the management of information are not simply obtaining, interpretation, use, communication, evaluation, storage of information as is commonly depicted in communication models. There are many action processes involved from the time an information is needed to the time that it is made use of and stored for future retrieval. The present study exposes these numerous information action processes in detail, which shows that management of information at the small farmers’ level is a more complex activity than described so far.

Keywords: Information action processes, Management of information, Small-scale cattle keepers, Working knowledge, OPI approach, Grounded theory, Information structures.

INTRODUCTION

Farmers manage information which they receive to produce their working knowledge, and this knowledge in turn helps them to use information to make decisions in achieving their goals. Working knowledge is information which have been acquired from elders / peers / outsiders and put into practice over a long period of time. Studies on information management by farmers can be theoretically represented as follows: Data is searched for, collected or acquired from the environment, organised in memory / print as a data base, processed to produce information, interpreted or understood, and evaluated or assessed (Eels, 1982, Rogers, 1983; Jones et al., 1987) followed by decision making on what to choose or select, implement, reject, transfer, leading to the formation of working knowledge. The stages in the management of information depict awareness, understanding, adopting, accepting or rejecting, and reversal or modifying of information (Rogers, 1983). A summary of the conceptual management of information at the farmer level (Figure 1) illustrates the model at the beginning of the study. The above studies however limit themselves to the use of new information, and do not show how farmers draw from the sources of information and do not give enough illustrations of what we call working knowledge. The present study is an attempt to extract the maximum number of action processes through close interaction with a category of farmers (small-scale cattle keepers) in order to shed more light on the communication processes and the management of information. A previous paper had looked at specific examples of working knowledge, which guide the activities of small-scale cattle keepers through the use of information structures (Naidoo, 2004). These were described by information content to show how cattle keepers used them as cognitive maps in their management of information. The present paper looks at a broader aspect of working knowledge by reporting on the action processes as well as the interaction between the information from sources and information embedded as working knowledge. The Information action processes go one step beyond the information structures to show the detailed processes undergone at farmer level to manage information.

METHODOLOGY

This study was conducted with small-scale cattle keepers in the village of Canot / Gros Cailloux involving a population of 59 cattle keepers. An in-depth study was carried out using the ‘OPI’ approach with the cattle keepers (Naidoo, 2003), involving discrete observation, participant observation, open ended interviews and confirmation of findings with respondents, and analysed using the method of concepts, categories and core categories.

Mapping the action processes involved in the management of information by farmers – a case of the small-scale cattle keepers in a village in Mauritius. G Naidoo

**Figure 1** A representation of the conceptual management of information at the farmer level at the start of the study

Open responses were also sought from respondents on what information they need to carry out their cattle keeping activities, their sources of information and what they do with the information received, how they get information on animal husbandry practices and on loan schemes, the documents they keep for their cattle keeping activities, their livestock keeping practices and knowledge of livestock keeping, as well as their interaction with other cattle keepers. The issues were meant to come from the respondents themselves instead of as variables imposed on them by initial theoretical propositions. The methodology was therefore geared towards bringing to light the processes of management of information by farmers, and showing its relation with their working knowledge.

**RESULTS AND DISCUSSION**

The findings from the study show that management of information is characterised by a series of processes (Figure 2). Some of these processes are categorised under the working knowledge as known information, whereas some processes are related to new information from sources. Other action processes are the products of the interaction between new information from sources and the working knowledge. Personal characteristics of the cattle keepers also influence management of information. The grounded theory (Glaser and Strauss, 1967) that emerge from the study lists the information management processes in a more extensive and detailed manner than what has been reported so far.

The information processes are placed in three columns in the grounded theory model depicting the different processes. It is not possible to show hierarchies in this model as some action processes are carried out independently, and not in a sequential order. This is an attempt to list the processes involving the working knowledge on the left hand side from the management of information from sources on the right hand side. The middle column depicts the stronger interaction processes between the working knowledge and the sources of information.

The processes associated with the working knowledge on the left hand side of the grounded theory model depict more aspects of farmer behaviour and showing the types of experience which are most influential in affecting decisions within the broad definition of working knowledge. These information processes need to be included in this management of information model for the small-scale cattle keepers, and cannot be dissociated from the purely action type information processes represented by verbs in the derived model of information management.

Mapping the action processes involved in the management of information by farmers – a case of the small-scale cattle keepers in a village in Mauritius. G Naidoo

**Figure 2** The action processes in the management of information

**MANAGEMENT OF INFORMATION ACTION PROCESSES**

**Personal Characteristics**

<table>
<thead>
<tr>
<th>Working knowledge</th>
<th>Interaction</th>
<th>Information from Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing</td>
<td>Assessing</td>
<td>Understanding</td>
</tr>
<tr>
<td>Optimising</td>
<td>Accepting</td>
<td>Seeking</td>
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<tr>
<td>Protecting</td>
<td>Resisting</td>
<td>Accessing</td>
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<tr>
<td>Sustaining</td>
<td>Resenting</td>
<td>Acquiring</td>
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<tr>
<td>Observing</td>
<td>Rejecting</td>
<td>Trusting</td>
</tr>
<tr>
<td>Experiencing</td>
<td>Modifying</td>
<td>Using</td>
</tr>
<tr>
<td>Supplementing</td>
<td>Perceiving</td>
<td>Organising</td>
</tr>
<tr>
<td>Income generating</td>
<td>Shifting</td>
<td>Recording</td>
</tr>
<tr>
<td>Economising</td>
<td>Producing</td>
<td>Transferring</td>
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<tr>
<td>Learning</td>
<td>Comparing</td>
<td>Updating</td>
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<tr>
<td>Task differentiating</td>
<td>Selecting</td>
<td>Storing</td>
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<tr>
<td>Equalising</td>
<td>Needing</td>
<td>Retrieving</td>
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<tr>
<td>Sharing</td>
<td>Associating</td>
<td>Disclosing</td>
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<tr>
<td>Pressuring</td>
<td>Copying</td>
<td>Losing</td>
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<tr>
<td>Budgeting</td>
<td>Discarding</td>
<td>Implementing</td>
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<tr>
<td>Marketing</td>
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<td>Implementing</td>
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<td>Transporting</td>
<td>Copying</td>
<td>Implementing</td>
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<tr>
<td>Problem solving</td>
<td>Copying</td>
<td>Implementing</td>
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<tr>
<td>Prioritising</td>
<td>Copying</td>
<td>Implementing</td>
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<td>Confronting</td>
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<td>Recalling</td>
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<td>Maximising</td>
<td>Copying</td>
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<tr>
<td>Using</td>
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<td>Implementing</td>
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<td>Conforming</td>
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<tr>
<td>Complying</td>
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</tr>
<tr>
<td>Feeling</td>
<td>Copying</td>
<td>Implementing</td>
</tr>
</tbody>
</table>

From the sources of information (Figure 2), there is information seeking, accessing, acquiring and understanding. Cattle keepers are generally pro-active in seeking information. The information sought is mostly of a technical and economic nature, covering inputs, markets and land, and including information on obtaining help from various institutions. The priority in the information sought is about using to the maximum the available resources. They seek help from children to understand information. If the information content is trusted such as for opportunity sales of milk or cattle, then the information is organised according to their needs for such information in terms of appropriate timing of production, and according to their sources. There is updating and implementing information content. The cattle keepers do not have complete control over the recording of their production as organised sale is done by external buyers. There is copying, disclosing, discarding and even losing information. There is also apprehending diseases. Managing the information obtained from new sources involves also transferring, storing, and retrieving information.
Discussions with the cattle keepers confirm that they manage working knowledge mainly by recall and use. They use information content about new norms of marketing of milk imposed by marketing agencies by shifting from cows to bull rearing to help generate income. Aspects of farmer behaviour are guided by observing and experiencing cattle husbandry practices for many years, such as economising by using freely available resources in their environment, learning from the elders in the family, having a positive or negative feeling about use of an information, and exhibiting task differentiation among different members of the family. Working knowledge which conforms to their needs is used much more than new information unless they are pressurised by market forces to innovate, when they will comply or not with the exigencies of new information such as levels of production from their cows. Working knowledge of farmers is guided by seeking to optimise and sustain their cattle production by not going for a high level of production as recommended by extension agents but with the objective of keeping their cows as many years as possible on their premises on a lower plane of production and reproduction. Working knowledge is used as acquired information for supplementing feeds with kitchen wastes and left overs to maximise locally available resources. It is extensively used as a characteristic of farmer behaviour to protect their farm animals through practices associated with their beliefs, and also to guard against their fear and suspicion of occult harm being caused to their animals by others in the village. To this end, they aim at equalising resources. It is extensively used as a characteristic of farmer behaviour to protect their farm animals premises on a lower plane of production and reproduction. Working knowledge is used as acquired information for supplementing feeds with kitchen wastes and left overs to maximise locally available resources. It is extensively used as a characteristic of farmer behaviour to protect their farm animals through practices associated with their beliefs, and also to guard against their fear and suspicion of occult harm being caused to their animals by others in the village. To this end, they aim at equalising resources.

The interactions between new information from information sources and working knowledge produce perceptions (i.e. the pictures they make of these in their mind) which lead to accepting, resenting, or rejecting information content among others. It also leads to assessing and modifying the information. There is comparing of information before selecting it for action. The perceiving of information by the cattle keepers is different as compared to that of the extension officers. For the former, only purposeful messages yielding benefits to their whole farming system are needed, instead of very technical and costly innovations. There is resisting to the factors leading to exclusion from fodder information and of the apparent imposition of artificial insemination for cattle by the authorities. However artificial insemination is not rejected being given the absence of breeding bulls on or near their premises. Information is also managed by associating one item with another to produce new information such as association of bank loans with debts.

Although the sources of information can include the farmer network, technology input, and economic opportunities, there are personal characteristics influencing information management other than farmer behaviour within their working knowledge. Such personal characteristics of the cattle keepers are their age group, dimension of land ownership, employment status, formal educational level, languages of communication with external agents, housing status of the cattle keepers and of their cattle sheds, extent of division of labour among household members, nature of disposal of cows’ milk, and rearing of other livestock on the farm premises. Their personal characteristics are guided to a large extent by their search for economic opportunities, although their working knowledge would restrain them to activities which are sustainable.

CONCLUSIONS AND RECOMMENDATIONS

The findings of the study show that cattle keepers are not the passive recipients of information, but manage new information in conjunction with their cattle keeping practices englobed in their working knowledge. The personal characteristics of the cattle keepers have also an important influence on their management of agricultural information. The study shows that working knowledge is not only a product of the information managed, but also reacts with information from sources in a complex process of information management. The cattle keepers adhere to their working knowledge because it is what they know and feel comfortable in doing. The verbs in the grounded theory (Figure 2) show what the cattle keepers do with information from sources, then how they draw from the information...
pool which has become part of the working knowledge to influence farm behaviour, and then there is an interaction process which triggers the decision making processes leading to the creation of new information for the working knowledge cognitive pool. Management of information has tended to concentrate on information from sources, but known information when tested becomes working knowledge, hence both are considered when discussing about management of information. This paper underlines the importance of the interaction between the newly available information from sources and the working knowledge, and puts forward the idea that it is the interaction which becomes new information to be embedded in the working knowledge. Therefore when doing extension work, it must be borne in mind that information from outside sources will go through a sieving process at the cognitive level of the farmer and what will come out will be interpreted and used as new information. No information is likely to be accepted per se by the small-scale farmers. This has implications for extension agents working closely with small farmers, as they may be put off by the reluctance of farmers to accept information which they bring. The latter may use the derived management of information model (Figure 2) as a checklist to understand the action processes which farmers undergo in their dealings with information which will help them to deliver advisory information to the farmers on a case by case basis in a more practical, acceptable and implementable way.

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INFORMATION TECHNOLOGY AS A TOOL TO IMPROVE THE UTILISATION AND MANAGEMENT OF SUGAR CANE GERMPLASM AT THE MSIRI

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Mauritius Sugar Industry Research Institute

ABSTRACT

The Mauritius Sugar Industry Research Institute (MSIRI) operates a breeding programme for the genetic improvement of sugar cane and maintains a broad collection of parent clones, which are used for hybridization. Each year, large populations of seedlings are raised and are subsequently screened through several stages of selection, spanning over 12 to 15 years, before a variety is released for cultivation. This programme generates an impressive amount of data, which needs to be processed for crossing and selection purposes and stored for future use. At the MSIRI, computerisation of breeding records started as early as the mid-1960s and data has been preserved in the form of electronic files and in databases. With the installation of the Local Area Network in the late 1990s, a computerised information management system, the PBMIS, has been developed to restructure and centralise all available data on a main server and provide access to information to several users simultaneously. The PBMIS comprises a relational database, the PBDB, and a menu-driven application programme which provides the necessary facilities for addressing queries or for recording new results. The PBDB database is organised in the form of tables grouped into 3 main components namely germplasm, hybridization and selection. The PBMIS has enabled information to be processed more efficiently and made readily available to breeders for rapid decision-making in the use and management of germplasm. This paper reviews the progress achieved in the use of information technology in the MSIRI sugar cane breeding programme and the development of the PBMIS with emphasis on the germplasm and hybridization components.

Keywords: hybridization, relational database, information system, Mauritius.

INTRODUCTION

The Mauritius Sugar Industry Research Institute (MSIRI) conducts its own sugar cane (Saccharum officinarum) breeding programme to produce commercial varieties, which are adapted to various agroclimatic conditions of Mauritius. The programme involves the production of a new population of genotypes each year through the hybridization of parent varieties, and its subsequent screening through several clonal stages of selection, spanning from 12 to 15 years, before a variety can be released for commercial cultivation. About 2 000 to 2 500 crosses are made during the flowering season which extends from May to July and 100 000 seedlings, each representing a new variety, are raised each year. The remaining seeds are stored in a seed bank at -20°C. About 2 000 genotypes are maintained in a germplasm collection from which potential parents are chosen for hybridization. These clones are made up of wild and noble species, early generations of interspecific and intergeneric hybrids, and commercial-type hybrids produced locally and imported from other breeding stations worldwide. A large volume of data is processed for the management and utilisation of the germplasm. The use of Information Technology (IT) is therefore fundamental to effectively handle the amount of data which is generated through this activity. Each breeding station has its own way to process its breeding data. The use of IT for monitoring crossing and selection data for a number of breeding stations has been reviewed (Meyer et al., 1974, Hogarth and Skinner, 1987, Wu, 1987, Nuss et al., 1989, Sun-Yuan Hsu et al., 1991).

This paper reviews the progress accomplished in the application of IT in the varietal development programme at the MSIRI during the past decades with an emphasis on the management and utilisation of sugar cane germplasm for hybridization.
HISTORY OF IT DEVELOPMENT

Automation of data recording and processing at the MSIRI was initiated as early as the mid-1960s when an automatic interpreter-puncher, a sorter and a tabulator were used to record data on punch cards (MSIRI, 1967). In the late 1980’s, a multi-user computer, IBM 6150, was installed with terminals throughout the institute. The operating system was UNIX and computer languages such as FORTRAN 77 and C+ were used for analysis of data. This was further supported by the NAG FORTRAN library (a comprehensive collection of routines for the solution of numerical and statistical problems) which contains numeric statistical algorithms necessary for performing analyses, and some of these algorithms are still in use today. For the first time, all data available from hybridization and selection activities were organised in relational databases namely, ‘checklist’, ‘availability’, ‘potential parents’, ‘archive’ and ‘final phase’ databases (MSIRI 1990, 1991). The ‘checklist’ database included information such as disease resistance and parentage of clones planted in final phase trials and multiplication nurseries. The ‘availability’ database stored information on the amount and location of breeding material in field. Data related to the agronomic, morphological and breeding characteristics of parent varieties were stored in the ‘potential parents’ database. All data in connection with the analysis of variety trials and the decision on varieties were stored in the ‘final phase’ database. Varieties which were discarded in selection or uprooted from the germplasm collection were directed to the ‘archive’ database. Improved system analysis and programming techniques were employed for retrieving data and managing databases with the use of a combination of C language and SQL (Structured Query Language). Consequently, two computer systems, one for crossing and the other for the final phase of selection, were designed. The relationship between the various databases and computer systems in the 1990’s is shown in Figure 1.

Figure 1 Inter-relationships between computer systems and databases for the cane breeding programme in the 1990’s. (MSIRI 1990)
In 1996, following the advent of Personal Computers (PC) and Microsoft Windows, the hardware environment changed completely with the installation of PCs linked to a Local Area Network (LAN) throughout the MSIRI. The IBM 6150 was phased out and a main server was installed with Microsoft Windows NT as operating system. The introduction of PCs and the LAN brought a whole range of new software. 'SQLBase’ was introduced as a Database Management Software (DBMS) and ‘SQLWindows’ was used as a development and query tool of relational databases. Consequently, all databases under the UNIX system were reviewed and converted to a new structure under the Windows environment. The four main databases were redesigned and regrouped under one single database, the ‘Plant Breeding Database’ (PBDB). This was necessary because linking of several databases for retrieval of information proved to be tedious and time-consuming. The new database structures together with the development tool provided the facilities to build user-friendly applications for simple data processing procedures. This enabled breeders to gain some autonomy for on-line data capture, thus replacing to a certain extent the use of recording sheets.

**THE PLANT BREEDING MANAGEMENT INFORMATION SYSTEM (PBMIS)**

Despite the design of the PBDB database and its accompanying query tool, the information system had some limitations. The database resided on a PC and the application was single-user and single-access. An integrated management information system, the PBMIS, was designed to centralise the PBDB database on a main server (MSIRI, 2002). This allowed work in a multi-user environment by connecting users to the database simultaneously. A computer software application with a friendly graphical interface was developed and installed on PCs connected to the Local Area Network. Cane breeders could thus use built-in queries and reports to retrieve data from the database. The main objective in developing the PBMIS was to improve the maintenance and utilisation of sugar cane germplasm in an efficient and accurate way by providing the following features:

- Developing a system that is user-friendly with a menu-driven framework
- Creating a multi-user environment where several end-users have access to information simultaneously
- Enabling online flexible querying of the database and speedy retrieval of data without the need for writing programming codes
- Generating reports faster
- Providing an interface for rapid and easy maintenance so that database always has up-to-date information
- Providing an efficient security management for maintaining the integrity of the database
- Enabling the storage of several years of past data in one single database

**Computer software and hardware**

The central component of the system is the relational PBDB database. A menu driven application program acts as a graphical interface between the database and the end-user. The application was developed by using SQLWindows 5.0, which is an object-oriented client / server application development system for Microsoft Windows environment. The system is linked to a multi-user relational database server (SQLBase) where the database can be accessed. The operating system is Microsoft Windows and the PC must have at least a Pentium I processor and a basic configuration of 32 MB of RAM (Random Access memory) and 32-bit virtual memory.

**Organisation of the database**

The PBDB database is organised in the form of tables grouped into 3 main components namely, Germplasm, Hybridization, and Selection.

The ‘germplasm’ component contains general information about past and present clones such as origin, parentage, morphological and agronomic traits, disease resistance, year and locations planted. The ‘hybridization’ component stores data on crosses made and the outcome of these crosses, namely, quantity of fuzz (seeds with remnants of flowers) and / or clean seeds obtained, amount and date sown and the number of seedlings produced for each genetic combination (grouping of crosses of same parents).
The ‘selection’ component is concerned with data from trials implemented at different stages of selection which include trials layout, type of data recorded and generated from statistical analyses and selection practices. The organisation of information within the three main components through the flow of activities of hybridization and selection is illustrated in Figure 2.

Figure 2 Flowchart of hybridisation and selection activities with respect to the three components of the PBDB

'Germplasm' component

A total of nine tables make up the ‘germplasm’ component as follows:

- SCVARIETY contains information on varieties which have been evaluated in the final stage of selection (variety trials) as well as those which have been imported from other breeding stations worldwide. All clones are given a variety name as well as a clone ID.
The variety name is the main reference for the variety and the clone ID is an entity used to interrelate the different tables in the PBDB database. The male and female parents and the species group to which the variety belongs (wild, noble or hybrid species) are kept in this table. A total of 4 500 varieties have been recorded to date and, out of these, around 2 000 are available as active parents in the breeding plots while the other entries have been discarded due to bad performance in variety trials or susceptibility to diseases.

- SCDISEASE contains data on the reactions of all the 4 500 clones to major diseases such as gumming, smut, leaf scald, yellow spot and rust.
- SCMORPH and SCAGRO store data on morphological and agronomic characteristics respectively. These traits are gathered from visual assessment of varieties in final phase trials and mainly describe growth habit, stalk number, stalk diameter, stalk height, ease of trashing, hairiness, germination potential, visual appreciation and regrowth.
- SCFLOWER regroups data on sex, date of flowering, flowering intensity and flowering span which are collected from flower surveys of clones present in the germplasm collection.
- SCLIFEHIST deals with information related to the life history of all varieties evaluated in the final phase of selection. It includes stage planted, year planted or uprooted in trials and year released for commercial cultivation.
- EXCIMPORT contains all varieties imported from other breeding stations, country of origin and year imported.
- EXCEXPORT contains an update of Mauritian varieties exported to other countries and year of export.
- MTBREED registers the exact location in breeding plots of varieties which can be currently used as parents for crossing.

‘Hybridization’ component

The ‘hybridization’ component is organized in seven tables as follows:

- HYCROSS records the crosses made each year and parents involved, cross code given according to the breeding objective, date cross performed and date fuzz collected.
- HYPOLFERT records the pollen fertility level of male varieties and the sampling site.
- HYCOMBI regroups crosses with same parents and same breeding objective in a specific year. Each group of crosses is given a combination number
- HYSOWING is concerned with sowing activities and records weight of fuzz and / or seeds sown, date sown and number of seedlings produced for each combination number in a specific year.
- HYSTOCK stores data in connection with the fuzz / seed bank namely, amount of fuzz / seeds available for each genetic combination.
- HYPOTTING records the number of seedlings potted per combination and its location in the greenhouse.
- HYSTAGE1 records data related to the planting of seedlings in the fields, such as number planted, site and date planted.

Utilisation and management of sugar cane germplasm

Figure 3 illustrates how data is processed in the PBGIS for an efficient utilisation of the germplasm in hybridization activities as described by Ramdoyal et al., (1999). At each step in the crossing programme, information is retrieved from the relevant tables and printed on field lists which provide guidance to breeders for decision-making. Thus, flower survey lists are printed with relevant information (e.g site and location of parent variety) for recording and updating flowering status of parent varieties. Flower collection lists regroups relevant information on potential varieties available for crossing following each flower survey and enables an up-to-date record of the actual number of flowers which can be collected from each parent variety on any crossing day. Computer programs assist in the formulation of appropriate crosses to meet specific objectives based on predefined search criteria (crossing list). In order to assist in sowing activities, queries on the status of the seed bank and the germination potential of all cross combinations (sowing list) can be easily retrieved.
Figure 3 Dataflow in the hybridization component of PBMIS – Components of the various database tables illustrated are described in the text.

Legend:
- Database table
- Update
Information technology as a tool to improve the utilisation and management of sugar cane germplasm at the MSIRI.

D Mundil et al.

Data collected from all crossing activities (e.g crosses made, registration number and parentage) and sowing activities (e.g germination potential, seedlings produced per genetic combination) are entered in spreadsheets such as Microsoft Excel and then loaded into the database tables via the application programme. A thorough validation of data is done to ensure that accurate information is being recorded for further processing and use. Concurrently, data on the germination potential of new crosses from the current crossing season is obtained and loaded in the databases. Reports on population produced for each project objectives (potting list) can be easily obtained and used for activities related to potting of seedlings and their transplanting in the field.

An efficient utilisation of sugar cane germplasm is tributary to a proper management of breeding collections. To this end, constant updating of information on the characteristics of parent varieties, obtained from selection trials or otherwise, and their breeding potential, obtained from the performance of progenies in selection and/or from cross evaluation trials, is necessary to enable the most appropriate choice of parents for crossing. Varieties no longer useful as parents are uprooted and new clones, both local and foreign, are added to the germplasm collection. Good parent varieties are multiplied on a larger scale and some collections may be replanted in order to regenerate the breeding material. Some clones may also be relocated to other sites where the environment is conducive to pollen shedding and fertility. The PBMIS provides the necessary tool to the breeder for managing the sugar cane germplasm. Any specific clone can be easily located, and the date planted and the plot size can be retrieved rapidly from the database with the help of the menu-driven application. Moreover, a good management of this database allows specific information on varieties to be obtained for any use. Such information may include genealogy, agro-morphological characteristics or disease reactions which are useful for export purposes.

CONCLUSION

The Plant Breeding Management Information System (PBMIS) is the result of a close collaboration between computer system analysts and cane breeders at the MSIRI. It has proved to be a valuable research tool for the use and management of germplasm in the varietal development programme. The regrouping of data into one single database and its centralisation on the main server has made access to information easier. Faster processing of data, including updating of database and generation of reports, has enhanced crossing efficiency by saving time and manpower during the crossing season. The PBMIS has speeded the availability of data to breeders via the user-friendly, menu-driven graphical interface which provides access to up-to-date, online information at any time of the year. Data is thoroughly validated before being loaded into databases.

Thirty years of past data on hybridization activities are currently stored in the PBDB database. This provides the basis for retrospective analysis of crossing data, which is particularly useful for assessing performance of crosses and formulating appropriate crossing policies.

The PBMIS is a modern, versatile system, which can adapt to changes in breeding procedures. In future, online analysis of the performance of crosses through the different stages of selection will be carried out when the selection component of the PBMIS is completed.

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Information technology as a tool to improve the utilisation and management of sugar cane germplasm at the MSIRI.

D Mundil et al.

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A GEOGRAPHICAL MARKETING INFORMATION SYSTEM FOR POTATO

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Agricultural Marketing Board

ABSTRACT

A Geographical Marketing Information System (GMIS) for potato is set up to increase the availability of marketing information and spatial arbitrage along the potato marketing chain. The GMIS builds on a potato suitability map to estimate production of potato for the first planting season. It also uses production and marketing choices to generate profitability estimates for different categories of potato planters.

Key words: GMIS, land suitability, cost of production, marketing costs, profitability

INTRODUCTION

The Non-Sugar-Sector-Strategic Plan emphasises the need to review and improve the local horticultural marketing system (MoAFTNR, 2003). One of the numerous weaknesses of the present system is a lack of transparency along the marketing chain. The proposed geographical marketing information system (GMIS) has been set up in this respect. It aims to provide different types of information, to cater to the needs of different stakeholders along the potato marketing chain so that they can take judicious production and marketing decisions. The GMIS also tries to ensure that the information needs of the weaker market participants are satisfied, in order to reduce the existing differences in the level of information.

It is proposed that the GMIS would first of all be set up for potato. Even though the price of potato has been liberalised, the AMB is still involved in potato production and marketing. It provides certified potato seed to producers and also buys potato seeds and wares at guaranteed producer prices. Owing to the nature of its activities, the AMB databases information on potato producers. These include the seed varieties, the locality, the acreage and the approximate date of plantation. Another reason behind choosing potato, is that locally it is cropped in two well defined seasons\(^1\) and it is therefore easier to forecast the timing of harvests as opposed to other less seasonal crops.

This system can be used by producers to have an indication of their profitability, based on their production, management and marketing choices. It can also be accessed by registered dealers who are going to be made aware of the locations of plantations, contact details of planters and potential harvest dates and quantities. The GMIS will also generate potato plantation forecasts that can be used by the AMB to plan imports.

The concept adopted in the generation of the GMIS is quite innovative in that it uses the attributes of a Geographical Information System (GIS) to database, layer and analyse production, economic and marketing data. Then relevant information that best suit the needs of planters, retailers and the AMB will be extracted and disseminated.

This paper explains the conception of the GMIS, and shows how the different sections fit together to generate information at levels required by the stakeholders. Here it needs to be highlighted that the GMIS is still work in progress and needs to be cross-checked. Nevertheless to illustrate its utility, the production characteristics of two hypothetical potato producers are input into the system to generate estimates of their potential profitability.

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\(^1\) The GMIS is being set up for the first potato season and will then be extended to the second one

AIMS

The main aim is to devise a production and marketing information system that is going to provide information to the stakeholders involved along the potato filière. This main objective comprises several sub-objectives, which are to:

1. Devise a land suitability map for potato
2. Map potato plantations
3. Provide production forecasts based on (1) and (2)
4. Estimate an imputed cost of production (COP) based on the guaranteed producer price, (1) and (2)
5. Provide information on locational marketing alternatives to potato producers and dealers
6. Estimate marketing costs (MC) based on marketing channels
7. Compute profitability estimates based on (4) and (6)

METHODOLOGICAL MATRIX

Each of the aims listed before has a specific methodological approach appended to it. These are given below and the subsequent findings are presented in the following section.

1 Determine the land suitability for potato

The procedure to determine the land suitability for potato is adapted from Jholy et al., (2001), who derived the suitability of potato and tomato for sugarcane lands. The methodology is based on the land resources and agricultural suitability map of Mauritius (Arlidge and Wong You Cheong, 1973). This map gives the various land units covering the island and their intrinsic characteristics. Six broad classes are identified based on these physical characteristics. They include agroclimatic conditions, availability of water, erosion susceptibility, availability of plant nutrients and land cultivability. These are presented in Table 1.

Land units with deep, fertile, free-draining and slightly acidic soils are more appropriate for potato production. Such characteristics are usually typified by the following soil types: Humic Latosols, Low Humic Latosols and Latosolic Brown Forests and to a lesser degree Humic Ferrigenous Latosols and Latosolic Reddish Prairie. Thus the land units with the above-mentioned characteristics are earmarked as suitable for potato production.

Using a GIS software, these land units are layered with a soil type map and a spatial rainfall map to generate different suitability classes for potato. One difference with the method adopted by Jholy et al. (2001) is that instead of using isohyets as rainfall delimiters, regional rainfall averages are used. The Meteorological Services (MS) divide the country into 9 regions and rainfall data are collected for each region. These are aggregated to constitute monthly averages. One attraction of using such data over mean annual isohyetal bands, is that they are on a monthly basis and can therefore be compiled to better reflect the amount of rainfall during the first potato season (mid-April to June). The monthly averages that are used are long-term ones spanning 1971-2001.

The approach adopted by Jholy et al., (2001) excluded the impact of pest and disease in devising suitability classes. They argued that the incidence of the major pests and diseases for potato occur in certain agroclimatic zones and that the production of potato in those areas will necessitate reactive management practices based on the particular pest and disease. Those preventive and or mitigative practices incur additional costs, which impact the COP. This is subsequently reflected in the profitability of potato production.

Another attraction of the GMIS is that it also takes into consideration the area under irrigation. As per Table 1, the overriding limitation of land classified as CS1 and CS2, is the inadequate availability of water. Therefore under irrigation such lands can be respectively reclassified as S1 and S2. This effectively increases the land that is suitable for potato production.
Table 1 Criteria used in defining suitability classes

<table>
<thead>
<tr>
<th>Suitability class</th>
<th>Availability of water</th>
<th>Limiting superhumid climate</th>
<th>Availability of plant nutrients</th>
<th>Erosion susceptibility</th>
<th>Land cultivability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly suitable (S1)</td>
<td>High</td>
<td>None</td>
<td>High</td>
<td>None to slight</td>
<td>Easy to fairly easy</td>
</tr>
<tr>
<td>Moderately suitable (S2)</td>
<td>Moderate</td>
<td>None to moderate</td>
<td>High to moderate</td>
<td>None to slight</td>
<td>Fairly easy to moderately difficult</td>
</tr>
<tr>
<td>Marginally suitable (S3)</td>
<td>Low</td>
<td>None to high</td>
<td>Moderate to low</td>
<td>None to moderate</td>
<td>Difficult</td>
</tr>
<tr>
<td>Conditionally highly suitable (CS1)</td>
<td>Low</td>
<td>None</td>
<td>High</td>
<td>None to slight</td>
<td>Easy to fairly easy</td>
</tr>
<tr>
<td>Conditionally moderately suitable (CS2)</td>
<td>Low</td>
<td>None</td>
<td>High to moderate</td>
<td>None to slight</td>
<td>Fairly easy to moderately difficult</td>
</tr>
<tr>
<td>Not suitable (N)</td>
<td>Low</td>
<td>None to high</td>
<td>Low to very low</td>
<td>None to strong</td>
<td>Very difficult</td>
</tr>
</tbody>
</table>

Source: Jhoty et al. (2001)

The colours used to represent the different suitability classes in the suitability map are almost the same as those used by Jhoty et al. (2001). The reason being, to limit confusion and facilitate comparison between the two maps.

2 Map potato plantations

The GMIS goes to a higher level of disaggregation by mapping potato plantations. This is made possible as informational data on potato producers are available at the AMB. Here it is proposed to make use of such data, without disclosing any information on planters. One shortcoming though is that the exact site of plantation is not known, as it is on a locality basis. The eastings and northings of such localities are entered into the GIS and layered onto the potato suitability map.

3 Provide production forecasts, based on land suitability for potato and mapped plantations

By layering the potato plantations on the potato suitability map, it is possible to allocate a suitability class and subsequently a potential yield to each of the planters. These yields estimates are based on the opinions of agronomists, extension officers, operation officers and planters. The estimates are preliminary in nature and are used to provide abaseline for production forecast. Both the yield estimates and the production forecast will be amended as a result of the verification process, which will give a degree of on-farm reality to the GMIS. The spunta variety is the most common one planted in the first season. The potential yields are presented in Table 2.

---

2 Some plantation sites can change between the registration of planters and the time they take delivery of their potato seeds. Any relocations and updates will be input into the GMIS.
3 Ideally a GPS could be used to exactly pin the plantation site of each planter.
4 A locality is not a point. Here, the central lettering of the locality name appearing on the map is used as a point representing a particular locality. The Y 682 (DOS 529) series 1:100 000 map is georeferenced and used to ascribe coordinates to localities.
5 A small number of planters also crop the variety mondial, but this proportion is around 8% of the total seed allocation. Here it is assumed that mondial achieves the same yields as in Table 1.

Table 2 Potential yields under the different suitability classes

<table>
<thead>
<tr>
<th></th>
<th>Yield under S1</th>
<th>Yield under S2</th>
<th>Yield under S3</th>
<th>Yield under CS1</th>
<th>Yield under CS2</th>
<th>Yield under NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big planters</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Small planters</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Based on the GMIS, the local potato production for the first season 2005 can be predicted. It should also forecast the timeliness of potato harvests, based on approximate planting dates. This information combined with prospective yields can be disseminated to registered dealers who can contact producers at plantation sites. This can effectively reduce transaction costs and also increase the involvement of both these stakeholders in spatial arbitrage.

4 Estimate an imputed cost of production based on the guaranteed potato producer price

Another objective of the GMIS is to estimate the COP to subsequently derive an estimate of profitability of potato production. Each year the National Potato Committee computes the COP for potato in order to generate the guaranteed producer price for the coming year. Such computations though are not for public scrutiny.

Therefore, an alternative approach is adopted here to obtain a normative estimate of the COP based on the guaranteed producer price. The latter is computed by adding a mark-up of 30% on the COP of potato per tonne. Therefore if this mark-up is deducted from the guaranteed price, this will provide a good indication of the COP per tonne. The cost of crop delivery of potatoes to the AMB is also included in the cost of production. Technically speaking this should be under MC and not production costs. The transport costs are also deducted from the imputed COP, so that it solely reflects the production costs. Another attraction of estimating the COP devoid of transport costs to the AMB, is that MC attributed to other marketing channels can also be computed and added to the COP.

A benchmark yield of 1.8 is used in the computation of the COP. Multiplying the COP per tonne by 8 thus gives an imputed COP per acre. This is going to be used as baseline in subsequent profitability computation scenarios.

The producer price for the coming year has not yet been finalised. It is proposed here to use the same % increase in producer price from 2003 to 2004, and compute a probable producer price for 2005. The producer price increased by around 5% from 2003 to 2004. Assuming it increases in the same proportion from 2004 to 2005, an estimate for the producer price can be derived.

5 Map the potato marketing chain and provide information on marketing alternatives to farmers

A commodity chain approach is adopted to map the potato marketing chain and its various links. All existing links are identified and illustrated in a potato marketing diagram. This can show stakeholders the various alternatives and potentialities that exist within the chain.

The AMB also carries out the registration of potato dealers. Their contact details and retail outlets are also recorded. As mentioned before the production details of potato producers are databased. To reduce the informational costs involved in obtaining information for both parties, the GMIS aims at being the interface that provides production and harvest information to dealers and contact details of dealers to planters. This can effectively reduce the information costs of these two market participants and the exchange of information can also increase bargaining and arbitrage. Therefore any transaction

6 1 acre =0.4046 hectares.

would ideally be based on a better information exchange, which would better reflect the offer of the planters and the utility of the dealers.

Given that practically the above involves the interchange of confidential information, a census is actually being carried out with the two parties to gauge their interest and their willingness to participate in the system.

6 Estimate of marketing costs

As illustrated in Figure 2 there are numerous alternative channels through which potato can be marketed. Here it is proposed to take three of the most common ones, that is potato marketed through the AMB, through the auction system and through dealers. The MC are identified and averages are obtained from 15 producers that market their potato along the three channels mentioned above. The transport costs per tonne vary depending on the location of the production sites, the wholesale and retail markets, but here for sake of simplicity they are assumed constant.

Here it is assumed that producers do not market their potato only along a particular channel, but rather use a mixture of two or more channels. The reason, being that potato harvest is not homogenous and consequently harvests are made up of tubers of differing sizes and quality, depending on suitability classes and management practices. Over-sized high quality tubers are usually bought by dealers and obtain higher than guaranteed prices. Medium-sized tubers are either bought by dealers or marketed through the AMB. Here it is assumed that half of medium-sized tubers go through the AMB and get the guaranteed price and the remainder are bought by dealers at the same price. But in the later case the planter saves on the transport cost that he would have incurred if he had brought his potato to the AMB. Under-sized potatoes find their way through the auction system, and the respective costs are presented in Table 3.

7 Compute profitability estimates based on (4)

Profitability (variable profits) is computed as aggregate revenue minus COP and MC for the two categories of producers, under different suitability classes (S1, S2, S3) and marketing strategies (AMB, auction, dealers).

8 Verification of the GMIS

It is proposed to cross-check the GMIS against yield figures collected during the forthcoming first season at 5 plantation sites in each suitability class. Therefore 30 producers will be surveyed. Fifteen of them (5*3 marketing channels) will be asked additional questions about their marketing choices to confirm the marketing costs assessed in the previous section.

RESULTS

This section shows how the different methodologies and respective results are coalesced to form the GMIS.

1. Land suitability for potato and mapping of potato plantations

Figure 1 gives the land suitability under rain-fed and irrigation conditions. Given that irrigation costs are taken into consideration when computing COP, the suitability map with irrigation is used as base map for further estimations.

Mapping of registered planters also reveals the suitability of their land for potato production.
2. Production forecasts

Production forecasts for the coming first potato season are estimated based on the amount of potato seeds allocated to particular planters, and the potential yields under their respective suitability classes. Predicted production for the first season is 5171 tonnes.

Figure 1 Land suitability under irrigation

[Map showing land suitability under irrigation with different colors for highly suitable, moderately suitable, marginally suitable, conditionally highly suitable, conditionally moderately suitable, not suitable, slopes, built-up area, main rivers, main roads, and waterbodies.]
3. The imputed cost of production

The estimated guaranteed price for potato for 2005 is Rs 13 320. The imputed COP is Rs 10 246, and the COP without transport costs is Rs 9 746. Thus the COP used in following sections is Rs 9 746 per tonne.

4. Marketing channels for potato

Figure 2 shows the commodity chain for potato, with the different links and cross-links. It demonstrates the marketing opportunities and alternatives available to the market participants. Figure 2 also reveals the potentialities for value addition and the prospective wholesale and retail outlets.

Figure 2 The potato marketing chain

5. Provide information on locational marketing alternatives to farmers

This component of the GMIS is not yet implementable given that the survey to gauge the participation of planters and producers is not yet completed.

6. Marketing costs based on marketing channels

Here there is need to emphasise that profitability estimates are for producers only, therefore only the average MC borne by them are presented in Table 3.

7. Profitability estimates

These profitability estimates are a function of total revenue, COP and MC. Total revenue is based on prices and the proportion of premium, acceptable and non-acceptable crops. As shown in Figure 3, if
potato is planted in the S1 suitability class it is assumed that 10%, 70% and 20% of the crop will respectively be premium, acceptable and non-acceptable. Using this assumption as basis, revenue per tonne is computed.

As mentioned in the introduction, the GMIS is run using the production and marketing characteristics of two hypothetical potato producers (a big and a small). Several factors differentiate small from big planters. These include acreage planted, irrigation facilities, availability of land, land improvements, extension facilities and production expertise. Here, to ease comparison between the small and the big planter, acreage is assumed the same at 2 acres. The total variable profitability for the big planter as shown in Figure 3 is Rs 92 546, and that of the small planter is Rs 43 870. These show that there is a real profitability gap between these two categories of planters.

**Figure 3** Computing profitability estimates using the GMIS

<table>
<thead>
<tr>
<th>acreage (acres)</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>price of premium crops</td>
<td>14 000</td>
</tr>
<tr>
<td>guaranteed price of acceptable crops</td>
<td>13 320</td>
</tr>
<tr>
<td>price of non-acceptable crops</td>
<td>5 000</td>
</tr>
<tr>
<td>theoretical yield</td>
<td>8</td>
</tr>
<tr>
<td>other yield</td>
<td>11</td>
</tr>
<tr>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>suitability class</th>
<th>S1</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of premium crops</td>
<td>10</td>
</tr>
<tr>
<td>% of acceptable crops</td>
<td>70</td>
</tr>
<tr>
<td>% of non-acceptable crops</td>
<td>20</td>
</tr>
<tr>
<td>output-other yield</td>
<td>22</td>
</tr>
<tr>
<td>revenue-other yield (T)</td>
<td>11 724</td>
</tr>
<tr>
<td>total revenue-other yield (T/acre)</td>
<td>128 964</td>
</tr>
<tr>
<td>total revenue-other yield (T/area planted)</td>
<td>257 928</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>guaranteed price - a mark-up of</th>
<th>3 073.85</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

| imputed cost of production (T) | 10 246.15 |
| imputed cost of production (T)-tc | 9 746.15 |
| imputed cost of production (arp) @ 1:8 | 77 969.23 |
| imputed cost of production (area planted) | 155 938.46 |
| imputed cost of production (T) @ other yields | 7 088.11 |

| marketing costs (Rs/T) | 429.25 |

<table>
<thead>
<tr>
<th>AB</th>
<th>Auction</th>
<th>Dealers</th>
</tr>
</thead>
<tbody>
<tr>
<td>640</td>
<td>835</td>
<td>85</td>
</tr>
</tbody>
</table>

| profitability (T) | 4 206.64 |
| profitability (acre) | 46 273.02 |
| total profitability (Rs/area planted) | 92 546.04 |
CONCLUSIONS

The GMIS has been devised to estimate the potential profitability of potato producers, based on the suitability class of their land, their management skills and their marketing choices. The profitability of two hypothetical planters is estimated and the profitability of the big planter is twice that of the small planter. Before pondering too much on these figures it needs to be highlighted that the GMIS is still work in progress and that profitability estimates will be amended and updated in light of assumptions made to suit different types of planters and their marketing choices. Those, wishing to alter their production and marketing could then make use of such estimates as baseline information and take a more informed decision.

ACKNOWLEDGEMENTS

I wish to thank the Director of the MSIRI for granting permission to make use of various physical MSIRI maps; and Mrs S B Joomun and Mr D Ramanah in helping to digitise such maps.

REFERENCES


MOAFTNR see under Ministry of Agriculture, Food and Natural Resources.

Table 3 Marketing costs borne by producers Rs/tonne

<table>
<thead>
<tr>
<th></th>
<th>AMB</th>
<th>Auctioneers</th>
<th>Dealers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorting</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td>25</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Bagging</td>
<td>25</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Cost of leno bags</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Handling</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport to AMB</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport to auction</td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auctioneers’ fees and commission</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total MC</td>
<td>640</td>
<td>835</td>
<td>85</td>
</tr>
</tbody>
</table>
AGRONOMIC PERFORMANCE AND TUBER CHARACTERISTICS AND QUALITY OF NEWLY-RELEASED LOCAL POTATO CLONE BELLE ISLE

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ABSTRACT

The Mauritius Sugar Industry Research Institute has been selecting potato varieties since the late 1960s. As from the mid 1980s few good varieties have been found. This prompted the initiation in 1989 of a potato breeding programme. Emphasis was initially on high yields, adaptation to local production conditions and tolerance to important diseases. The first four clones were released in 1998 but, to date, only two have been grown on a limited scale, mainly because of poor acceptance. Emphasis was therefore shifted to tuber morphological characteristics such as size and shape which are the main determinants of acceptance, to adaptation to the second season when retailers are less choosy, and to tuber quality. The first local clone to possess the additional attributes was released recently under the name Belle Isle. It originates from a cross made in 1990 and has been tested under the code number 90005. It is only as from last year that several growers have shown interest in the variety, thus prompting its release.

Belle Isle has five attributes of interest. Firstly, it is a good yielder. In 65 replicated yield trials predominantly on growers' fields in Mauritius, its total tuber yield has been 17% more than that of Spunta in the first season and 47% more than that of the same control in the second season. In 14 replicated yield trials in Rodrigues, its total tuber yield was 48% higher than that of Spunta. Secondly, its yield is more stable than that of other clones in Mauritius as well as in Rodrigues. Thirdly, it is resistant to late blight whose incidence on variety Spunta has increased dramatically in several localities in 2004. Fourthly, the seeds are smaller and cheaper. And finally, the tubers have a relatively higher dry matter content which, combined with good colour after frying, makes it suitable for crisp production. However, the tubers of Belle Isle are smaller than those of Spunta. Growers are therefore advised to pay attention to its marketing.

Keywords: Yield stability, Late blight, Cooking quality, Potato crisps

INTRODUCTION

There is a need in Mauritius to increase the productivity and reduce the cost of production of potato as well as of most other crops. One of the most cost-effective ways to improve productivity is to grow superior cultivars. For this reason, the Mauritius Sugar Industry Research Institute (MSIRI) has been evaluating potato varieties since the inception of its potato programme in the late 1960s. At first, the work was limited to the evaluation of foreign commercial varieties with emphasis on yield and adaptation to climate, soils, pests, diseases and cropping systems. As from the mid-1980s, acceptable foreign varieties started to become rare and, in 1989, the programme was enlarged to encompass the breeding of local clones.

The first four clones produced by the breeding programme were released in 1998 (MSIRI, 1998a). Two were later withdrawn after planters reported having problems marketing the relatively small tubers. The remaining two clones – Belle Mare and Belle Vue – have not been grown to any extent either. In the first season when they have been tried, the main variety on the market is Spunta whose large and oblong tubers are preferred by growers and retailers alike. The clones’ smaller and round tubers are not appreciated. Their other characteristics, such as higher and more stable yields (Govinden and Wong Yen Cheong, 1997), resistance to late blight (Saumtally et al, 1997) and cheaper seeds have all gone unnoticed. Like the local clones, Scottish variety Stirling, recommended because of its good yield and resistance to late blight (Wong Yen Cheong and Govinden, 1995), has also not been accepted in spite of its large tuber size.

However, the situation is starting to change following the observation of late blight on variety Spunta in 2004 (Saumtally, 2004). Since its release in 1975, Spunta has been observed to be tolerant to late blight. Suddenly, in 2004, heavy incidences of the disease were seen on the variety in many localities, not only in the superhumid zone where the disease is quite common on susceptible varieties, but also in the sub-humid zone where it is seldom observed. The fact that local clone Belle Mare, previously rated resistant (Saumtally et al., 1997), has also now been observed to be susceptible suggests the emergence of a new strain of the disease.

Moreover, the cost of seeds has increased dramatically in the recent past. All planter groups are complaining about it. In Rodrigues most planters cannot afford these seeds.

The incidence of late blight on variety Spunta and the increase in the cost of seed may be expected to erode growers’ preference for the variety. Local clones now stand a better chance of adoption, especially if efforts are made to popularise them. This paper is a small contribution to this end.

**MATERIAL AND METHODS**

The first stages in the breeding of potato, starting with crosses have been described elsewhere (Govinden and Wong Yen Cheong, 1997). Replicated yield trials with variety Belle Isle were planted the fourth year (1994) at a single site - Réduit Experiment Station - and at two sites in the fifth year. Multi-locational yield trials on growers’ fields started in the sixth year (1996). To date, 62 such multi-locational yield trials have been completed in Mauritius, bringing the total number of trials considered in this paper to 65. In Rodrigues 14 trials have been completed, 6 in 2003 and 8 in 2004.

The trials were established within commercial potato plantations on growers’ fields in the different climatic zones where potato is grown. Most trials in the subhumid zone were irrigated, as were also some of those in the humid zone. The trials in the superhumid zone were rainfed. In Rodrigues, where there is no superhumid zone, some trials were watered occasionally.

Eighteen out of the total of 65 trials in Mauritius were in pure stands and the rest (47) in interrows of sugar cane to reflect the relative importance of the two main potato cropping systems. All the trials in Rodrigues were pure stands since sugar cane is not grown there. In pure stands the plots consisted of 2 to 4 rows of 15 to 20 plants at a density of 41 660 plants per hectare. In interrows of plant sugar cane, the potato was planted as single rows in every cane interrow at a density of 20 800 plants per hectare. Plots consisted of 2 rows of 15 to 20 plants. Most trials had three replicates arranged in randomized complete blocks.

The trials comprised several controls including variety Spunta and many clones. These changed throughout the years as some clones were retained and others were rejected.

Normal growers’ cultural practices for sugar cane and potato were followed. Fertilizers in the form of 13:13:20:2 were applied in the furrows at planting at rates of about 1000 to 1200 kg ha$^{-1}$ in pure stands in Mauritius and 600 to 1000 kg ha$^{-1}$ in Rodrigues and about 600 kg ha$^{-1}$ in intercropping. The most recent recommendations were followed in the management of weeds, pests and diseases (e.g MSIRI, 1998b; MSIRI, 2003).

The crops were lifted at full maturity, normally between 90 and 110 days after planting. At harvest the tubers were graded. Tubers weighing more that 50 g are considered marketable.

To calculate average yields, the yields of intercropped potato were doubled in order to arrive at their pure stand equivalents.

The sites in Mauritius do not constitute a balanced set and, consequently, the comparisons of first with second season and intercropping with pure stands are only indicative. Since the evaluation of the yield of the new clone involves a comparison with the control, it is valid under all environmental conditions. Yield stability was estimated by the linear regression method of Eberhart and Russell (1966). The yields of individual varieties were regressed on an environmental index represented by the mean of all varieties. However, the regressions are only useful if a common set of variety is chosen to represent the environment. Thus, only 30 trials could be included in the stability analysis for Mauritius. The dataset for Rodrigues comprised 14 yield trials.
Tuber cooking and processing quality tests were done on freshly-harvested tubers. Specific gravity was measured with a hydrometer on samples of 3.6 kg. Small samples of 5 tubers were peeled and boiled until they were cooked. Colour was noted immediately after boiling. After 24 hours, after-cooking darkening, tuber disintegration and texture were rated. Crisps were prepared using standard methods from samples of 10 tubers. Colour was rated after frying.

RESULTS AND DISCUSSION

Tuber yield

The average yield of control variety Spunta in the set of trials in Mauritius was lower than normal (Table 1), in part because of the choice by some collaborators of relatively poor soils in the first season and additionally, because of late planting in the second season. The yield of Spunta was definitely lower in the second season, which may be attributed to physiological ageing of the seeds. Since the yield of Belle Isle was similar in both season, its superiority over Spunta was more pronounced in the second season. On average across seasons, Belle Isle was superior to Spunta by 29% in total yield and by 11% in marketable yield; the difference is clearly due to the smaller size of Belle Isle tubers. The effect of cropping system was negligible, Belle Isle being superior to Spunta in intercropping as well as in pure stands.

Table 1 Comparative tuber yield of potato varieties Belle Isle and Spunta in multi-locational yield trials

<table>
<thead>
<tr>
<th>No. of trials</th>
<th>Yield (tha⁻¹) pure stand equivalent</th>
<th>Marketable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Actual</td>
<td>% of control</td>
</tr>
<tr>
<td>Belle Isle</td>
<td>Spunta</td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>36</td>
<td>28.3</td>
</tr>
<tr>
<td>Second</td>
<td>29</td>
<td>28.3</td>
</tr>
<tr>
<td>Both seasons</td>
<td>65</td>
<td>28.3</td>
</tr>
<tr>
<td>Cropping System</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercropping</td>
<td>47</td>
<td>28.4</td>
</tr>
<tr>
<td>Pure Stand</td>
<td>18</td>
<td>28.2</td>
</tr>
<tr>
<td>Both systems</td>
<td>65</td>
<td>28.3</td>
</tr>
</tbody>
</table>

In Rodrigues there is a single planting season extending from May to August. On average over the 14 trials, Belle Isle gave a total yield of 18.7 t ha⁻¹ compared to 12.6 t ha⁻¹ for Spunta. The superiority of Belle Isle of 48% over Spunta was therefore more marked than on average in Mauritius and of the same order of magnitude as in the second season.

Yield stability

The range of mean yields in the dataset from Mauritius used in the yield stability regression analysis varied from less than 10 tha⁻¹ to more than 40 tha⁻¹ (Figure 1). Of the five well-known varieties, Spunta had the lowest coefficient of determination ($r^2 = 0.73$) and Belle Isle the largest ($r^2 = 0.86$). This means that as the environment changed, the yield of Belle Isle was more predictable by the linear model. Belle Isle also had the highest regression coefficient (b = 1.07) and Spunta the smallest (b = 0.93). This means that as the environment improved, the yield of Belle Isle increased relative to the mean whereas that of Spunta decreased.
Belle Isle is therefore to be preferred to Spunta since it is better able to capitalize on the conditions, such as better soils, cooler temperatures, more rain, less diseases and pests, and better crop management etc, underlying the improvement of the environment.

The range of yields in the dataset from Rodrigues used in the regression analysis was much smaller than in the one for Mauritius, which casts some doubt on the applicability of the technique. The yield of Belle Isle was much more predictable by the linear regression model ($r^2 = 0.79$) than that of other varieties, especially Spunta whose yield was totally unpredictable ($r^2 = 0.07$) (Table 2). The regression coefficients of the two varieties were also much more divergent than in Mauritius, with Belle Isle having a very positive response ($b = 1.88$) to an improvement in the environment and Spunta a negative response ($b = 0.52$). The causes of this divergence are not known. In Rodrigues, where late blight was not reported in the trials under review, the single factor which has a strong influence on yield of potato is seasonal rainfall. Perhaps the two varieties emerged poorly or did not develop a good canopy early in the season because of drought. Later, when the rains fell, Belle Isle was able to produce a flush of leaves whereas Spunta could not. This hypothesis is grounded in the observation that, under local conditions, all foreign commercial potato varieties are determinate whereas all local clones are semi-determinate to various degrees. In fact, Belle Isle is one of the least semi-determinate local clones.

**Figure 1** Yield stability of selected potato varieties.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Regression equation</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>95006</td>
<td>$Y = 0.91x + 0.94$</td>
<td>0.74</td>
</tr>
<tr>
<td>Belle Isle</td>
<td>$Y = 1.88x - 7.98$</td>
<td>0.79</td>
</tr>
<tr>
<td>Belle Vue</td>
<td>$Y = 0.83x + 1.39$</td>
<td>0.48</td>
</tr>
<tr>
<td>Mondial</td>
<td>$Y = 0.86x + 0.53$</td>
<td>0.45</td>
</tr>
<tr>
<td>Spunta</td>
<td>$Y = 0.52x + 5.13$</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Table 2** Yield stability parameters of some potato varieties in 14 trials in Rodrigues (2003 & 2004)
Tuber morphological and quality characteristics

In a study on potato consumption patterns and consumer preferences, consumers in Mauritius rated tuber size as the most important quality characteristic (Govinden et al. 1997). Then came skin colour, eye depth, taste and shape, in this order. A majority of consumers prefer medium to large tubers. Consequently, the smaller size of Belle Isle tubers is a major drawback of the clone. Even in Rodrigues there is a preference for large tubers, but collaborating growers reported the selling prices of the two varieties to be similar. Whether the preferences have evolved since 1997 or not, it will be necessary to promote the variety more aggressively.

Since the withdrawal from cultivation of Dutch variety Exodus more than a decade ago, there is no variety on the market which is particularly suitable for processing into crisp. The small artisanal crisp industry must make do with what it can get. Belle Isle is an excellent crisping variety (Table 3) and can therefore capture the market for which its relatively smaller tuber size is in fact an advantage. Moreover, the higher specific gravity – an indicator of dry matter content – of Belle Isle (Table 3) leads to a higher industrial yield of crisps and, more relevant still to the health-conscious consumers, to a lower oil content after frying (Talburt and Smith, 1967).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Shape</th>
<th>Eye depth</th>
<th>Specific gravity</th>
<th>Cooking quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sloughing</td>
</tr>
<tr>
<td>Belle Isle</td>
<td>Round</td>
<td>2-3</td>
<td>1.088</td>
<td>1</td>
</tr>
<tr>
<td>Belle Mare</td>
<td>Round</td>
<td>2-3</td>
<td>1.100</td>
<td>1</td>
</tr>
<tr>
<td>Belle Vue</td>
<td>Oval</td>
<td>2</td>
<td>1.092</td>
<td>1</td>
</tr>
<tr>
<td>Mondial</td>
<td>Oval</td>
<td>1-2</td>
<td>1.068</td>
<td>1</td>
</tr>
<tr>
<td>Spunta</td>
<td>Oval</td>
<td>1</td>
<td>1.074</td>
<td>1</td>
</tr>
</tbody>
</table>

Rating scales:

1Eye depth: 1= shallow; 5 = deep 2Sloughing: 1 = firm; 5 = complete disintegration
3Darkening: 1 = nil; 5 = dark 4Texture: 1 = hard; 5 = soft 5Crisp colour: 1= white; 5 = dark

Other pertinent varietal characteristics

The variety preference of potato growers also depends on other characteristics beside yield and tuber quality. For many growers in the superhumid zone who find it difficult to control late blight, the reactions of varieties to the disease are of paramount importance. Saumtally et al. (1997) have reported that Belle Isle had no late blight infection in one trial in which the highly-susceptible control variety Up-to-Date showed 100% infection. More recent results following the 2004 epidemic in Mauritius confirmed that Belle Isle is resistant to late blight whereas Spunta is highly susceptible (Saumtally, 2004). This is also our observation in the 2004 multi-locational trials in Mauritius, but in Rodrigues the reaction of the varieties could not be assessed because the incidence of the disease was too low.

Wholesalers, retailers and growers like Spunta because of its large tubers. However, the seeds are also large and the variety performs poorly when the seeds are cut. As long as the cost of seeds was heavily subsidized, growers did not see much interest in using small seeds, or varieties with small seeds, but now that subsidies have been reduced, the cost of seeds has become a major concern. The price of Spunta seeds is now MUR 31 000 t⁻¹. At the normal density of 41 600 plants ha⁻¹ and the average seed size of 125 g, the cost of Spunta seeds amounts to MUR 161 000 ha⁻¹, a large investment by any count. In contrast, the average size of Belle Isle seed is 55 g, and the Agricultural Marketing Board has been selling seeds at MUR 18 000 ha⁻¹. Thus, the cost of Belle Isle seed amounts to MUR 41 250 ha⁻¹, or just a quarter of that of Spunta. The advantage of Belle Isle is therefore clear. Moreover, there is also a possibility of cutting the larger seeds because Belle Isle has more eye buds per seed than Spunta.
CONCLUSION

Local potato clone Belle Isle is being released for cultivation in Mauritius and Rodrigues for four reasons. Firstly, its yield is higher than that of popular variety Spunta and more stable too. Secondly, the variety is resistant to late blight. Thirdly, the seeds are cheaper and, fourthly, the tubers are appropriate for crisps. However, the tubers of Belle Isle are smaller than those of Spunta. Growers are therefore advised to pay careful attention to marketing. The Agricultural Marketing Board will organize the production of seeds.

ACKNOWLEDGEMENTS

Thanks are presented to the Agricultural Marketing Board for the donation of seeds for the trials. We acknowledge sugar estates and other growers for land, labour and other resources and for their help in the management of trials. We are pleased to record our appreciation of the collaboration of the Agricultural Services of Rodrigues.

REFERENCES


THE EFFICIENCY OF TRANSPLANTING SUGAR CANE SEEDLINGS DIRECTLY IN THE FIELD AND ITS IMPACT ON THE SELECTION CYCLE AND RESOURCES

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Mauritius Sugar Industry Research Institute

ABSTRACT

A trial was carried out to study the feasibility and efficiency of transplanting sugar cane seedlings directly in the field without potting and to assess its impact on the selection cycle and on resources. Seedlings of five families were transplanted on one-metre wide raised beds at three spacing: close, intermediate and wide, at population densities of seven, five and three seedlings/m$^2$ respectively. Selection was practised when the crop was aged ten months and all genotypes that produced enough planting material, including those that would have been rejected on visual assessment, were evaluated in two-metre row plots at the 1st clonal stage.

Mortality rate of seedlings was highest in the closest spacing. Significant differences between spacing were found for stalk number and stalk height but not for stalk diameter. Families differed significantly for all the characters measured and family x spacing interaction was detected for stalk diameter and stalk height.

Phenotypic correlation coefficients between the seedling stage and the 1st clonal stage were very low for stalk number, stalk diameter and stalk height. Broad-sense heritability for stalk number at the seedling stage was very low, while stalk diameter and stalk height were more heritable. Among genotypes that would have been rejected at the seedling stage based on visual appreciation, only a few were re-selected at the 1st clonal stage. Stalk diameter and stalk height were the most reliable criteria for selecting seedlings.

The intermediate spacing was most appropriate for transplanting seedlings directly in the field. At this planting density, 8.5 hectares of land, 890 man-days and other resources such as transport and potting medium can be saved each year. In addition, this new technique of evaluating seedlings shortens the selection cycle by one year and impacts favourably on the next stage of selection with respect to planting and selection periods.

Keywords: Potting, spacing, visual selection, genotypes, heritability.

INTRODUCTION

Sugar cane breeding programmes typically commence by evaluating large numbers of seedlings derived from true seeds. The starting population used for selection varies on breeding stations and ranges from 10 000 to 1.2 million seedlings (Mamet and Travaileur, 1998). These are screened through a series of selection stages and multiplied clonally, their numbers being reduced at each stage, with the best genotypes being tested in larger plots. The time taken to release a sugar cane variety ranges from eight to twenty years (Skinner et al., 1987).

The variety selection programme at the Mauritius Sugar Industry Research Institute (MSIRI) extends over eleven to fifteen years, with an initial population of 100 000 seedlings produced every year. Seed is sown in September and seedlings are transferred in polythene bags at the age of six to eight weeks before they can be transplanted to the field in March of the following year at the age of three to four months. Most breeding stations first transfer seedlings to polythene bags, flats, beds or peat pots for better survival and growth before transplanting them in the field. However, this involves transplanting the seedlings twice, which increases the time lapse from sowing to evaluation in the field. Seedlings are also transferred directly from sowing trays to airbricks, as in South Africa (Thomas, 1989) or transplanted in raised beds, as practised in India and Reunion Island.
The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources.

H Mungur et al.

Seedlings at MSIRI are visually screened in April/May, 13 to 14 months after transplanting in the field. Selection criteria are number and size of stalks, growth habit and absence of defects. Individual seedling selection is of low efficiency given the low broad-sense heritability for the majority of traits (Skinner, 1971, Skinner et al., 1987). Selection should therefore be lenient. Genotypes which are selected at the seedling stage are planted in two-metre row plots in the 1st clonal stage. Selection at this stage is done after 14 to 15 months, in July of the following year. The time at which selection is practised at both the seedling and 1st clonal stages affects the quality of planting material for establishing the 1st and 2nd clonal stages respectively.

A lot of resources are devoted to evaluate large populations of genotypes in small plots. At MSIRI, eleven hectares of land are required every year for evaluating the seedling population. Since most activities are carried out manually, the selection process at this stage is laborious and expensive. In the context of the new strategic plan for the sugar sector (MAFTNR, 2001), the sugar industry is called upon to operate in an environment of reduced labour. Alternative methods of evaluating seedlings under resource constraints need to be considered without jeopardising the efficiency of selection.

This paper investigates the feasibility of transplanting sugar cane seedlings directly in the field without potting and its impact on the selection cycle and on resources.

MATERIALS AND METHODS

The seedling stage

Seeds of five bi-parental families were sown thinly in July to produce vigorous seedlings. These were transplanted without potting, in November of the same year, on one-metre wide raised beds at Réduit Experimental Station at three different spacing:

- Close - three rows of seedlings at 0.40 m between rows and 0.30 m within rows
- Intermediate - three rows of seedlings at 0.40 m between rows and 0.40 m within rows
- Wide - two rows of seedlings at 0.40 m between rows and 0.40 m within rows

at population densities of seven, five and three seedlings/m² respectively. The statistical design was a split plot with two replicates, with spacing as main plot and families as sub-plots. Each family was represented by 368 seedlings.

An assessment of mortality of seedlings was done at regular intervals for the first three months after transplanting and at the time of selection, ten months after the establishment of the crop. At selection time, the following characteristics were measured on all genotypes that produced enough planting material, ten three-eyed cuttings, to establish the 1st clonal stage: number of millable stalks per stool, stalk diameter (mm), stalk height (cm) and visual grade (select or reject). A total of 717 genotypes, 260 from close spacing, 245 from intermediate spacing and 212 from wide spacing were selected for further evaluation at the 1st clonal stage. The genotypes selected also included those that would have been rejected on visual assessment but which produced enough planting material for planting in the 1st clonal stage.

1st clonal stage

At the 1st clonal stage genotypes were planted in two-metre row plots, using a row and column design with the control variety, R 570, planted every five to six rows of test genotypes. Same criteria were measured as for the seedling stage, ten months after establishment. In addition, Brix (total dissolved solids correlated with sucrose content) was measured in the field, using a hand refractometer, on all genotypes, including the control. Selection was based on a threshold level for Brix and on visual assessment. A total of 173 genotypes were promoted for further evaluation in the 2nd clonal stage.
The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources. H Mungur et al.

Statistical analyses

With unequal number of progenies assessed for the five families, analysis of the unbalanced data was done by Residual Maximum Likelihood using the software ASREML (Gilmour et al., 2001) and GenStat for windows, Release 4.2.

Estimates of phenotypic correlation coefficient for stalk number, stalk diameter and stalk height between the seedling stage and the 1st clonal stage were obtained through covariance analysis according to the formula:

\[ r_{12} = \frac{\text{Cov}(\text{seedling}, \text{clonal})}{\sqrt{\text{Var}(\text{seedling})\text{Var}(\text{clonal})}} \]

where \( r_{12} \) is the estimate of correlation coefficient for the trait measured at the seedling and 1st clonal stages, \( \text{Cov}(\text{seedling}, \text{clonal}) \) is the covariance between the mean of the trait measured at the seedling stage and the mean of the trait measured at the 1st clonal stage. \( \text{Var}(\text{seedling}) \) is the phenotypic variance of the trait at seedling stage and \( \text{Var}(\text{clonal}) \) is the phenotypic variance of the trait in the 1st clonal stage (Steel and Torrie, 1980).

Broad-sense heritability \( (h_b^2) \) values for stalk number, stalk diameter and stalk height at the seedling stage was estimated using the variance components generated by REML analysis. The formula used was:

\[ h_b^2 = \frac{V_g}{V_p} \]

where \( V_g \) is the genetic variance and \( V_p \) is the phenotypic variance (Falconer and Mackay, 1996).

Estimates of land and labour resources were based on a population of 100,000 seedlings and an average selection rate of 20%.

RESULTS AND DISCUSSION

Mortality in seedlings

Mortality rate was highest (27%) in the closely transplanted seedlings, compared to that observed in intermediate (16%) and widely (8%) spaced ones (Figure 1). This suggests that high competition is detrimental in the high density treatment. It has been observed that vigorous seedlings in sowing trays tend to establish better in the field, indicating that they are more likely to have a significant competitive advantage in small plots. To ensure survival in the field, seed sowing should be done as lightly as possible to produce robust seedlings for direct transplanting without potting.

Visual selection

Selection rate varied with families and spacing treatments (Figure 2). Highest selection rate was obtained in the wide spacing treatment (43%) followed by intermediate spacing (28%) and close spacing (23%). Out of 717 genotypes selected at the seedling stage, 535 were visually suitable on a select/reject basis (Table 1), whereas the rest, although visually unsuitable, produced enough cuttings for planting at the next stage of selection.
The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources. H Mungur et al.

**Figure 1** Survival (%) of sugar cane seedlings transplanted at three spacing

![Graph showing survival (%) of sugar cane seedlings transplanted at three spacing]

**Crop stage**

<table>
<thead>
<tr>
<th>Transplanting</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
<th>10 months (selection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide spacing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate spacing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Close spacing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2** Percentage of seedlings selected visually in five sugar cane families transplanted at three spacing

![Bar chart showing percentage of seedlings selected visually in five sugar cane families transplanted at three spacing]

When all the 717 selected genotypes were evaluated on larger plots at the 1st clonal stage, 174 (33%) of the 535 genotypes (select grade) were again selected and provided most of the genotypes promoted to the 2nd clonal stage (Table 1). Among those that were visually unsuitable (reject grade) at the seedling stage, only 19 (10%) were promoted for further evaluation at the 2nd clonal stage (Table 1). This shows that if only the attractive genotypes are selected, there is a risk of losing some good ones. However, due to high environmental influence, selection should be lenient as the consequences of eliminating good genotypes are more serious than continuing to test the inferior ones.

The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources.

H Mungur et al.

Table 1 Number of genotypes categorised on select/reject basis at the seedling and 1st clonal stages and number of genotypes selected for the 2nd clonal stage

<table>
<thead>
<tr>
<th>Genotypes selected</th>
<th>717</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual grade at the seedling stage</td>
<td>Select (S) 535</td>
</tr>
<tr>
<td>Visual grade at the 1st clonal stage</td>
<td>S 174  R 361</td>
</tr>
<tr>
<td>Genotypes selected for 2nd clonal stage</td>
<td>154 (29%)</td>
</tr>
</tbody>
</table>

* Genotype that is not visually attractive

Cane yield components

Stalk number in the seedling population varied from two to eleven, substantiating the large coefficient of variation of 38-40% observed in the three spacing. Lower coefficient of variation of the order of 14-15 and 17-18 were obtained for stalk diameter and stalk height respectively, showing less variability for these two characters as compared to stalk number. Similar coefficients have been reported by Nagarajan (1997) and Nair (1989) in sugar cane seedling populations.

Families differed significantly for all the characters measured (Table 2) showing the superiority or inferiority of some families over the others for any of the three characters. Mean values for the cane yield components are given in Table 3. With regards to spacing, significant differences were observed for stalk number and stalk height but not for stalk diameter, implying that planting density did not have an impact on stalk thickness. The majority of genotypes in the close spacing treatment produced two to six millable stalks, as compared to three to seven in the low planting density treatments showing that larger spacing tends to favour the production of stalk. However, mean stalk number observed at intermediate and wide spacing were not significantly different as revealed by an LSD test. Irrespective of the family, stalk height was significantly taller at intermediate spacing. The absence of family x spacing interaction for stalk number shows that the relative performance of sugar cane families for that character would not change with respect to spacing (Table 2).

Table 2 Mean square values for stalk number, stalk diameter and stalk height for five families transplanted at three spacing

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Stalk number</th>
<th>Stalk diameter</th>
<th>Stalk height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f</td>
<td>m.s</td>
<td>d.f</td>
</tr>
<tr>
<td>Family (F)</td>
<td>4</td>
<td>16.84**</td>
<td>4</td>
</tr>
<tr>
<td>Spacing (S)</td>
<td>2</td>
<td>25.85**</td>
<td>2</td>
</tr>
<tr>
<td>F x S interaction</td>
<td>8</td>
<td>2.84ns</td>
<td>8</td>
</tr>
<tr>
<td>Residual</td>
<td>701</td>
<td>2.35</td>
<td>2033</td>
</tr>
</tbody>
</table>

*: Significant at P= 0.05
**: Significant at P= 0.01
ns: Not significant


139
The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources.

*H Mungur et al.*

**Table 3** Mean and S.E for stalk number, stalk diameter (mm) and stalk height (cm) for five families transplanted at three spacing

<table>
<thead>
<tr>
<th>Family</th>
<th>Spacing</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Close</td>
<td>Intermediate</td>
</tr>
<tr>
<td><strong>Stalk number</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>3.46 ± 0.18</td>
<td>3.89 ± 0.22</td>
</tr>
<tr>
<td>F2</td>
<td>3.86 ± 0.18</td>
<td>4.61 ± 0.23</td>
</tr>
<tr>
<td>F3</td>
<td>3.17 ± 0.15</td>
<td>3.66 ± 0.19</td>
</tr>
<tr>
<td>F4</td>
<td>3.56 ± 0.21</td>
<td>3.91 ± 0.21</td>
</tr>
<tr>
<td>F5</td>
<td>4.12 ± 0.22</td>
<td>4.06 ± 0.21</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>3.63 ± 0.09</td>
<td>4.03 ± 0.10</td>
</tr>
<tr>
<td><strong>Stalk diameter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>23.4 ± 0.55</td>
<td>25.4 ± 0.56</td>
</tr>
<tr>
<td>F2</td>
<td>25.0 ± 0.46</td>
<td>25.5 ± 0.46</td>
</tr>
<tr>
<td>F3</td>
<td>25.1 ± 0.43</td>
<td>24.5 ± 0.52</td>
</tr>
<tr>
<td>F4</td>
<td>23.2 ± 0.39</td>
<td>23.7 ± 0.43</td>
</tr>
<tr>
<td>F5</td>
<td>22.8 ± 0.36</td>
<td>22.1 ± 0.38</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>23.9 ± 0.21</td>
<td>24.2 ± 0.22</td>
</tr>
<tr>
<td><strong>Stalk height</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>153.4 ± 3.3</td>
<td>170.4 ± 4.3</td>
</tr>
<tr>
<td>F2</td>
<td>135.1 ± 2.4</td>
<td>154.8 ± 3.6</td>
</tr>
<tr>
<td>F3</td>
<td>166.8 ± 4.1</td>
<td>175.8 ± 5.6</td>
</tr>
<tr>
<td>F4</td>
<td>160.3 ± 4.2</td>
<td>167.7 ± 3.8</td>
</tr>
<tr>
<td>F5</td>
<td>159.2 ± 3.7</td>
<td>166.1 ± 2.8</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>155.0 ± 1.7</td>
<td>167.0 ± 1.8</td>
</tr>
</tbody>
</table>

In light of these results and taking into consideration land resources, the intermediate spacing appears to be the optimum planting density for transplanting seedlings directly in the field.

**Heritability**

The broad-sense heritability for stalk number was very low (0.04) at the seedling stage, indicating a very high component of environmental variation. Julien (1988) reported that stalk number is generally the character which is mostly affected by environment in a seedling population. Low to moderately low narrow-sense heritability values of 0.24 and 0.48 have been reported by (Singh and Singh, 1994) and (Singh et al., 1995) in seedlings evaluated in rows 0.60 m apart with 0.40 m within row. Stalk number is not a reliable selection criterion in seedlings in the range of spacing investigated in this study.

Stalk diameter and stalk height were more heritable with heritability values of 0.54 and 0.58 for the two traits respectively. A narrow-sense heritability value of 0.59 has been observed by (Singh et al., 1995) for stalk diameter and stalk height while Singh and Singh (1994) reported values of 0.71 and 0.91 for the two characters. Phenotypic expression of these two traits can constitute reliable selection criteria for selection purposes.
Correlation between seedling and 1st clonal stages

Correlation coefficients between the seedling and the 1st clonal stages were generally significant for all the traits, though they were very low (Table 4). Consistent correlation coefficients of 0.24, 0.24 and 0.23 for stalk number between the two selection stages were obtained in the close, intermediate and wide spacing treatments respectively, showing no advantage to any of the spacing. Higher correlation coefficients for stalk number, ranging from 0.32 to 0.61, between seedlings transplanted directly in the field and the 1st clonal stage have been reported by Tripathi et al. (1977), Sundaresan et al. (1979), Nagarajan et al. (1983) and Nair (1989). However, these studies were carried out at larger spacing at the seedling stage.

Low correlation coefficients for stalk diameter were obtained between the seedling stage and the 1st clonal stage. The highest repeatability for this trait (0.32) was observed in the intermediate spacing treatment. However, the repeatability values were lower than those (0.39 to 0.63) reported by Tripathi et al. (1977), Sundaresan et al. (1979), Nagarajan et al. (1983) and Nair (1989) between the first two selection stages. A better expression of stalk diameter can therefore be expected when genotypes are widely spaced in the field.

<table>
<thead>
<tr>
<th>Spacing</th>
<th>Stalk number</th>
<th>Stalk diameter</th>
<th>Stalk height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close</td>
<td>0.24** ± 0.06</td>
<td>0.15* ± 0.06</td>
<td>0.25** ± 0.06</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.24** ± 0.06</td>
<td>0.32** ± 0.06</td>
<td>0.25** ± 0.06</td>
</tr>
<tr>
<td>Wide</td>
<td>0.23** ± 0.07</td>
<td>0.09* ± 0.07</td>
<td>0.38** ± 0.06</td>
</tr>
<tr>
<td>Pooled population</td>
<td>0.21** ± 0.04</td>
<td>0.24** ± 0.04</td>
<td>0.26** ± 0.04</td>
</tr>
</tbody>
</table>

*: Significant at P= 0.05
**: Significant at P= 0.01
ns: Not significant

Correlation coefficients of 0.25 to 0.38 were observed for stalk height between the two selection stages in the three spacing treatments. Tripathi et al. (1977) reported coefficients ranging from 0.13 to 0.65 in progenies evaluated at a spacing of 0.6 m in rows 0.9 m apart in the first two stages of the selection programme.

The correlation coefficients observed for the three characters in the intermediate spacing treatment were consistent and indicate that selection for the three yield components can be favourably considered at that spacing.

Impact on resources

A seedling which is directly transplanted in the field at the intermediate spacing will occupy 0.2 m² compared to 0.9 m² with the conventional method of transplanting potted seedlings. Only 2.5 hectares of land area will be required every year, instead of eleven hectares to accommodate a typical seedling population of 100 000 in the MSIRI selection programme. In addition, direct transplanting does not require the preparation of the soil medium (soil, farmyard manure, filter mud and factory ash) for the potting of seedlings. An economy of 50 tonnes of soil can therefore be made each year. Similarly, the labour that is used for potting and that required for the transport of potted seedlings from the nursery to the field is no longer needed. Estimates of labour requirements at the seedling stage under the conventional and direct transplanting methods show that a substantial saving of 890 mandays can be achieved with the direct transplanting method (Table 5). In addition, seedling trays occupy less space and are more conveniently transported to the fields. Therefore, about 70 lorry trips are no longer required for the transport of potted seedlings to the field.
The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources.

H Mungur et al.

**Table 5** Estimated labour requirements (mandays) for the main activities at the seedling stage for the conventional (potting) and direct transplanting methods of transplanting seedlings

<table>
<thead>
<tr>
<th>Activities</th>
<th>Potting</th>
<th>Direct transplanting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potting</td>
<td>520</td>
<td>-</td>
</tr>
<tr>
<td>Management practices</td>
<td>250</td>
<td>230</td>
</tr>
<tr>
<td>Field preparation</td>
<td>110</td>
<td>250</td>
</tr>
<tr>
<td>Transplanting in field</td>
<td>570</td>
<td>180</td>
</tr>
<tr>
<td>Selection of seedlings</td>
<td>550</td>
<td>450</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2000</strong></td>
<td><strong>1110</strong></td>
</tr>
</tbody>
</table>

**Impact on selection cycle**

The calendar of activities for the first two selection stages of the MSIRI selection programme, for both the conventional and direct transplanting methods of transplanting seedlings, shows a gain of one year in the selection cycle (Figure 3). For the direct transplanting method, the best time for sowing seed is July for transplanting seedlings in the field in November of the same year, compared to March of the following year with potted seedlings. Selection in seedlings planted without potting can be done in September when the crop is aged 10 months instead of 13 to 14 months, in April/May of the following year, for seedlings transplanted by the conventional method. At this crop age, planting material of better quality is obtained for establishing the 1st clonal stage compared to that obtained when selection is practised on seedlings transplanted from pots. Concurrently, the change in the planting calendar for the 1st clonal stage enables selection at this stage to be done on a 10 months’ crop compared to 14 to 15 months for the conventional method. Planting material of good quality is thus obtained for establishing the 2nd clonal stage. It has been observed that with the conventional method, many attractive genotypes are lost every year on account of poor quality of canes (bulging buds, sprouting) by the time selection is carried out.
The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources.  
*H Mungur et al.*

**Figure 3** Calendar of activities for the conventional and direct transplanting methods of transplanting seedlings for the first two stages of selection

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Conventional method</th>
<th>Direct transplanting method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>May - July</td>
<td>CROSSING</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>September</td>
<td>SOWING</td>
<td>SOWING</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>POTTING</td>
<td>Transplanting seedlings</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>directly in field</td>
</tr>
<tr>
<td>2</td>
<td>March</td>
<td>Field transplanting</td>
<td>Selection of seedlings &amp;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>planting 1st clonal stage</td>
</tr>
<tr>
<td>3</td>
<td>April - May</td>
<td>Selection of seedlings &amp; planting 1st clonal stage</td>
<td>Selection of 1st clonal stage</td>
</tr>
<tr>
<td>4</td>
<td>July</td>
<td>Selection of 1st clonal stage</td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION**

This study has shown that direct transplanting of sugar cane seedlings without potting in the initial stage of the selection programme at MSIRI is viable. Sowing of seed should be done as thinly as possible to produce robust seedlings to ensure a good establishment in the field. Intermediate spacing at a population density of five seedlings/m² appears to be the optimum spacing for evaluating seedlings with this method. Visual selection integrating stalk number, stalk diameter and stalk height as selection criteria can be practiced but should be lenient at this stage of selection. However, less emphasis should be placed on stalk diameter and only clones with thin stalks should be rejected at this stage. Selection efficiency is improved as selection at both the seedling and the 1st clonal stages can be done on ten months’ old crops, ensuring a better quality of planting material for establishing the next stages of selection. Concurrently, substantial saving on land, labour, transport and other resources can be realised yearly with this transplanting technique. This also impacts on the calendar of activities for the first two stages of selection and results in the shortening of the selection cycle by one year.

**ACKNOWLEDGEMENTS**

The authors are grateful to the personnel of the Plant Breeding department for their contribution and thank the Director of the MSIRI for his constant support and for reviewing this paper.

The efficiency of transplanting sugar cane seedlings directly in the field and its impact on the selection cycle and resources.

H Mungur et al.

REFERENCES


ABSTRACT

Sugarcane production in the high altitude areas of Mauritius is limited by the relatively lower solar radiation regime compared to the coastal zone. Closer row spacing has been adopted in these regions to improve on light interception and increase radiation use efficiency. Equally important is the need to decrease intra-row competition, which in practice can be achieved through increasing furrow width and widening the placement of the cuttings in the furrow. The latter approach has not been encountered in the literature and the effect of increasing furrow width was assessed using commercial varieties with different canopy characteristics. Field trials were implemented at Eau Bleue and Belle Rive using three furrow widths. At Eau Bleue, the treatments also included two interrow spacings and three sugarcane varieties while at Belle Rive two planting densities were used on two varieties. Light interception was measured to assess conversion efficiency. At Eau Bleue, there was no significant difference in cane yield between the two interrow spacings. Sugarcane grown in the widest furrow gave the highest cane yield with an advantage averaging 5.9 t ha\(^{-1}\) over the narrowest furrow. This yield difference was more pronounced in varieties M 52/78 and R 579 compared to M 3035/66. Differences could not be definitely attributed to higher radiation interception. At Belle Rive, the best cane yield was obtained when furrow width was increased and planting density doubled. Sugarcane grown in the widest furrow at the higher planting density gave 7.5 t ha\(^{-1}\) and 12.8 t ha\(^{-1}\) more cane in varieties M 1400/86 and M 52/78 respectively than the narrow furrow at the commercial planting density. The higher cane productivity resulted from higher tiller densities, which contributed to a larger leaf area. The latter intercepted and converted more solar radiation into biomass giving a higher radiation use efficiency of 1.44 g MJ\(^{-1}\) compared to 1.21 g MJ\(^{-1}\) for the narrow furrow at the commercial planting rate.

Keywords: furrow width, planting density, light interception, competition.

INTRODUCTION

In Mauritius, sugarcane grown in the super humid zone accounts for about 27% of the total area under cane. Sugarcane yield is determined to a large extent by the solar radiation incident on the crop, rainfall and temperature. The super humid regions are characterised by heavy rainfall, reduced solar radiation and low air temperature (Le Borgne, 1987). Sugarcane grown in these highlands has been observed to have gappy stands and low stalk populations. Thus, the crop has a poorly developed canopy with a reduction in the amount of light intercepted and biomass produced (Inman-Bamber, 1996). The gappy condition also favours rapid proliferation of weeds, which compete with the crop for soil moisture and nutrients. The overall effect may be seen at harvest with a sparse number of short millable stalks resulting in relatively lower cane yields.

Crop biomass is considered to be a function of the amount of radiation intercepted and many studies have shown that there is a linear relationship between the two attributes (Monteith, 1977; Muchow et al., 1994; Sinclair and Muchow, 1999). In the high altitude areas where solar radiation is the major limiting factor to sugar cane production, any cultivar that attains full canopy early in the season, is expected to intercept higher amounts of radiation and produce more biomass.

Therefore, one possible solution to the low cane productivity in the super humid zone would be to develop specific cultivars capable of intercepting maximum light in this zone. Moreover, cane productivity could be increased by amending cultural practices such as reducing the row spacing, increasing the planting density and redefining the planting pattern. Trials on sugarcane have shown that the effect of reducing row spacing to improve productivity was more pronounced at high altitudes.
Options for raising radiation use efficiency in the superhumid zone of Mauritius. SS Koonjah and AR Nayamuth.

than in the tropics (Irvine and Benda, 1980; Bull, 1975). Cane productivity increased by over 50% when high planting density was used in sugarcane clones (Bull and Bull, 1996). Crops grown at high planting density tend to be composed mostly of primary stalks which grow rapidly, compete actively with weeds and exploit soil water and nutrient reserves more efficiently than conventionally planted crops.

Efforts to decrease inter- and intra-row competition for light by the sugarcane plant and also to improve canopy closure early in the season are likely to increase both stalk density and stalk height, thus improving cane yield. At the same time, weed infestations are checked thereby minimising the cost involved in weed control. Thus, the objectives of the study were to evaluate the effect of increasing furrow width and placement distance between cuttings together with doubling the planting density, on radiation interception and cane productivity in the high altitude areas.

MATERIALS AND METHODS

The field study was conducted at Eau Bleue and Belle Rive in the super humid zone. Three furrow widths were used in both trials and the cuttings were placed 15, 30 and 45 cm apart (W_{15}, W_{30} and W_{45}). At Eau Bleue, three commercial varieties were used, namely M 3035/66, M 52/78 and R 579 at two interrow spacing of 1.3 and 1.6 m. (S_{1.3} and S_{1.6}). At Belle Rive, M 1400/86 and M 52/78 were the two commercial varieties used together with a further treatment of doubling the usual planting rate, that is 28,000 and 56,000 setts per hectare (D_1 and D_2). The varieties chosen had known difference in canopy architecture. Varieties M 1400/86 and R 579 are characterised by narrower leaf lamina and oblique leaf arrangement. M 3035/66 and M 52/78 have broader leaf lamina with horizontal leaf arrangement.

Each plot consisted of 4 rows of 10 m length. The experiment was managed as per commercial practice. Weeds were adequately controlled using herbicides and fertilizer was applied at a rate to avoid nutrient stress. A split-split plot design with row width as the main treatment was used. At Eau Bleue, the sub treatment was interrow spacing whereas at Belle Rive it was planting density. In both trials, variety constituted the sub-sub treatment and four replicates were used.

Tiller density, stalk height and leaf area index were followed throughout the crop cycle at both sites together with light interception using a ceptometer (AccuPAR, Decagon Devices, Inc., USA). At Belle Rive, a pair of tube solarimeters (Type TSL, Delta-T Devices, Cambridge, UK) was also used in 12 plots comprising one replication of all the treatments i.e., three row widths, two planting density and two varieties.

In each plot, one solarimeter was placed at ground level diagonally across the row width and the other one diagonally across half the interrow. The lower dry leaves were removed from time to time to ensure that radiation interception by green leaves only was measured. A reference tube solarimeter was placed in the nearby meteorological station to measure incoming radiation.

Prior to harvest, above ground crop biomass was determined by sampling whole cane stalks with the dry leaves from each plot. The fresh and dry weights were determined and the net above-ground biomass was calculated. Cane yield was assessed by harvesting and weighing all canes in the plots.
RESULTS AND DISCUSSIONS

The trial at Eau Bleue was followed during the plant cane and three ratoons whereas that at Belle Rive was followed during the plant cane and two ratoons. But in this paper, only the mean results of all the crop years will be presented and discussed.

Agro-climatic parameters

The meteorological conditions during the study period are summarised in Table 1. At both sites, the cumulative rainfall was above 3000 mm and well distributed such that the crop did not suffer from any water stress. The maximum and minimum temperatures were close to their respective normal. The annual solar radiation was 5130 MJ m\(^{-2}\) at Eau Bleue and 5530 MJ m\(^{-2}\) at Belle Rive, and close to the normal at both sites.

Table 1 Summary of weather conditions during the study period

<table>
<thead>
<tr>
<th></th>
<th>Eau Bleue</th>
<th>Belle Rive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant cane</td>
<td>1st ratoon</td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>3035</td>
<td>3030</td>
</tr>
<tr>
<td>Max Temp (^{\circ})C</td>
<td>28.3</td>
<td>27.9</td>
</tr>
<tr>
<td>Min Temp (^{\circ})C</td>
<td>17.1</td>
<td>17.0</td>
</tr>
<tr>
<td>Solar radiation (MJ m(^{-2}))</td>
<td>5100</td>
<td>5187</td>
</tr>
</tbody>
</table>

Since there were no significant differences in agronomic measurements between cane grown under row width 15 cm and 30 cm, thus all the results will be discussed on the basis of the two extreme row widths, i.e. W\(_{15}\) and W\(_{45}\).

Eau Bleue trial

Tiller Density

Tiller population per m\(^2\) in row width W\(_{45}\) was generally higher than that grown in W\(_{15}\) over all the years. The trend in tiller density between the two row widths averaged over the interrow spacings and varieties is illustrated during the second ratoon in Figure 1. Evolution of tiller density did not follow the classical pattern. A clear overproduction of tillers and a peak tiller density followed by tiller death phase was not prominent, most probably because of limited resources in terms of radiation and low temperatures. However, by the 23\(^{rd}\) week there were more tillers in row width 45 cm than in 15 cm. At harvest, stalk density was significantly higher by 0.35 tiller m\(^{-2}\) in W\(_{45}\) as compared to W\(_{15}\). This difference in tiller population between the two row widths could be attributed to lower intra-row competition in W\(_{45}\) as compared to W\(_{15}\), which enabled more stalks to survive until harvest. Tiller population in W\(_{45}\) within the 1.3 and 1.6 m interrow spacing was higher than in W\(_{15}\) by 0.29 and 0.49 tiller m\(^{-2}\) respectively. W\(_{45}\) gave higher tiller population than W\(_{15}\) within all three varieties under test.
Cane grown under interrow spacing of 1.3 m averaged over row width and varieties had a significantly higher tiller population of more than 1.0 tiller m\(^{-2}\) as compared to those grown at 1.6 m. Therefore, closer interrow spacing supported higher tiller densities than at wider interrow spacing which is in line with that reported elsewhere (Inman-Bamber, 1996; Bull and Bull, 2000). Among the varieties, it was M 52/78 that maintained a significantly higher tiller population than R 579 and M 3035/66. There were no significant interactive effects of row width x interrow spacing, row width x variety, interrow spacing x variety, and row width x interrow spacing x variety.

**Leaf Area Index and light interception**

A higher tiller population implies a larger leaf area exposed for light interception. During the second ratoon, leaf area index (LAI) recorded in W\(_{45}\) was above that of W\(_{15}\) as from week 21 and this difference was maintained until harvest when LAI in W\(_{45}\) exceeded that of W\(_{15}\) by 0.4 (Figure 2a). This advantage in LAI was due to higher tiller density in W\(_{45}\) than in W\(_{15}\) as the leaf area per stalk in W\(_{45}\) was slightly below that of W\(_{15}\). Within the two interrow spacings and the three varieties, LAI of crop grown in W\(_{45}\) was higher than in W\(_{15}\). The LAI between cane grown at S\(_{1.3}\) and S\(_{1.6}\) averaged over row widths and varieties was not significantly different although a slight advantage in favour of cane grown at S\(_{1.3}\) was noted. Among varieties, a higher crop LAI was obtained in R 579 followed by M 3035/66 and M 52/78. But the difference in LAI between W\(_{45}\) and W\(_{15}\) among the three varieties was not consistent.

In second ratoon only there was a slight though not significant difference in light interception between cane grown under W\(_{45}\) and W\(_{15}\) (Figure 2b). The difference in light interception occurred around the same time as that noted for leaf area index. Within both interrow spacings S\(_{1.3}\) and S\(_{1.6}\), more light was intercepted in W\(_{45}\) than in W\(_{15}\). Similarly, the same trend was observed among the three varieties used. A comparison of light intercepted between S\(_{1.3}\) and S\(_{1.6}\) averaged over all row widths and varieties showed that slightly more light was captured in crops in S\(_{1.3}\) than S\(_{1.6}\). Within variety, the difference in light interception between W\(_{45}\) and W\(_{15}\) was not consistent except for R 579, which intercepted more radiation when grown in W\(_{45}\).
Figure 2  Trend in (a) leaf area index and (b) light interception between the two row widths averaged over interrow spacings and varieties at Eau Bleue
Cane yield

Row widths
Cane yields between the two row widths were variable over the four years with the best performance generally obtained with the widest furrow (Figure 3). The mean gain over the four crop cycles in cane productivity averaged over planting density and varieties was 5.9 t ha\(^{-1}\) in favour of W\(_{45}\) as compared to W\(_{15}\). Therefore, using 45 cm row width gave a cumulative benefit of nearly 24 tonnes more cane per ha over the four years as compared to the current row width.

Figure 3 Mean cane yield for row widths and interrow spacings over four crop cycles at Eau Bleue

![Figure 3](image)

Interrow spacings
Averaging all treatments, there was a slight non-significant difference in cane productivity in favour of 1.3 m (Figure 3). The mean cane productivity over the four years was 1.1 t ha\(^{-1}\) more in S\(_{1.3}\) compared to S\(_{1.6}\). Within varieties again it was in S\(_{1.3}\) that more cane was harvested than in S\(_{1.6}\). Trials carried out elsewhere have also shown an increase in cane yields at closer row spacings whenever soil moisture is not a severe limiting factor (Boyce, 1968; Freeman, 1968; Bull, 1975; Irvine and Benda, 1980; Inman-Bamber, 1996; Bull and Bull, 2000).

Varieties
Considering the four years mean results among the three varieties (Table 2), the widest furrow under M 52/78 and R 579 gave 10.1 and 7.4 t ha\(^{-1}\) more cane than the narrowest furrow. In M 3035/66, only a slight increase of 3.7 t ha\(^{-1}\) was obtained in the widest row compared to the narrowest row. Within each variety, the gain in cane productivity using W\(_{45}\) compared to W\(_{15}\) was not consistent over the years. None of the interactive effects between row width, interrow spacing and variety were significant.

Table 2 Mean cane yield (t ha\(^{-1}\)) over four crop cycles between the two row widths at Eau Bleue

<table>
<thead>
<tr>
<th>Varieties</th>
<th>W(_{15})</th>
<th>W(_{45})</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 3035/66</td>
<td>82.6</td>
<td>86.5</td>
<td>3.7</td>
</tr>
<tr>
<td>M 52/78</td>
<td>90.7</td>
<td>100.8</td>
<td>10.1</td>
</tr>
<tr>
<td>R 579</td>
<td>90.5</td>
<td>97.9</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Options for raising radiation use efficiency in the superhumid zone of Mauritius. SS Koonjah and AR Nayamuth.

The differences in cane yield between the treatments were not consistently related to higher light conversion efficiency. Probably the way light interception was measured using a Decagon Ceptometer and making point measurement was not concise enough for detecting differences between the treatments. Higher cane yields were thus attributed to either a higher tiller population, reduced intra-row competition among tillers, higher leaf area index or a combination of these factors.

**Belle Rive trial**

**Tiller density**

The trend in tiller population for cane grown in W_{45} was always higher than in W_{15} in all the three crop years. The difference was more pronounced in the plant cane and is depicted in Figure 4a. In the plant cane, the tiller density averaged over planting density and varieties was significantly higher by 1.7 tillers m^{-2} in W_{45} than in W_{15}. The same tendency was obtained when comparing the tiller density for the higher planting density used compared to the commercial rate. Variety M 52/78 had a better tiller population compared to that of M 1400/86. In both varieties, the tiller density of the combined treatment W_{45}D_{2} was significantly higher than that of W_{15}D_{1} throughout the crop cycle (Figure 4b and 4c). Bull (1975) also recorded a higher stalk population using higher planting density and the advantage was maintained until the final harvest.

**Figure 4** Trend in tiller density between row widths and between combined treatments in M 1400/86 and M 52/78 during plant cane at Belle Rive
Stalk population at harvest averaged over the three crop cycles was higher in the widest furrow at the higher planting density compared to the smallest row width at the commercial planting density (Figure 5). The difference in stalk density between $W_{45}D_2$ and $W_{15}D_1$ was significant in both varieties at the plant cane harvest only. This difference in stalk density decreased with crop cycles giving a mean yearly advantage of 1.35 tillers m$^{-2}$ in M 1400/86 and 1.50 tillers m$^{-2}$ in M 52/78.
Figure 5 Mean stalk density at harvest over three crop cycles in the two combined treatments at Belle Rive

Leaf Area Index

The capacity to capture solar radiation for maximising the process of photosynthesis relies mostly on the green leaf area of the crop. From week 22 to 42, the leaf area index for sugar cane grown in the widest row exceeded that of the narrowest row (Figure 6) by more than 0.32 (10%). The LAI values in the plant cane for the combined treatment W_{45}D_{2} was higher by more than 0.50 (17%) in both varieties as compared to that of W_{15}D_{1}.

Figure 6 Trend in leaf area index between row widths and between combined treatments in M 1400/86 and M 52/78 during plant cane at Belle Rive.
In general, canopies with greater LAI intercepted more incident light during the major part of the crop cycles, that is, until senescence started towards about six weeks before harvest. This eventually contributed to a higher increment in cane productivity obtained in widest row width with double planting density compared to the narrowest row at commercial planting density. In maize, the rate of leaf area development and maximum leaf area index was found to increase with higher plant population densities (Westgate et al., 1997; Maddonni et al., 2001).
Options for raising radiation use efficiency in the superhumid zone of Mauritius. SS Koonjah and AR Nayamuth.

Light interception

Over the three years, sugarcane grown in the widest row intercepted more light than that in W15. Light interception in the combined treatment W45D2 was always higher than that in W15D1 as illustrated in the plant cane (Figure 7). The average difference in light interception between these two treatments amounted to 9% in M 52/78 and 12% in M 1400/86. The better response to light interception in W45D2 compared to W15D1 was attributed to more tillers which contributed to a higher leaf area index. In both maize and sugarcane, higher planting densities and closer interrow spacing have been found to improve light interception (Bull and Bull, 1996; Bull, 1975; Inman-Bamber, 1996; Madonni, 2001; Westgate, 1997).

**Figure 7** Trend in light interception during the plant cane in both varieties at Belle Rive

![Graph showing light interception trend for M 1400/86 and M 52/78 varieties.](image)

Cane yield

Results obtained from the three crop cycles (Figure 8) confirmed that increasing both row width and planting density gave higher cane yields. The mean cane productivity over the three years for cane grown in the widest row out yielded the narrowest one by 5.5 t ha\(^{-1}\) (8.4 %) and the higher planting density gave 4.1 t ha\(^{-1}\) (6.1 %) more cane than the commercial planting rate. But it should be noted that the margin gain in cane productivity with increasing row width and planting density was not consistent over all cycles with the highest margin obtained in the plant cane.

Figure 8 Mean cane yield for row width and planting density over three crop cycles at Belle Rive

![Figure 8](image)

The best cane productivity was generally obtained under the combined treatment of widest row width and double planting density (Table 3). The advantage in mean cane yield over the three years for \(W_{45}D_2\) was 7.5 t ha\(^{-1}\) (11.2 %) in M 1400/86 and 12.8 t ha\(^{-1}\) (21.4%) in M 52/78 compared to that in \(W_{15}D_1\). The difference in yield between the two extreme combinations was more consistent in M 52/78 than in M 1400/86 over the three crop cycles. Interactive effects of row width x planting density, variety x row width, variety x planting density, and row width x planting density x variety were not significant.

Table 3  Cane yield (t ha\(^{-1}\)) for the different treatments in the two varieties at Belle Rive

<table>
<thead>
<tr>
<th>Variety</th>
<th>(W_{15}D_1)</th>
<th>(W_{45}D_1)</th>
<th>(W_{15}D_2)</th>
<th>(W_{45}D_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 1400/86</td>
<td>67.1</td>
<td>72.0</td>
<td>68.0</td>
<td>74.6</td>
</tr>
<tr>
<td>M 52/78</td>
<td>59.7</td>
<td>64.9</td>
<td>67.4</td>
<td>72.5</td>
</tr>
</tbody>
</table>

The higher cane yields were attributed to the combination lower intra-row competition and a better use of available space. This was reflected in the higher tiller densities being supported throughout most of the crop cycles and maintained until harvest in the treatments that had higher cane productivities. Higher tiller densities stemmed from a higher planting density also. The number of harvestable stalks being a direct component of cane yield, this led to higher cane productivities as reported by Bull (1975) and Bull and Bull (2000). The higher tiller densities were principally responsible for the maintenance of a higher LAI, which in turn intercepted more solar radiation. This eventually contributed to a higher total biomass accumulation as well as for the harvestable cane portion. In maize, the rate of leaf area development and maximum LAI was found to increase with higher plant population densities (Westgate et al., 1997 and Maddoni et al., 2001). The same authors reported the beneficial effects on yield.
Total biomass Accumulation

Sugar cane grown at the widest furrow width gave 4.9 t ha⁻¹ more total above-ground dry matter than those grown in narrow furrow width and similarly an advantage of 1.9 t ha⁻¹ of biomass was obtained with higher planting density over the commercial planting rate (Figure 9). Total biomass production was superior in the combined treatment W₄₅D₂ in both varieties over the three crop cycles. The gain in total biomass averaged over the three years for W₄₅D₂ was 2.9 t ha⁻¹ in M 1400/86 and 13.1 t ha⁻¹ in M 52/78 as compared to W₁₅D₁. The advantage in biomass accumulation per unit land area in W₄₅D₂ was largely due to the high stalk population and leaf area index, which intercepted and converted more solar radiation into biomass.

Figure 9  Total above-ground biomass between row widths and planting density at harvest over three crop cycles at Belle Rive

Radiation Use Efficiency

Radiation use efficiency (RUE) is a measure of the efficiency of the crop to convert solar radiation into biomass (Monteith, 1977; Muchow et al., 1994; Sinclair and Muchow, 1999). The combined treatment of widest furrow and double planting density gave higher RUE values compared to those of the narrow furrow width at commercial planting density over all three crop cycles. In M 1400/86, the mean RUE over the three years for W₄₅D₂ was 1.44 g MJ⁻¹ compared to 1.23 g MJ⁻¹ in W₁₅D₁ and in M 52/78, W₄₅D₂ gave a RUE of 1.45 g MJ⁻¹ compared to 1.21 g MJ⁻¹ in W₁₅D₁ (Figure 10). The advantage in RUE for W₄₅D₂ was attributed to higher tiller density and canopy cover together with a better radiation interception over the season with improved biomass accumulation at harvest. In maize, it was reported that RUE and grain yield were improved using a combination of narrower interrow spacing and plant population densities greater than typically used (Wesgate et al., 1997).
Figure 10  Mean radiation use efficiency over three crop cycles at Belle Rive for the two combined treatments at Belle Rive

CONCLUSION

Sugarcane productivity in the super humid zone can be improved by increasing the furrow width from 15 cm to 45 cm while placing the cane setts at the extremities. Similarly, increasing the planting sett density also leads to higher cane production in plant cane crop. However, the maximum gain in cane productivity can be achieved through using the combined effect of wider spacing of cuttings in larger furrows and doubling the planting density. The advantage in yield was due to a higher number of tillers per unit area, a rapid canopy cover and higher interception of solar radiation. Thus, the combined treatment gave higher radiation use efficiency in both varieties. This practice apart from improving cane productivity in the high altitude areas where solar radiation is a limiting factor can also reduce costs of production through lower weed proliferation and herbicide usage.

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MICROBIAL BIOFERTILISERS: A SOURCE OF NITROGEN FOR SUGAR CANE IN MAURITIUS?

G. Umrit and K.F. Ng Kee Kwong

Mauritius Sugar Industry Research Institute

ABSTRACT

Sugar cane production in Mauritius is intensive with 11,000 tonnes fertiliser nitrogen (N) applied annually to maintain productivity. This represents an annual investment of more than MUR 220 million by the sugar cane farming community and with further likely increases in the price of synthetic N fertilisers research effort is focused on alternative technologies that will reduce our reliance on mineral N fertiliser. In this context field trials were conducted at four locations (Pamplemousses, Réduit, Belle Rive and Union Park) during 2002-2004 period to determine the contribution of three commercially available microbial biofertilisers (Gluconacetobacter, Azotobacter and Azospirillum) to the N nutrition of sugar cane receiving different levels of fertiliser N (70 and 140 kgNha⁻¹). Data obtained showed that inoculation of cane setts with the biofertilisers at planting had no significant effect (P=0.05) on N uptake and biomass produced by six month-old cane at any of the four sites. Consequently, total N uptake, cane and sugar yields at harvest did not differ significantly (P=0.05) between inoculated and uninoculated treatments. Concurrently, pot experiments carried out at Réduit did not provide any evidence of a significant (P=0.05) difference in root and shoot biomass or N uptake between inoculated and uninoculated treatments. The prospects of using the three microbial biofertilisers studied as an alternative means of harnessing N by sugar cane are therefore remote.

Keywords: Sustainable sugar cane farming, N fertilization, Gluconacetobacter, Azotobacter, Azospirillum.

INTRODUCTION

Nitrogen (N) remains by far the most important nutrient in sugar cane fertilization, with 11,000 tonnes fertiliser N applied annually to maintain productivity. This represents an annual investment of more than MUR 220 million by the sugar cane farming community. The efficiency of N utilization by sugar cane is however low, not exceeding 40% (Ng Kee Kwong and Deville, 1987) with substantial amounts being either immobilised in soil or lost into the atmosphere by denitrification (Ng Kee Kwong et al., 1999). With the inevitable price rises of N fertilisers that must occur over the coming decades as signalled by the declining availability of fossil fuels, there is a pressing need for developing alternative and cheaper sources of N supply that will reduce reliance of the sugar industry on synthetic N fertilisers. This will not only reduce cost of production but also further minimize any potential environmental impacts of fertiliser N not used by the crop. In this context, biological N fixation (BNF) technology, such as the use of N₂-fixing inoculant biofertilisers will have an important role to play as an alternative source of N (Kennedy et al., 2004).

There has in fact been, during the last decade, a resurgence of interest in microbial biofertilisers as a source of N following studies on the effect of non-symbiotic N₂-fixing organisms on plant growth (Kennedy and Islam, 2001). Though a large number of microbial biofertilisers have been produced in countries such as India, Pakistan, China and Egypt and tested on several cereal crops, reliable data showing conclusive impacts on yield and non-symbiotic N₂ fixation are still lacking (Kennedy and Roughley, 2002; Giller and Merckx, 2002). Similar studies with the sugar cane crop is even more limited and the few studies done have yielded inconsistent results: from no apparent effect (Anon, 1990/2000) to the ambitious claim that inoculation of cane setts with the biofertilisers completely substituted for the recommended dose of 275 kgNha⁻¹ (Muthukumarasamy et al., 1999).
Microbial biofertilisers: A source of Nitrogen for sugar cane in Mauritius? G Umrit and KF Ng Kee Kwong.

The present study was therefore initiated to elucidate this uncertainty regarding the potential of inoculant biofertilisers as a source of N for sugar cane. The main objectives were to determine (i) the contribution of commercially available microbial biofertilisers to the N nutrition of sugar cane and (ii) whether the use of microbial biofertilisers could be an alternative to mineral N for sugar cane in Mauritius.

MATERIALS AND METHODS

Field trials

Field trials were laid down at four locations (Table 1) during the 2002-2004 period involving the following biofertiliser and inorganic N treatment combinations:

<table>
<thead>
<tr>
<th>Biofertiliser</th>
<th>N fertiliser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no biofertiliser)</td>
<td>0 kgNha⁻¹, 70kgNha⁻¹, 140 kgNha⁻¹</td>
</tr>
<tr>
<td>Gluconacetobacter</td>
<td>70 kgNha⁻¹, 140 kgNha⁻¹</td>
</tr>
<tr>
<td>Azotobacter</td>
<td>70 kgNha⁻¹, 140 kgNha⁻¹</td>
</tr>
<tr>
<td>Azospirillum</td>
<td>70 kg ha⁻¹, 140 kgNha⁻¹</td>
</tr>
</tbody>
</table>

*Gluconacetobacter* and *Azotobacter* were obtained from two suppliers namely Vasantdada Sugar Institute (VSI), Pune, India and Nafed Biofertilisers, New Delhi, India while *Azospirillum* was obtained from VSI. At each site the 13 treatments were replicated four times in a randomized complete block design, with each treatment plot consisting of four rows of sugar cane 3 m long spaced 1.6 m apart.

Prior to planting, cane setts were inoculated with the biofertilisers *Gluconacetobacter*, *Azotobacter* and *Azospirillum* by dipping in a suspension containing 10 kg lignite-based inoculant per 100L water for one hour as recommended by the supplier. Fertiliser nitrogen, as ammonium sulphate, was applied at planting time at the rates of 70 and 140 kgNha⁻¹. Adequate phosphorus as triple superphosphate and potassium as muriate of potash were also applied to the sugar cane based on soil test values.

The dry matter, total N uptake and ¹⁵N natural abundance of sugar cane at the logarithmic growth stage and at harvest were determined from 10 sugar cane plants sampled from each plot. The sugar cane plant samples were separated into green tops, trash and stalks.

All plant samples were dried at 90 °C, weighed, ground in a Wiley mill to pass through a 0.5mm sieve and then ball-milled to <150 microns. Total N and ¹⁵N natural abundance in the ground material were measured on an ANCA 20-20 GSL isotope ratio mass spectrometer (PDZ Europa Ltd., Cheshire, UK).

The cane stalks from the central two rows of each treatment plot were also weighed at harvest to obtain cane yield and then sampled for the determination of sucrose using an automatic saccharimeter.

Pot Experiment

The experiment in pots was undertaken in parallel to the field trials and comprised a completely randomized design involving the same treatments as described for the field trials with four replications of each treatment on a Low Humic Latosol from Réduit. Two one-eyed cuttings of sugar cane were planted in pots containing 20kg soil following inoculation of the cuttings with biofertilisers as described in the preceding section. Nitrogen was applied as ammonium sulphate at rates equivalent to 70 and 140 kgNha⁻¹. Adequate phosphorus and potassium were also applied to the soil. Plants were allowed to grow under field conditions. Three months after planting, pots were destructively sampled for root and shoot dry weight and N content.
**Table 1** Characteristics of soils at the four experimental sites

<table>
<thead>
<tr>
<th>Annual rainfall (mm)</th>
<th>Réduit</th>
<th>Belle Rive</th>
<th>Union Park</th>
<th>Pamplemousses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Humic Latosol</td>
<td>1500</td>
<td>3800</td>
<td>3850</td>
<td>1500</td>
</tr>
<tr>
<td>Humic Ferruginous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latosol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH (H$_2$O)</td>
<td>5.7</td>
<td>5.6</td>
<td>5.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Organic C, (g kg$^{-1}$)</td>
<td>20.7</td>
<td>23.4</td>
<td>49.0</td>
<td>21.4</td>
</tr>
<tr>
<td>Total N (g kg$^{-1}$)</td>
<td>2.8</td>
<td>2.5</td>
<td>5.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

**Biomass production and N accumulation**

Biomass production and N accumulation by sugar cane at the logarithmic growth phase varied widely from one experimental site to another, reflecting the influence of climate and soil type on sugar cane growth (Table 2). The data obtained showed that there were significant responses ($P = 0.05$), in terms of plant N accumulation to N fertilization at all four sites. Similarly dry matter production increased with N fertilization, though such increases fell short of significance ($P = 0.05$) at Belle Rive and Union Park. However, inoculation of cane setts with *Gluconacetobacter*, *Azotobacter* or *Azospirillum* at reduced fertiliser N (half recommended rate) had no significant effect ($P = 0.05$) on either N uptake or dry matter accumulation by 6 month-old cane. Our results are in contradiction with those of Shankariah and Hunsigi (2001) who observed higher dry matter accumulation and N uptake in 6 month-old cane following soil application of *Azotobacter* and *Azospirillum*, but they concur with findings reported elsewhere e.g. Anon. (1997/1998).

**Biological nitrogen fixation**

The $^{15}$N natural abundance technique was used to determine whether sugar cane inoculated with the different diazotrophs obtained any significant input of N through BNF. The technique is based on $\delta^{15}$N signatures of inoculated plants relative to the $\delta^{15}$N of the uninoculated control plants. Generally, plants deriving most of their N requirement from N$_2$ fixation have negative $\delta^{15}$N values ($-4.40$ to $0.00$ %) while positive $\delta^{15}$N values ($> 5.00$ %) suggest insignificant or no N$_2$ fixation. Plants with mixed N sources tend to have $\delta^{15}$N values between $0.00$ and $3.00$ % (Biggs et al., 2000). Data obtained in the present study showed no difference in $\delta^{15}$N measurements between inoculated and uninoculated treatments (Table 2) indicating that there was no BNF contribution to sugar cane.
**Table 2** Effect of biofertilisers on N uptake, biomass produced and δ¹⁵N of sugar cane at the logarithmic growth stage at Réduit (RES), Belle Rive (BRES), Union Park (UPES) and Pamplemousses (PSES).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N Uptake (kg N ha⁻¹)</th>
<th>Biomass (t DM ha⁻¹)</th>
<th>δ¹⁵N (‰) (Mean of 4 sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RES</td>
<td>BRES</td>
<td>UPES</td>
</tr>
<tr>
<td>No N, No biofertiliser</td>
<td>82</td>
<td>31</td>
<td>60</td>
</tr>
<tr>
<td>70 kg N ha⁻¹, No biofertiliser</td>
<td>121</td>
<td>39</td>
<td>72</td>
</tr>
<tr>
<td>140 kg N ha⁻¹, No biofertiliser</td>
<td>139</td>
<td>49</td>
<td>81</td>
</tr>
<tr>
<td>70 kg N ha⁻¹ + Gluconacetobacter</td>
<td>108</td>
<td>40</td>
<td>64</td>
</tr>
<tr>
<td>70 kg N ha⁻¹ + Azotobacter</td>
<td>116</td>
<td>42</td>
<td>65</td>
</tr>
<tr>
<td>70 kg N ha⁻¹ + Azospirillum</td>
<td>123</td>
<td>41</td>
<td>77</td>
</tr>
</tbody>
</table>

*Vertical bars represent standard error of treatment means*

Microbial biofertilisers are believed to increase crop yield not only by BNF but also through various mechanisms including the secretion of plant growth regulators that promote root morphology and development thereby enhancing nutrient and water uptake (Wani and Lee, 2002; Cocking, 2003). Our data from the pot experiment, designed to determine the effect of biofertiliser treatment on root yield, showed that inoculation of cane setts with biofertilisers had no significant effect on root/shoot yield and consequently on N uptake by 3 month-old sugar cane plants (**Figure 1**) thereby indicating the non-effectiveness of the products used.

**Figure 1** Effect of inoculation with biofertilisers Gluconacetobacter, Azotobacter and Azospirillum on shoot biomass (■), root biomass (■) and N uptake (▲) by 3-months old sugar cane grown in pots at Réduit.
N uptake and cane/sugar yields at harvest

Consistent with the lack of any significant effect of inoculation on N uptake and vegetative growth during the active growth stage, total N uptake and cane and sugar yields at harvest (12 months) did not respond to biofertiliser application though significant responses to N fertilization were observed (Table 3). Our results are in contradiction with the findings of Muthukumarasamy et al. (1999) who claimed that inoculation of cane setts with *Gluconacetobacter* could completely substitute for the recommended 275 kgN ha\(^{-1}\). However, our data concur with findings at the Copersucar Technology Center, Brazil (Anon, 1999/2000) where significant responses to N treatments were observed but treatment of cane setts with *Gluconacetobacter* had no apparent effect. Shankariah and Hunsigi (2001) reported a 4-6% increase in cane yield due to inoculation with *Azotobacter* and *Azospirillum* but no significant effect of *Gluconacetobacter*.

Table 3 Effect of biofertilisers on N uptake (kg N ha\(^{-1}\)) cane and sugar yields (t ha\(^{-1}\)) at Réduit (RES), Belle Rive (BRES), Union Park (UPES) and Pamplemousses (PSES).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cane</th>
<th>Sugar</th>
<th>N Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RES</td>
<td>BRES</td>
<td>UPES</td>
</tr>
<tr>
<td>No N, No biofertiliser</td>
<td>144</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td>70 kgN ha(^{-1}), No biofertiliser</td>
<td>176</td>
<td>45</td>
<td>59</td>
</tr>
<tr>
<td>140 kgN ha(^{-1}), No biofertiliser</td>
<td>189</td>
<td>54</td>
<td>71</td>
</tr>
<tr>
<td>70 kgN ha(^{-1}) + <em>Gluconacetobacter</em></td>
<td>174</td>
<td>47</td>
<td>59</td>
</tr>
<tr>
<td>70 kgN ha(^{-1}) + <em>Azotobacter</em></td>
<td>158</td>
<td>46</td>
<td>64</td>
</tr>
<tr>
<td>70 kgN ha(^{-1}) + <em>Azospirillum</em></td>
<td>161</td>
<td>47</td>
<td>66</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>24</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

Though in this study the colonization of plant tissues by the different N\(_2\)-fixing bacteria were not examined, the observed lack of response to inoculation seem to suggest a failure of the diazotrophs to infect the cane tissue. In fact, as reported by Boddey *et al.* (1995), infection of cane tissue by *Gluconacetobacter* is rare except when inoculated “in vitro”.

As reviewed by Wani and Lee (2002) the success of inoculation with microbial biofertilisers depends on the ability of the inoculated bacteria to survive in the rhizosphere or plant tissue, which in turn depends on a number of factors including the host plant and the biological and environmental factors. The fact that only a limited number of diazotrophic strains are capable of effectively colonizing specific sugar cane genotypes indicates a strong genotype x diazotrophic strain interaction (Urquiaga *et al.*, 1992). Identifying the optimum combination of sugar cane genotype and diazotrophic strain probably holds the key to success with biofertilisers.

CONCLUSION

Our results showed that application of the commercially available biofertilisers *Gluconacetobacter*, *Azotobacter* and *Azospirillum* showed no significant effect on N uptake, growth and yield of sugar cane. There was no evidence of any BNF contribution to the N nutrition of sugar cane following inoculation with the biofertilisers tested. Consequently the prospects of using the three microbial biofertilisers studied as an alternative means of harnessing N by sugar cane are remote.
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EVALUATION AND POTENTIAL OF BIOLOGICAL NITROGEN FIXATION BY FRENCH BEAN (PHASEOLUS VULGARIS CV LONG TOM)

S. Sunassee

Agricultural Research and Extension Unit

ABSTRACT

Bean is the most commonly grown vegetable legume in Mauritius. As part of a study to determine the potential benefits of rhizobial inoculation by bean, four sets of field assessments and trials were conducted. In the first set of trials, the nodulation status of bean was evaluated at the Crop Research Stations in Richelieu, Réduit and Wooton under three nitrogen fertiliser levels: 0, 25 and 50 kg N ha\(^{-1}\). The experiments were repeated to evaluate the effect of previous cropping on nodulation. In the second set of activities, the nodulation status of bean was assessed in six localities during the period January 2001-April 2001. This was followed by a series of trials to assess the effect of liming on nodulation at Dubreuil, Grande Chartreuse and Plaine Sophie from June 2001 to September 2001. In the fourth set of activities, the population level of indigenous rhizobia present in the soil that could nodulate bean was estimated using the Most Probable Number-Plant Infection Technique.

Results show that bean was poorly nodulated in the field and nodules present were ineffective. The effect of nitrogen levels on nodulation could not be detected because of poor or non nodulation in most trials. Liming did not have any positive effect on nodulation in the acid soils of the uplands. Most probable number counts revealed that the number of rhizobia were less than 50 cell g\(^{-1}\) of soil. This indicates that inoculation with the appropriate strains of rhizobia is required.

Keywords: Phaseolus vulgaris, nitrogen, liming, nodulation, indigenous rhizobia, most probable number technique.

INTRODUCTION

The excessive use of chemical fertilisers causes higher production costs as well as adverse effects on the environment especially in terms of contamination of ground and subsurface water with nitrates. The optimisation of biological nitrogen fixation through the use of legumes in cropping systems is an alternative to reduce the use of nitrogenous fertilisers. In Mauritius, the most commonly grown vegetable legume in the field is the French bean (Phaseolus vulgaris L.) which is grown over an area of 400 hectares with an average annual production of around 2010 tonnes (MEDPRD, 2003). It has been estimated that the French bean can fix up to 50 kg N ha\(^{-1}\) (Erdmann, 1967) under favourable environmental conditions. In Mauritius, information about the nodulation status of bean fields is limited to work undertaken by Pillay and Mamet (1972) and Vencatasamy (1980). Field trials and observations made by Pillay and Mamet in 1972 revealed that there were insufficient numbers of Rhizobium phaseoli in the soils to enable successful nodulation of Phaseolus vulgaris. Observations made at various locations by the author when this study was initiated confirmed that P. vulgaris was indeed poorly nodulated in the field. Vencatasamy (1980) found that R. Phaseoli strains differ in their capacity to provide nitrogen for the bean plant and that cultivar Long Tom can fix up to 9.5 kg N ha\(^{-1}\) per crop and that under conditions of extended vegetative growth, the cultivar can double its N\(_2\) fixing capacity which will provide about 25% of the N\(_2\) requirements necessary for maximum yield.
OBJECTIVES

Studies were conducted from 2000 to 2002 to assess the nodulation status of bean under field conditions in order to determine whether the bean/rhizobium symbiosis is effective in existing vegetable cropping system. The effect of soil acidity and nitrogen on nodulation were examined. To decide whether inoculation is necessary, the population level of indigenous rhizobia present in the soil that could nodulate bean cv. Long Tom was also estimated using the Most Probable Number-Plant Infection Technique.

MATERIALS AND METHODS

On-station evaluation of nodulation in bean

The nodulation status of the bean crop was assessed at three levels of nitrogen (0, 25 and 50 kg ha\(^{-1}\)) under three field trials. These were performed from April 2000 to September 2000 at the Crop Research Stations in Richelieu, Réduit and Wooton, which respectively represent the sub-humid, humid and super-humid zones of the island. The experiments were repeated soon after final harvest from October 2000 to December 2000 to investigate the effect of a second cropping on nodulation.

The treatments were allocated in a randomized block design and replicated four times. Plot size was 2m50 x 2m25 and contained 5 rows of bean spaced 50 cm apart and bean seeds cv Long Tom were sown 15 cm within the row. At each site composite soil samples were taken and analysed for pH, organic C, total N, available P and available K. At Wooton the soil pH was adjusted for acidity through addition of cement at the rate of 6.5tha\(^{-1}\) in the furrows. Nitrogen in the form of ammonium sulphate was added in the furrows at 25 and 50kg ha\(^{-1}\). A basal application of 40 kg ha\(^{-1}\) P\(_2\)O\(_5\) as triple superphosphate and 60kg ha\(^{-1}\) K\(_2\)O as potassium sulphate were used at all sites. Cultural practices were as recommended for commercial plantations (AREU, 1998). Five randomly selected plants were carefully lifted from inner rows at flowering, pod formation and late pod fill to examine nodulation. Number of nodules was assessed on a 0 to 4 nodule score basis as described by CIAT (1988). Nodule size, shoot fresh and dry weights were measured. Nodule shape and colour as well as presence of leghaemoglobin inside the nodule were also noted. Leghaemoglobin is a reddish to pink pigment present in nodules which are effectively fixing atmospheric nitrogen. Green pods at early pod fill stage were also harvested and weighed.

Assessment of nodulation of bean (Phaseolus vulgaris cv. Long Tom) in selected localities of Mauritius

The nodulation status of bean cv Long Tom were examined in six regions during the period January-April 2001. Six to ten bean fields (>0.01ha each) were visited and five bean plants at flowering and/or pod formation stage were carefully lifted and the roots examined. The presence or absence, degree of nodulation on the root system, shape, size and colour of the interior of mature nodules were noted. Degree of nodulation was assessed on 0 to 4 nodulation score basis (CIAT 1988). Effective nodules were characterised by the presence of pink to red coloration (leghaemoglobin) inside the nodule. The soil pH and total nitrogen of each field were analysed as already described.

Effect of liming on nodulation of bean (Phaseolus vulgaris cv. Long Tom)

Twelve on-farm trials were set at Dubreuil, Grande Chartreuse and Plaine Sophie to assess the effect of liming and nitrogen on nodulation in bean between June to September 2001. Treatments consisted of liming with cement at 0 and 6.5tha\(^{-1}\) and nitrogen application at 0 and 25 kg ha\(^{-1}\). The soil pH was raised to between 6.0 and 7.5. The treatments were allocated in a complete randomized design without replication. There were three rows of bean spaced 50cm apart and 20 cm within rows of 2.0 m long. At pod filling stage five randomly selected inner plants were carefully uprooted and nodulation assessed based on the 0 to 4 nodule score basis.
Quantification of rhizobia in selected soils of Mauritius using the Most Probable Number-Plant Infection Technique

In order to determine the population level of indigenous rhizobia that could nodulate French bean (*Phaseolus vulgaris* cv *Long Tom*) soil samples were taken from research stations and bean growing localities from August 2001 to October 2002 and the number of rhizobia in the soil estimated using the Most Probable Number (MPN)-Plant Infection Technique (Woomer et al., 1990). Three fields were selected per locality and a composite soil sample taken for the MPN counts. Surface sterilised bean seeds were transplanted singly into Leonard bottles containing sterile rocksand/bagasse in a 2:1 volume ratio. Jensen’s N free nutrient solution was provided to the plants. 100g of soil from each sample collected was diluted with 400 ml of sterile water, the mixture filtered and five-fold dilution up to $5^{-6}$ were made and 1.0 ml aliquot was used to inoculate the bean. Four Leonard bottles were used as replicates per dilution level. The number of plants positively nodulated was noted and the MPN calculated from tables. Two sets of observations were taken from each field. The Rhizobia from nodules were isolated in the standard manner (Vincent, 1970), their growth performance on yeast mannitol agar observed. To confirm that the isolates were rhizobia, Congo red and bromothymol blue methods, biochemical tests (peptone glucose agar and ketolactose tests) and plant inoculation tests were carried out.

RESULTS AND DISCUSSION

On-station evaluation of nodulation in bean

Results of the first set of trials are shown in Table 1. At Richelieu CRS bean plants remained non-nodulated at all stages of the crop cycle and thus no relationship could be drawn between N levels and nodulation. However, increasing the nitrogen levels increased shoot and pod weight significantly. The positive response of bean to fertiliser N indicates that inoculation by the appropriate competitive rhizobia could prove useful.

At Réduit CRS nodulation was observed as from the vegetative stage in all treatments but no significant differences in nodulation were found between the treatments. Nodules were roundish, white in colour, small (less than 2.0 mm in diameter) and scattered over the whole of the secondary root system.

At Wooton CRS nodulation was very poor both at flowering and pod filling stages. Nodules were small usually less than 2.0 mm in diameter, round to oval, white in colour and were ineffective since they did not contain any leghaemoglobin. Nodules were found near the first formed secondary root. Nitrogen significantly increased shoot weight and pod weight However, nitrogen had no effect on nodulation.

When the experiments were repeated on the same plots no significant improvement in nodulation was observed. The results were similar to those obtained during the first set of trials. Bean was poorly nodulated at all three sites and when present they were small, white (on average 1.0 mm) and distributed over the whole of the secondary roots. No reddish or pinkish coloration could be seen when the nodules were cut. Such type of nodulation is ineffective (Moraby, 1976). The above results indicate that the bean/rhizobium relationship is not functional and that the bean/rhizobium symbiosis is not really contributing to atmospheric nitrogen fixation and to improving soil fertility.
**Table 1** Fertiliser N effects on nodulation, shoot fresh weight and pod fresh weight of bean.

<table>
<thead>
<tr>
<th>Fertiliser N (kg ha(^{-1}))</th>
<th>Shoot fresh weight (g/plant)</th>
<th>Pod fresh weight (g/plant)</th>
<th>Nodulation</th>
<th>Nodule size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Richelieu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>22.75(^b)</td>
<td>46.58(^b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>59.08(^a)</td>
<td>61.29(^b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>61.74(^a)</td>
<td>104.21(^a)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(P≤0.05)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>SE</td>
<td>3.91</td>
<td>11.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Réduit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>41.14</td>
<td>64.28</td>
<td>1.80</td>
<td>1.43</td>
</tr>
<tr>
<td>25</td>
<td>33.91</td>
<td>71.70</td>
<td>1.35</td>
<td>1.00</td>
</tr>
<tr>
<td>50</td>
<td>37.38</td>
<td>65.80</td>
<td>1.70</td>
<td>1.15</td>
</tr>
<tr>
<td>(P≤0.05)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>SE</td>
<td>2.87</td>
<td>6.70</td>
<td>0.32</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Wooton</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15.93(^c)</td>
<td>26.80(^c)</td>
<td>1.00</td>
<td>0.90</td>
</tr>
<tr>
<td>25</td>
<td>31.38(^b)</td>
<td>50.36(^b)</td>
<td>0.95</td>
<td>0.90</td>
</tr>
<tr>
<td>50</td>
<td>46.77(^a)</td>
<td>76.76(^a)</td>
<td>0.80</td>
<td>0.90</td>
</tr>
<tr>
<td>(P≤0.05)</td>
<td>*</td>
<td>*</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>SE</td>
<td>2.27</td>
<td>7.17</td>
<td>0.49</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the same column were not significantly different (P≤0.05)

**Assessment of nodulation of bean (Phaseolus vulgaris cv. Long Tom) in selected localities of Mauritius**

**Table 2** Nodulation status of bean cv Long Tom in six bean growing areas in 2001

<table>
<thead>
<tr>
<th>Locality</th>
<th>No. of fields</th>
<th>Soil pH</th>
<th>Soil Total N (%)</th>
<th>Nodulation Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belle/Mare/Palmar</td>
<td>6</td>
<td>8.1±0.12</td>
<td>0.37±0.06</td>
<td>0.50±0.54</td>
</tr>
<tr>
<td>Carreau Laliane</td>
<td>6</td>
<td>5.7±0.13</td>
<td>0.27±0.03</td>
<td>0.33±0.55</td>
</tr>
<tr>
<td>La Ferme/St Martin/Petite Riviere</td>
<td>10</td>
<td>6.4±0.21</td>
<td>0.27±0.13</td>
<td>0.67±0.69</td>
</tr>
<tr>
<td>Rose Belle</td>
<td>6</td>
<td>5.8±0.10</td>
<td>0.17±0.02</td>
<td>0.50±0.55</td>
</tr>
<tr>
<td>Dubreuil</td>
<td>8</td>
<td>5.9±0.56</td>
<td>0.98±0.12</td>
<td>0.75±0.52</td>
</tr>
<tr>
<td>Plaine Sophie</td>
<td>8</td>
<td>7.1±0.42</td>
<td>0.66±0.16</td>
<td>0.75±0.89</td>
</tr>
</tbody>
</table>
Results indicated that bean was non nodulated in 21 fields out of 44 fields visited and even where the roots were nodulated the nodules present were most of the time not effective at fixing nitrogen. Ineffective nodules were generally small (less than 1.0 mm), numerous and widely distributed over the root system and did not contain any red or pink colouration. Bean plants were generally poorly nodulated in the fields with a maximum score of two (10-50 nodules/plant) in five fields only. Effective nodules were noted in two fields one at Dubreuil and the other one at Petite Riviere. Nodulation was poor even under the optimum pH (pH 6.8) (Munns, 1978) for the Rhizobium/bean symbiosis (Table 2). High soil mineral nitrogen of farmers’ fields can also explain the ineffective nodulation. It has been reported that an optimum level of soil mineral N is required for nitrogen fixation to occur and that excess soil mineral N decreases nodulation and nitrogen fixation (Vencatasamy, 1980, Cao Ngoc Diep et al., 2001). Under conditions of high soil mineral nitrogen inoculation of rhizobia will be useless. Ineffective nodulation can also be due to bean varieties, which are difficult to nodulate. Pacovsky et al. 1984, found that more than 40% of the available electron flow to nitrogenase was used to reduce H\(^+\) to H\(_2\), rather than N\(_2\) to NH\(_4^+\). They concluded that the copious production of \(\text{H}_2\) and the associated energy loss limited the productivity of the bean symbiosis. Thus there is a need to assess nitrogen fixation by different bean cultivars. The need for inoculation will arise where there are no native rhizobia in the soil, the soil mineral N is low and when there is a positive response to fertiliser nitrogen.

**Effect of liming on nodulation of bean (Phaseolus vulgaris cv. Long Tom)**

The effects of liming and nitrogen on nodulation are shown in Table 3. No significant differences in nodulation were noted at all sites. Effective nodulation was observed in one plot at Grande Chartreuse and two plots at Plaine Sophie. At other sites where nodulation was observed, the nodules were tiny, round, white and scattered throughout the secondary roots. The effect of nitrogen levels on nodulation could not be detected because of poor or non nodulation in most trials.

**Table 3  Nodulation status of bean cv. Long Tom**

<table>
<thead>
<tr>
<th>Nodulation score</th>
<th>No Liming</th>
<th>Liming @6.5 cement/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dubreuil/G/Chartreuse</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No nitrogen</td>
<td>1.33</td>
<td>1.33</td>
</tr>
<tr>
<td>Nitrogen @ 25 kg/ha(^1)</td>
<td>1.33</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Plaine Sophie</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No nitrogen</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Nitrogen @ 25 kg/ha(^1)</td>
<td>1.17</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Liming did not have any positive effect on nodulation on the uplands at Dubreuil, Grande Chartreuse and Plaine Sophie. Other factors are affecting nodulation since even under optimum soil pH nodulation was poor.

**Quantification of rhizobia in selected soils of Mauritius using the Most Probable Number-Plant infection technique**

The results of the most probable number counts performed in order to assess the level of indigenous bean rhizobium population in the soils of selected localities of Mauritius are shown in Table 4. It was observed that all the soils examined contained less that 50 cells per gram. This is the minimum soil rhizobial population below which inoculation with appropriate rhizobia is required (Giller, 2001 Personal Communication). Low number of *Rhizobium leguminosarum* bv. *Phaseoli* could be due to various soil factors including pollution by pesticides and fungicides. It thus appears that inoculation is required for bean in most soils. However, there will be a need to rationalize the use of mineral fertilisers and crop protectants if it is intended to introduce rhizobia in soils.
Table 4  Most Probable Number of rhizobia in soils.

<table>
<thead>
<tr>
<th>Locality</th>
<th>MPN (cells/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wooton</td>
<td>4.3 ± 0.98</td>
</tr>
<tr>
<td>Réduit</td>
<td>24.3 ± 6.62</td>
</tr>
<tr>
<td>Richelieu</td>
<td>8.0 ± 2.77</td>
</tr>
<tr>
<td>Flacq</td>
<td>2.0 ± 0.79</td>
</tr>
<tr>
<td>Plaisance</td>
<td>6.0 ± 4.14</td>
</tr>
<tr>
<td>Mapou</td>
<td>13.7 ± 6.86</td>
</tr>
<tr>
<td>Grande Chartreuse</td>
<td>7.0 ± 9.54</td>
</tr>
<tr>
<td>Dubreuil</td>
<td>4.7 ± 2.08</td>
</tr>
<tr>
<td>Plaine Sophie</td>
<td>7.7 ± 5.68</td>
</tr>
<tr>
<td>La Marie</td>
<td>2.3 ± 0.58</td>
</tr>
<tr>
<td>St Martin</td>
<td>11.3 ± 3.57</td>
</tr>
<tr>
<td>Carreau Laliane</td>
<td>6.8 ± 3.67</td>
</tr>
<tr>
<td>Belle Mare</td>
<td>1.2 ± 1.31</td>
</tr>
</tbody>
</table>

CONCLUSION

The status of the French bean/Rhizobium symbiosis, which can potentially fix up to 20 tonnes of atmospheric nitrogen annually in Mauritius was evaluated. It was found to be most of the time ineffective in current field conditions. A complex of factors including soil mineral nitrogen, soil pH, crop varieties, absence of soil rhizobia and possibly pollution by crop protectants could be involved in limiting effective nitrogen fixation by French bean. MPN study revealed that the number of *Rhizobium leguminosarum* bv. *Phaseoli* present in the soil is quite low. This confirms observations made by Pillay and Mamet (1972). Inoculation by appropriate rhizobia is required. However, all other factors will have to be optimised in order to improve biological nitrogen fixation by bean so that this system could contribute positively to reduction of nitrogenous fertilisers. This will be important from both the economic and environmental viewpoint.

ACKNOWLEDGEMENTS

I am grateful to the Food and Agricultural Research Council for partial funding of the project on French bean/legume symbiosis. I am also indebted to the Biometrician of the AREU for his criticisms and advice on data presentation. I also wish to thank Mr P.Hanoomanjee for reviewing the paper. Many thanks also go to the staff of the Agricultural Chemistry Division of the Ministry of Agriculture, Food Technology and Natural Resources for plant and soil analysis.
REFERENCES


EFFECTS OF TWO COMMERCIALEY AVAILABLE COMPOSTS ON SOIL PROPERTIES, AND YIELD AND MINERAL CONTENT OF BEAN (PHASEOLUS VULGARIS)

B. Lalljee

Faculty of Agriculture, University of Mauritius

ABSTRACT

The Government of Mauritius’ agricultural policy, as enunciated in The Non-Sugar Sector Strategic Plan, envisages the diversification and broadening of the agricultural base, and increasing exportation potential of the country into niche markets through high-value-added agriproducts, such as organic products. One of the constraints to the realization of this policy is the present lack/shortage of viable and effective technologies that can be used by organic farmers. Hence it is imperative that locally developed, site-specific technologies be researched, and made available to farmers who are interested in converting to organic cultivation. Such technologies can also be used by conventional farmers to help make agriculture more environment-friendly and sustainable.

The present paper reports one such study that is part of a larger project on the development of environmentally-friendly agrochemicals that can be used in organic agriculture. Three rates of two commercially available composts were evaluated for their effects on soil quality, and yield and mineral content of bean (Phaseolus vulgaris, var. Long Tom), after 2 production cycles, in comparison with soil treated with a synthetic 17:8:25 NPK fertiliser, applied at the recommended rate.

Results showed that both these organic fertilisers significantly improved the following soil characteristics as compared to the synthetic fertiliser: total N (+7%), organic matter (+14.2%), CEC (+22%), available Cu (+5%), Zn (+10%), Fe (+13%), Mn (+9%), pH (1.5 units), electrical conductivity (+9%), respiration rate (+18.5%), total microbial count (+22%), water holding capacity (+28%) and Collembola count (+23%). There was no statistical difference between the two organic fertilisers (i.e. the composts) with respect to the above studied parameters. In potted plant studies, bean plants grown in the organic fertiliser-amended soil showed higher mineral content (+12%), protein content (+8%), ether extract (+4%), Ca (+8%) and P (+6%). On the other hand, dry matter yield of bean plants was 15% higher in the synthetic fertiliser treatment, as compared to the organic treatment. These two organic fertilisers have potential for sustainable crop nutrition and also for sustainable soil management in organic systems.

Keywords: compost, beans, soil physical, chemical and biological properties
INTRODUCTION

The utilization of compost and manure in agriculture is an age old practice. However, during the Green Revolution, the high yielding varieties of seeds used required heavy nutrient input and chemical synthetic fertilisers largely superseded the use of organic materials. The extensive use of synthetic fertilisers is not without problems. In many places, degradation of the environment has been linked to the injudicious use of fertilisers.

There is a paradigm shift in agriculture from extensive agriculture to sustainable agriculture, using an integrated nutrient management approach to crop fertilization, and relying on rational cultural and biological soil fertility methods. The Government of Mauritius is encouraging the use of alternative forms of nutrient supply and promoting Organic Agriculture, as clearly spelt out in the Sugar Sector Strategic Plan (2001) and the Non-Sugar Sector Strategic Plan (2002).

There is an increasing interest in composting of wastes of domestic, factories, agricultural, and industrial (e.g. sugar industry wastes such as vinasse, scum, bagasse, ash, etc.) origins. Mauritius produces 375,000 tonnes of solid waste annually (Budget Speech, 4th April, 2005). Although considered uneconomic in the past, it is now increasingly being recognized that landfill and other waste containment processes bring its own financial and environmental problems. As stated by the Vice-Prime Minister and Minister of Finance during this speech, apart from recycling, composting is the other approach advocated by the Government as a way of reducing the wastes and enabling exploitation of the wastes for commercial and other uses. It is well known that synthetic chemical fertilisers, no doubt, increase the fertility of the soil in terms of nutrient levels, but organic matter, such as compost, manures or other biosolids, not only increases the fertility of soil in terms of nutrient levels, but also improves the physical and biological properties of the soil.

Bar–Tal et al. (2004) showed that various composts, such as from sewage sludge and cattle manure compost increased total dry matter and improved nitrogen, phosphorus and potassium taken up by plants. Furthermore, the quantity of inorganic N increased in soil with increased application of compost. A large number of studies have shown that organic amendments lead to higher soil quality and more soil biological activity (Cardelli et al., 2005). Drinkwater et al. (1995) reported higher pH, organic C and N, mineral N and actinomycetes abundance and density in organically managed fields as compared to conventionally managed fields. Joann et al. (2003) found that water soluble aggregates (< 4 mm) were greater in compost- amended soil than in unamended soils. Furthermore, they also reported that the mean weight diameter (MWD) of aggregates increased with increased rates of compost application. A high level of organic matter maintains structural stability, however, organic matter from different sources differ in their effectiveness. Nemati et al. (2000) reported that the sludge application increased structural stability by 15-17% and decreased bulk density by 4-10%, depending on soil texture, and increased transmission and storage pore sizes and hydraulic conductivity. They further reported that these effects were short lived and therefore annual application is necessary to obtain long-term positive effects on structural stability of the soil.

Fertilisation with compost can result in more organic C and organic N, dehydrogenase activity, biomass and higher dehydrogenase/biomass ratio (Abele, 1976), higher amounts of organic matter, microbial biomass and respiration rates (Goldstein, 1986), higher populations of protozoans and nematodes, and greater respiration rate and enzyme activity (Foissner, 1987), lower bulk density, more total organic C, higher respiration rate, greater amounts of mineral N and a higher ratio of mineral N : C (Reganold, 1988; Reganold et al., 1994) as compared to neighbouring conventionally managed mineral fertiliser plots. The rate of decomposition and release of inorganic elements from compost varies according to the quality of the compost incubated farm compost and cattle manure and found that the inorganic release of nitrogen ranged from 2-11% of the total N content of the manure.

This paper reports the results of an experiment conducted to study the effects of two commercially available composts at 3 rates, on the soil physical, chemical and biological properties and also their effect on dry matter yield of beans.
Effects of two commercially available composts on soil properties, and yield and mineral content of bean (*Phaseolus vulgaris*).

**B Lalljee**

**MATERIALS AND METHODS**

The soil used for this study was a Tropeptic Haplustox (USDA) Low Humic Latosol (Reading) family collected from the University of Mauritius farm. Plastic pots (15 cm diameter and 20 cm height) were filled with 2 kg soil previously amended with 2 commercial composts (Floradur and Compost Sans Souci at rates of 10t/ha, 20t/ha and 30t/ha, and NPK fertiliser at the rate of 50 kg/ha. Required amount of amendments were based on weight of soil rather than on surface area of soil in pots. Each treatment was replicated 3 times and each replicate consisted of 3 pots. Thus, there were 72 pots for the experiment (8 treatments x 3 replicates x 3 pots).

The amended soil was left in the pots for 30 days at field capacity moisture in the greenhouse. Three seeds of bean (*Phaseolus vulgaris* var. Long Tom) obtained from the Agricultural Marketing Board were sown in the pots and at 15 days, these were thinned to 2 seedlings/pot. The plants were watered with deionised /distilled water. Plants were harvested at 90 days and dry matter analyses were conducted with whole plants (without roots) after drying the plants at 72°C for 72 h. in an oven. Chemical and physical and biological properties of the soil were evaluated by standard methods of analyses (FAO, 1980; Rowell, 1993; Jackson, 1976).

pH was determined on a soil : water (1:2.5) suspension using a Phillips electronic pH meter after calibration with standard buffers. Electrical conductivity was determined on a soil: water (1:1.25) solution using a Jenway conductivity bridge. Cation exchange capacity (CEC) was determined by saturating the soil with 1M Ammonium acetate (pH 7) in a leaching tube and washing with 85% ethanol followed by removal of NH$_4^+$ by 1M KCl solution. Total N and organic matter were determined by Kjeldahl method and the Walkley and Black method (Jackson, 1976). Available P was determined by the Truog method. Exchangeable K, Ca, Mg was determined by leaching with 1M ammonium acetate (Jackson, 1976; FAO, 1980).

Extractable Cu, Zn, Fe and Mn were determined by extraction with EDTA ammonium acetate and triethanolamine with a soil : extractant ratio of 1:10 and shaking time of 60 min. (Lalljee, 2005).

Water holding capacity (WHC) was determined by placing a core of the soil on water and calculating the amount of water uptake by capillary action after 24 h of equilibrium time. Bulk density was determined by the core method and Particle density by the Pyrometer method. Porosity was calculated from the values of Bulk density and Particle density (Chopra and Kanwar, 1976).

Total soil biomass was determined by the fumigation technique (Anderson and Ingram, 1993). Respiration rate was calculated by determining CO$_2$ produced after inoculating the soil at field capacity for 1 week (Rowell, 1993). Collembola counts were made after extracting with a Berlese –Tullgren funnel (Cassagne et al., 2003).

**RESULTS AND DISCUSSION**

The chemical properties of the soil after amendment with Floradur (Compost A) and Compost Sans Souci (Compost B) and fertilisers are shown in Table 1, pH increased from 5.3 to 6.9 through addition of compost, whereas the pH increase resulting from addition of synthetic fertiliser was negligible (5.3 to 5.1). Furthermore, the rate of increase was more or less proportionate to the amount of compost added (Table 1). During composting, the C: N ratio narrows due to decomposition. pH has been shown to increase through addition of wastes by several workers (Gineviri et al., 1991; Maynard and Hill, 1994; Shiralipour et al., 1992). pH increase will in turn bring about a change in soil chemistry, for e.g. P, K, Ca, Mg may become more available. Similarly, if there is toxicity due to metals, these will be reduced through the buffering action of organic matter on pH (Von Willert and Stehouwer, 2003). No significant change in the fertiliser-treated soil was observed, probably due to the effect of acidification due to the nitrification of nitrogenous fertilisers.

The EC gives an indication of the concentration of soluble ions in the soil solution. EC depends on charges of these ions as well as the mobility and presence of other ions. Multiple cations carry more charges than univalent cations. The EC increased from 2.4 to 3.0 for the fertiliser treatment and 2.4 to 2.8 for the compost treatment (Table 1). All the fertiliser and compost treatments increased soluble cations in the soil.
CEC increased significantly from 22.1 cM/kg for the fertiliser treated soil to 30.0 cM/kg for the compost-treated soil, an increase of about 37%. This is a very significant contribution to soil fertility, as it increases the soil capacity to retain nutrients, especially cations. Most of the increases in the negative charges are pH-dependent as they are mostly due to dissociation of functional groups such as OH, COOH, C6H5OH, etc. However, since the compost will decompose (mineralise) the increase in CEC will not be a permanent feature. Further additions of compost will be required to maintain the high CEC depending upon the rate of decomposition of the compost. Similar increased in CEC due to addition of compost has also been reported by other workers (Gardiner and Willer, 1998; Lax, 1991).

There was a significant increase in organic matter with addition of compost, being proportional to the amount of compost added (Table 1). However, there was no significant increase in organic matter on addition of the chemical fertilisers. Organic matter has been shown to have beneficial effects on soil, chemical, and physical properties and is reported to be the most reliable index of soil fertility (Hauck, 1982). Compost addition increased total organic C in the soil (Aoyama, 1993), organic matter, which remained for several years (Maynard and Hill, 1994), and dissolved organic carbon (DOC), which in turn increased Ca solubility (Stehouwer et al., 1995; Hue and Licudine, 1999). Clark et al (1988) demonstrated significant improvement in soil chemical properties through increase in organic matter. The increase in organic matter is not a permanent effect. It depends upon the rate of decomposition of the organic matter which in effect also depends on cultural practices, such as tillage, crop rotation as well as soil properties such as texture, structure, porosity, WHC, etc.

Total N, available P and exchangeable K increased significantly with compost and fertiliser addition (Table 1). However, the increase in total N was higher with compost than with chemical fertiliser, and furthermore, was proportionate to the rate of compost applied. There was no significant difference between increase in total N from the addition of the 2 different comports at the 3 rates applied. The N in the chemical fertiliser is mostly in soluble form (NH₄⁺ or NO₃⁻), and this is easily taken up by plants and soil organisms. But it is also very easily leached from the soil. On the other hand, the N in the compost is mostly in the organic form and is mineralised slowly due to microbes, depending on the C:N ratio. Hence the extent of leaching of soluble N is less. Bar-Tal et al. (2004) reported that most of the mineralised N from compost addition remains in the upper few cm. of the soil. The same authors reported that the rate of mineralisation of compost increased with time and that successive applications of compost resulted in P and K accumulation in the soil profile. Bahman and Power (1999) estimated N availability to be 40% from manures, and 15% from compost addition in the first year, which, however, decreased to 18% and 8% respectively in the second year.

In the present study, there was no significant difference in change in available P and exchangeable K between the fertiliser and the compost treatments. Barruzini and Delzan (1992) found that compost increased availability of P, whereas Ginevari et al. (1991) reported that it decreased available P. Availability of P is affected by a complexity of factors including the pH of the soil, phosphorus fixation by organic matter as well as by clay minerals and Fe, Al, Mn, Ca and Mg as insoluble phosphates. Increase in available P may be due to any 1 or more of these factors.

The extractable Cu, Zn, Fe and Mn showed significant increases (about 10-14%) on application of both comports at the 3 rates compared to the chemical fertiliser. The increase is due primarily to the inherent content of these trace elements in the compost and also to changes in soil chemistry, soil biology and biochemistry brought about by the addition of the compost. It is well known that compost addition increases heterotrophic microbial population and this increase leads to an acceleration in the decomposition of the inherent organic matter in the soil as well as in the added compost. Chemical fertilisers are high analysis fertilisers containing very small amounts of impurities and hence trace elements and does not significantly change trace element levels. Compost addition too does not always increase trace element content in the soil. Schnitzer and Skinner (1964) reported that addition of compost led to the formation of a very strong complex of Fe and organic matter, and the Fe became unavailable.
**Table 1** Soil chemical properties following addition of compost and chemical fertilisers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>EC dS/m</th>
<th>CEC cM/kg</th>
<th>Total N %</th>
<th>Organic Matter %</th>
<th>Available P mg/kg</th>
<th>Exchangeable K mg/kg</th>
<th>Extractable Cu mg/kg</th>
<th>Extractable Zn mg/kg</th>
<th>Extractable Fe mg/kg</th>
<th>Extractable Mn mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>6</td>
<td>2.6</td>
<td>29.1</td>
<td>0.16</td>
<td>8.14</td>
<td>58</td>
<td>126</td>
<td>8.2</td>
<td>6</td>
<td>110</td>
<td>106</td>
</tr>
<tr>
<td>Rate 2</td>
<td>6.4</td>
<td>2.7</td>
<td>30.6</td>
<td>0.18</td>
<td>9.22</td>
<td>59</td>
<td>128</td>
<td>8.4</td>
<td>6.1</td>
<td>126</td>
<td>109</td>
</tr>
<tr>
<td>Rate 3</td>
<td>6.6</td>
<td>2.8</td>
<td>31.4</td>
<td>0.2</td>
<td>10.21</td>
<td>59.5</td>
<td>129</td>
<td>8.6</td>
<td>6.4</td>
<td>127</td>
<td>110</td>
</tr>
<tr>
<td>Compost B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>6.3</td>
<td>2.5</td>
<td>28.7</td>
<td>0.17</td>
<td>8.64</td>
<td>60</td>
<td>121</td>
<td>8.3</td>
<td>6</td>
<td>125</td>
<td>103</td>
</tr>
<tr>
<td>Rate 2</td>
<td>6.8</td>
<td>2.7</td>
<td>29.5</td>
<td>0.18</td>
<td>9.16</td>
<td>61</td>
<td>125</td>
<td>8.6</td>
<td>6.3</td>
<td>126</td>
<td>105</td>
</tr>
<tr>
<td>Rate 3</td>
<td>6.9</td>
<td>2.8</td>
<td>31.0</td>
<td>0.19</td>
<td>9.9</td>
<td>63</td>
<td>128</td>
<td>9.1</td>
<td>7</td>
<td>129</td>
<td>107</td>
</tr>
<tr>
<td>Fertiliser</td>
<td>5.1</td>
<td>3</td>
<td>22.1</td>
<td>0.16</td>
<td>8.62</td>
<td>58</td>
<td>124</td>
<td>8.1</td>
<td>5.6</td>
<td>110</td>
<td>98</td>
</tr>
<tr>
<td>Untreated control</td>
<td>5.3</td>
<td>2.4</td>
<td>21.1</td>
<td>0.13</td>
<td>8.69</td>
<td>38</td>
<td>78</td>
<td>6.7</td>
<td>5.8</td>
<td>108</td>
<td>90</td>
</tr>
</tbody>
</table>
Significant improvement in soil physical properties was also observed (Table 2) on addition of the two composts at the 3 rates. There was an improvement of 23% in the WHC of the compost-treated soil as compared to the fertiliser-treated soil (an increase of about 23%). Furthermore, there was a reduction in the bulk density and an increase in the porosity. Soil physical properties are very important factors contributing to overall soil productivity. Guiquiani et al., (1995), Avnimelech et al. (1994), and Shiralipour et al. (1992) reported significant improvement in soil physical properties, namely total porosity and aggregate stability due to addition of compost. The main mechanism by which compost addition in soil influences aggregate stability and porosity is cementation with polysaccharides and other organic compounds (Trisdall and Oades, 1988). Compost is rich in divalent cations, such as Ca and Mg which also help in clay flocculation. Furthermore, gums and hyphae of some organisms help soil particles to bind into crumbs and granules. Miller and Jastrov (1992) reported the additional effects of microorganisms on soil aggregate stability through the binding effect and physical emmeshment by hyphae of some fungi and. Gotaas (1956) reported that aggregation is also brought about by cellulose (cellulose acetate, methyl and ethyl cellulose) resulting from bacterial metabolism. Donahue (1961) reported that organic matter addition leads to an increase in earthworms, which burrows into the soil, and the passage thus created lowers the bulk density of the soil and increase porosity and permeability. Tester (1990) also showed that increase in total porosity and aggregate stability results in a reduction in bulk density which leads to easier seed germination, root penetration and percolation of water, while Boyle et al. (1989) showed that compost also leads to a change in pore size distribution which in turn leads to easier down ward flow of excess water. Avnimelech et al. (1994) reported a linear relationship between organic carbon in the soil and the soil moisture content. This type of relationship was also obtained in the present study, where WHC increased proportionately to increase in level of compost added. Several other workers (Gotaas, 1956; Flaig, 1975; Shiralipour et al., 1992; Maynard and Hill, 1994) have reported increase in WHC with compost addition.

Table 2 Soil physical properties following addition of compost and chemical fertiliser

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WHC %</th>
<th>Bulk density x103 kg/m3</th>
<th>Particle Density x103 kg/m3</th>
<th>Porosity %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compost A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>37</td>
<td>0.94</td>
<td>2.43</td>
<td>61.3</td>
</tr>
<tr>
<td>Rate 2</td>
<td>39</td>
<td>0.92</td>
<td>2.41</td>
<td>61.8</td>
</tr>
<tr>
<td>Rate 3</td>
<td>42</td>
<td>0.9</td>
<td>2.39</td>
<td>62.3</td>
</tr>
<tr>
<td><strong>Compost B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>37</td>
<td>0.95</td>
<td>2.45</td>
<td>61.2</td>
</tr>
<tr>
<td>Rate 2</td>
<td>38</td>
<td>0.93</td>
<td>2.43</td>
<td>61.7</td>
</tr>
<tr>
<td>Rate 3</td>
<td>40</td>
<td>0.91</td>
<td>2.39</td>
<td>62</td>
</tr>
<tr>
<td>Fertiliser</td>
<td>32</td>
<td>1.1</td>
<td>2.51</td>
<td>55.8</td>
</tr>
<tr>
<td>Untreated control</td>
<td>30</td>
<td>1.06</td>
<td>2.53</td>
<td>58</td>
</tr>
</tbody>
</table>

The biological characteristics studied, namely soil biomass C, respiration rate and Collembola population, all increased significantly due to the addition of compost (Table 3). As with the other parameters observed, the increase was more or less proportionate to the amount of compost added. In the fertiliser treatment, no such difference in these properties was observed in comparison with the untreated control. The populations of various soil organisms are linked functionally through their roles in the degradation of various forms of organic materials. Microorganisms play a fundamental role in establishing biochemical cycles in the soil (Harris and Birch, 1989), in the retention, release and recycling of plant nutrients (Paul and Varoney, 1989). Gardiner and Willer (1988) reported that soil microorganisms improved soil structure and secreted polysaccharides and other cementing agents, which helped, bind soil particles.
Composts also help inhibit undesirable microorganisms such as soil borne pathogens (Ozones-Hampton et al., 1994). According to Goldstein (1998), disease control with compost is attributable to 4 main mechanisms:
1. Competition for nutrients among pathogens and beneficial microorganisms;
2. Antibiotic production by microorganisms in the compost;
3. Predation and parasitism; and
4. Systemic resistance induced in plants in response to compost.

Table 3 Soil biological properties following addition of compost and chemical fertilisers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total soil biomass mg/g soil</th>
<th>Respiration rate x 10-9 g CO2/g soil/sec</th>
<th>Collembola counts Nos./pot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>322</td>
<td>3.21</td>
<td>590</td>
</tr>
<tr>
<td>Rate 2</td>
<td>350</td>
<td>3.51</td>
<td>640</td>
</tr>
<tr>
<td>Rate 3</td>
<td>378</td>
<td>3.8</td>
<td>690</td>
</tr>
<tr>
<td>Compost B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>258</td>
<td>3.01</td>
<td>640</td>
</tr>
<tr>
<td>Rate 2</td>
<td>310</td>
<td>3.33</td>
<td>680</td>
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<tr>
<td>Rate 3</td>
<td>390</td>
<td>3.92</td>
<td>710</td>
</tr>
<tr>
<td>Fertiliser</td>
<td>190</td>
<td>1.98</td>
<td>36</td>
</tr>
<tr>
<td>Untreated control</td>
<td>195</td>
<td>2</td>
<td>41</td>
</tr>
</tbody>
</table>

Yepsen (1976) reported that the increase in respiration that results from the addition of compost also helps to destroy some pathogenic organisms and toxic compounds. Microarthropods, with their numerous species and huge numbers of individuals, occupy a wide range of ecological niches, which is a good representation of soil biodiversity. The mesofauna present in a habitat depend upon many factors, including pH (Loranger et al., 2001), pedoclimate (Seastedt and Crossley, 1981), aeration (Vreeken-Buijs et al., 1998), organic matter composition (Merila and Ohtohen, 1997), nutrient availability (Bird et al., 2000), humus type (Theenhaus and Schaefer, 1995). A large proportion of this fauna plays an important part in the decomposition of organic matter and nutrient cycling (Faber, 1992). Soil arthropods such as Collembola are considered useful bioindicators of change in soil quality (Detsis et al., 2000). The present study showed that all the 3 bioindicators improved significantly through compost addition and therefore had positive consequences on soil quality and support previous findings of many workers (Fraser et al., 1988; Angers et al., 1995).

Dry matter yield of beans increased significantly following addition of chemical fertiliser as well as compost (Table 4). The other plant parameters analysed, namely fat percentage (ether extract), Ca, P did not show any significant difference between the fertiliser and the compost, but there was significant difference between each treatment compared to the untreated control. The increase in yield as well as the other plant parameters, such as fat percentage, Ca and P may not all be due directly to the content of these in the compost. The compost may indirectly affect yield and quality by neutralizing certain toxics, such as Al\(^{3+}\) by surface chelation (Mortense, 1963) or they can chelate some trace elements making them more soluble and therefore more available (Gardiner and Willer, 1998). Wolkowski (1996) reported that compost always increased levels of plant nutrients and increased growth and yield above untreated control, but that the highest yields were obtained when recommended fertilisers were applied. Gotass (1956) found that organic acids from metabolic breakdown of organic materials form a complex with phosphates and makes them more available to plants. Robinson (1983) reported that compost addition increased yield of green bean.
Effects of two commercially available composites on soil properties, and yield and mineral content of bean (Phaseolus vulgaris). B Lalljee

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry matter yield %</th>
<th>Fat content (ether extract) %</th>
<th>Ca %</th>
<th>P %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>26.4</td>
<td>1</td>
<td>0.27</td>
<td>0.05</td>
</tr>
<tr>
<td>Rate 2</td>
<td>28.7</td>
<td>1.06</td>
<td>0.28</td>
<td>0.07</td>
</tr>
<tr>
<td>Rate 3</td>
<td>32.6</td>
<td>1.21</td>
<td>0.31</td>
<td>0.09</td>
</tr>
<tr>
<td>Compost B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate 1</td>
<td>24.1</td>
<td>1.11</td>
<td>0.25</td>
<td>0.06</td>
</tr>
<tr>
<td>Rate 2</td>
<td>25</td>
<td>1.23</td>
<td>0.29</td>
<td>0.08</td>
</tr>
<tr>
<td>Rate 3</td>
<td>29.3</td>
<td>1.25</td>
<td>0.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Fertiliser</td>
<td>26.4</td>
<td>1.01</td>
<td>0.28</td>
<td>0.09</td>
</tr>
<tr>
<td>Untreated control</td>
<td>18.2</td>
<td>1</td>
<td>0.23</td>
<td>0.06</td>
</tr>
</tbody>
</table>

CONCLUSION

The 2 composts tested, namely Floradur, and Compost SansSouci, significantly improved soil physical, chemical and biological properties and also improved dry matter yield and quality of Phaseolus vulgaris. The two composts were generally better in improving soil properties than the chemical fertiliser. However, the quality and dry matter yield was not significantly different among the various treatments.

The findings in this study are in agreement with other studies carried out on the beneficial effects of compost on soil and crop quality.

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Effects of two commercially available composts on soil properties, and yield and mineral content of bean (*Phaseolus vulgaris*).

*B Lalljee*


Effects of two commercially available composites on soil properties, and yield and mineral content of bean (Phaseolus vulgaris).

B Lalljee


ROBINSON RG. 1983. Yield and composition of field bean and Adzaki bean in response to irrigation compost and nitrogen. Agronomical J 75(1) : 31-34.
Effects of two commercially available composts on soil properties, and yield and mineral content of bean (*Phaseolus vulgaris*).


THE DEVELOPMENT OF A DROUGHT-TOLERANT MAIZE VARIETY FOR RODRIGUES

N. Govinden

Mauritius Sugar Industry Research Institute

ABSTRACT

Maize is still a very important crop in Rodrigues although its production has regressed dramatically in the past decade. The regression is due to several causes including low yield, which is itself due in part to drought. A research project was therefore undertaken from 1998 to 2003 to breed a drought-tolerant variety.

The procedure involved first hybridising MSIRI 3B, the most widely planted variety, with a drought-tolerant donor from the International Maize and Wheat Improvement Centre (CIMMYT). This was followed by four cycles of selection and backcrossing to MSIRI 3B, the recurrent parent, to recover its useful traits such as preferred kernel characteristics and resistance to Maize Streak Virus. The population was grown under controlled drought in plastic rain-out shelters, and selection was practised for short anthesis-silking intervals (ASI), a proxy for drought tolerance. Four cycles of mass-selection then followed in order to recombine and concentrate drought tolerance genes. Thus, after eight cycles of crossing and selection, a new population was developed. It has been named MSIRI 3C.

MSIRI 3C was evaluated in replicated yield trials in Mauritius and Rodrigues. In the absence of drought in the rainy season in Mauritius and Rodrigues, MSIRI 3C was marginally higher in yield than MSIRI 3B, by an average of 14% in 6 trials. Under severe drought conditions in Mauritius, the yield of MSIRI 3C was better than that of MSIRI 3B by 125% in one trial and by 74% in another. In the latter trial its yield was not significantly different from some of the best drought-tolerant varieties from CIMMYT. All the 13 field trials laid down in Rodrigues in the 2003 main planting season to evaluate drought tolerance were destroyed by a cyclone and no results were obtained. More trials were planted in 2004 but the results were compromised by rain.

Two indirect consequences of selection have been observed in the new variety. Firstly, under drought, but not under humid conditions, MSIRI 3C matures slightly earlier, by about one week, than MSIRI 3B. Secondly, it has a large proportion of stay-green plants. At ear maturity, such plants have several green leaves. They are useful in areas such as Rodrigues where growers often feed maize stover to livestock. The kernels of MSIRI 3C are orange-yellow and flint, as is preferred in Rodrigues. Seeds were produced and distributed to growers at the end of 2003 and at the beginning of 2004. Early reports indicate good acceptance of the variety by growers in Rodrigues.

Keywords: Anthesis-Silking Interval, Backcrossing, Mass selection

INTRODUCTION

Maize is still an important crop in Rodrigues although its production has declined steadily during the past two decades. In a drive to arrest this decline the Agricultural Services conducted a producer survey in 1995 in order to identify the causes of the decline, to prioritise the problems of growers and to seek appropriate solutions. Growers rated drought as the most important constraint to production (Govinden et al, 1998a).

Drought occurs on maize in Rodrigues because rainfall is insufficient and is poorly distributed. Sixty percent of the mean island-wide annual rainfall of about 1200 mm falls in the first five months from January to May (Padya, 1989). This first and main planting season corresponds to the cyclonic season. If the crop is planted early to avoid drought, the risk of cyclone damage increases. Conversely, if it is planted late to avoid cyclones, then drought is inevitable as from flowering when its effect on yield is
The Development of a Drought-Tolerant Maize Variety for Rodrigues. N Govinden

more marked. In the second planting season starting in May, rainfall is inadequate for maize and other annual crops except in the more humid areas with 1600 mm or more rainfall. Even there, late season drought is the rule.

Since there is no water to irrigate maize, the best approach to minimize the risk of drought is to grow drought-tolerant varieties. In a workshop following the 1995 survey, growers ranked the development of drought-tolerant varieties as the most appropriate solution to their problem, and indicated that the availability of such varieties was a precondition to increasing production (Govinden et al., 1998b). They solicited the help of the Mauritius Sugar Industry Research Institute (MSIRI). This paper reports the outcome of a project to develop an acceptable drought-tolerant maize variety for Rodrigues.

MATERIALS AND METHODS

Physiological basis of selection

Severe drought at flowering causes maize plants to become barren through an effect on the female organs. Tassel emergence, pollen shed and pollen fertility are much less affected that silk extrusion and ear and ovule development. Under normal field conditions the plants which produce an ear under drought at flowering can be either truly drought-tolerant plants or escapes, and it is not possible to distinguish one type from the other. It is therefore necessary to manage the drought stress carefully in order to minimize escapes. Even then, selection for yield per se is not as effective as selection using an index comprising yield and certain secondary traits (Fisher et al, 1989). The International Maize and Wheat Improvement Centre (CIMMYT) had used such an index to develop drought-tolerant source populations. Contrary to expectations, several secondary traits associated with improved plant water status under moisture stress, such as leaf elongation rate, leaf temperature and canopy senescence, did not show good responses to selection for drought tolerance (Bolanos et al., 1993). Of the secondary traits which were correlated with yield under water-stress, the best was reduced anthesis-silking interval (ASI). ASI is defined as the time in days between 50% silking (silk is visible on half of the plants) and 50% anthesis (half of the plants have started to shed pollen). For instance, Bolanos and Edmeades (1993) observed that grain yield decreased by 90 percent with an increase in ASI from –0.4 day to 10 days. They recommended selection for short ASI as an effective and rapid method to higher yield under water stress. An important advantage of selecting for short ASI compared to other characters such as kernel number, for instance, is that ASI can be seen at flowering. Hence, one cycle of selection can be completed in one season.

The breeding approach

The actual breeding was done in two phases, each of two years’ duration at the rate of two cycles of selection per year: first, backcrossing; then, mass selection.

Backcrossing was used to transfer drought tolerance from a donor from CIMMYT - population Tuxpeno Sequia C6 – to the recurrent parent – MSIRI 3B – which is the most widely planted maize variety in Rodrigues. The method aims at transferring one or a few traits while retaining the essential characteristics of the recurrent parent.

The two populations were first hybridised. Then, selection was practised for short ASI in the F2 progeny. Selected plants were pollinated with bulk pollen collected from MSIRI 3B. The same procedure was repeated in each cycle. Four cycles of backcrossing were completed in 1999 and 2000, by which time the resultant population was expected to be essentially similar to MSIRI 3B in all characters except drought tolerance.

The objective of the next four cycles of mass selection in 2001 and 2002 was to recombine and concentrate the genes for drought tolerance.

The resultant population was evaluated in field trials in 2002, 2003 and 2004.
Selection procedures

The selection plots were planted at Réduit where rainfall is too high in the first season, March to June, for drought-tolerant plants to be identified under natural conditions. Even in the second season, July to October, there are risks of one or more heavy showers during the crop cycle. Consequently, the crops were covered with rain-out shelters. These consisted of a plastic roof over a structure of metallic tubes. Each shelter covered 8 rows of 30 maize plants. Nine shelters were used to protect a total population of 2160 plants. The crop was planted at the usual density of about 60 000 plants per hectare. In the absence of rain, it was irrigated at fortnightly intervals as from sowing. The shelters were placed in the field one month after planting. As from the emergence of the first tassels at about 7 weeks in the first season and 8 weeks in the second season, the crop was inspected daily and plants with short ASI were selected. Their silk and tassel were covered with paper bags. Selection intensity varied from 10 to 15 percent depending on the level of drought stress. In the first week of selection when drought stress was still mild, only plants with ASI of 0 or 1 day were kept. As drought developed, plants with ASI of 2 days were also retained.

Several precautions were taken to ensure good results. First, small canals were dug around the shelters in order to drain rain water away from the selection plots. Secondly, selection was pursued for three weeks in order to avoid retaining early-maturing genotypes only. Thirdly, at each cycle of selection, a minimum of 200 plants were kept in order to avoid genetic drift in the population. Fourthly, in order to avoid inbreeding, pollen was collected from one group of selected plants, for instance in one shelter, bulked, and used to pollinate another group of plants. No plant was selfed. And finally, to maximize seed production, each silk was pollinated at least twice at 3-day intervals. To this effect, tassels which had been removed from the plants were conserved in jars of water in the laboratory, and pollen shed during the night was used the following morning.

The silk covers were left in place after pollination. When selection was completed, the developing ears were covered with plastic bags to protect them from rain and birds, and the selection plot was rewatered.

After harvest, the ears were hand-shelled individually and an equal number of kernels from each – normally thirty – was mixed to constitute the new population.

In the first four selection cycles (the backcrossing phase), selection was practised solely for short ASI. But, in the subsequent mass-selection cycles, negative selection was also made on kernel type. After harvest, ears with dent kernels were rejected. After shelling, white and yellow kernels were discarded before reconstituting the population.

Evaluation of the new population

In order to check that selection was being effective, evaluation of yield started in the first season of 2002 with an intermediate population (BC\textsubscript{4}S\textsubscript{2}) obtained after the second mass selection cycle. A small yield trial was planted at Réduit. Plots consisted of 2 rows of 15 plants replicated 12 times. Seeds were also sent to Rodrigues for trials in growers’ fields.

The evaluation was pursued at Pamplemousses in the second season of 2002, also with an intermediate population, this time the BC\textsubscript{4}S\textsubscript{3}. Plots consisted of 2 rows of 40 plants replicated 6 times.

Evaluation of the final population, renamed MSIRI 3C, started in the first season of 2003. In one trial planted at Pamplemousses, the plots consisted of 2 rows of 40 plants replicated 6 times. In 13 trials planted in Rodrigues on growers’ fields, the plots consisted of 4 rows of 30 plants replicated 4 times.

Two more trials were planted in the second season of 2003, one at Pamplemousses and one at Belle Rive. This time, in addition to the control variety MSIRI 3B, the trials included eight varieties of different maturity and with variable levels of drought tolerance obtained from CIMMYT. Plots consisted of 4 rows of 30 plants replicated 4 times. Maturity was assessed as number of days to 50% anthesis and 50% silking from which the anthesis – silking interval (ASI) was calculated. The final evaluation was made in the first season of 2004 at Pamplemousses, Belle Rive and Rodrigues.
All the evaluation trials were done under rainfed conditions, except at Pamplemousses where the field was irrigated at planting to promote uniform germination. Normal cultural practices were followed.

RESULTS AND DISCUSSION

The final mass selection cycle was completed at the end of 2002, and the new population (BC$_4$S$_4$) was named MSIRI 3C.

Maturity and Anthesis-Silking Interval (ASI)

The varieties obtained from CIMMYT belong to four maturity groups as seen from the days to 50% anthesis and 50% silking at Pamplemousses and Belle Rive in the second season of 2003 (Table 1). They ranged from very early (ZM 305), through early (400 series) and intermediate (500 series) to late (600 series). Both MSIRI 3B and MSIRI 3C were as early-maturing as the CIMMYT 400 series.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Pamplemousses</th>
<th>Belle Rive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anthesis</td>
<td>Silking</td>
</tr>
<tr>
<td>ZM 305</td>
<td>63.0</td>
<td>67.8</td>
</tr>
<tr>
<td>ZM 421</td>
<td>68.0</td>
<td>72.8</td>
</tr>
<tr>
<td>ZM 423</td>
<td>67.0</td>
<td>71.3</td>
</tr>
<tr>
<td>ZM 521</td>
<td>67.5</td>
<td>73.8</td>
</tr>
<tr>
<td>ZM 523</td>
<td>69.5</td>
<td>76.5</td>
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<tr>
<td>ZM 611</td>
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<tr>
<td>MSIRI 3C</td>
<td>66.8</td>
<td>71.3</td>
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</tbody>
</table>

The season was humid at Belle Rive and the reaction to drought could not be assessed. At Pamplemousses, however, drought stress occurred, and the ASI of the varieties gave an indication of the reaction to drought. All the CIMMYT varieties rated early or intermediate had similar ASI at Pamplemousses under drought as at Belle Rive under humid conditions. In contrast, the ASI of two of the late-maturing varieties (ZM 621 and ZM 623) increased under drought. Since drought stress increased as the season progressed, the early varieties flowered before drought stress became severe enough to affect flowering while the late-maturing varieties experienced severe drought stress before flowering and hence, their ASI was lengthened. The control variety (MSIRI 3B) and the test variety (MSIRI 3C) had contrasting reactions to drought. Under humid conditions at Belle Rive, they flowered at the same time and they had similar ASI. This shows that selection had had no effect on maturity. Under drought at Pamplemousses, flowering was delayed slightly in MSIRI 3B and, more importantly, the ASI of MSIRI 3B increased considerably whereas that of MSIRI 3C barely changed. This shows that selection for short ASI had been effective in MSIRI 3C. The variety may be expected to tolerate drought stress rather than avoid it through earliness.

Yield under humid conditions

The evaluation trials were planted under rainfed conditions, but rain interfered with the evaluation of drought tolerance and, in the end, the conditions were humid.

In 6 trials completed to date, MSIRI 3C was marginally (+14%) higher than MSIRI 3B (Table 2). This is an important result because, as is well-known, planters do not normally adopt new drought-tolerant varieties unless they yield at least as well as their own local varieties in the absence of drought.
The Development of a Drought-Tolerant Maize Variety for Rodrigues. N Govinden

Table 2  Grain yield of maize varieties MSIRI 3B and MSIRI 3C in trials under humid conditions

<table>
<thead>
<tr>
<th>Location</th>
<th>MSIRI 3B</th>
<th>MSIRI 3C</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Réduit, 1st season 2002</td>
<td>4.70</td>
<td>5.17</td>
<td>+10</td>
</tr>
<tr>
<td>Pamplemousses, 1st season 2003</td>
<td>3.44</td>
<td>4.44</td>
<td>+29</td>
</tr>
<tr>
<td>Belle Rive, 2nd season 2003</td>
<td>3.07</td>
<td>3.24</td>
<td>+6</td>
</tr>
<tr>
<td>Pamplemousses, 1st season 2004</td>
<td>4.53</td>
<td>5.87</td>
<td>+30</td>
</tr>
<tr>
<td>Reposoir, 1st season 2004</td>
<td>4.98</td>
<td>5.53</td>
<td>+11</td>
</tr>
<tr>
<td>Caverne, 1st season 2004</td>
<td>3.87</td>
<td>3.77</td>
<td>-3</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>4.10</td>
<td>4.67</td>
<td>+14</td>
</tr>
</tbody>
</table>

The CIMMYT varieties were present in two of these trials. Several of them were better than MSIRI 3C, especially at Belle Rive (Table 3). Their superiority under humid conditions may be attributed to better standability, the trial at Belle Rive having experienced a small cyclone during which the local varieties lodged slightly. At Pamplemousses, the best CIMMYT varieties were only marginally superior to MSIRI 3C.

Yield under drought stress

No results have been obtained in the trials planted in Rodrigues in 2002 because of rain and in the 13 trials planted in 2003 because of cyclone Kalunde. To date, drought occurred in only two trials, both at Pamplemousses in the second season. In the first, MSIRI 3B gave 0.58 t ha⁻¹ while MSIRI 3C yielded 1.31 t ha⁻¹, that is, 125% higher. In the second, the yield of MSIRI 3C was higher than that of MSIRI 3B by 74% (Table 3). This last trial included the CIMMYT drought-tolerant varieties. The yield of MSIRI 3C was better than that of several of these varieties and not significantly different from that of the best.

Table 3  Grain yield (t ha⁻¹ at 14% m.c) of maize varieties under contrasting humid and dry conditions

<table>
<thead>
<tr>
<th>Variety</th>
<th>Humid</th>
<th>Dry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Belle Rive 2003</td>
<td>Pamplemousses 2004</td>
</tr>
<tr>
<td>ZM 305</td>
<td>4.31</td>
<td>6.29</td>
</tr>
<tr>
<td>ZM 421</td>
<td>3.49</td>
<td>5.85</td>
</tr>
<tr>
<td>ZM 423</td>
<td>3.99</td>
<td>6.55</td>
</tr>
<tr>
<td>ZM 521</td>
<td>4.82</td>
<td>6.45</td>
</tr>
<tr>
<td>ZM 523</td>
<td>4.00</td>
<td>5.90</td>
</tr>
<tr>
<td>ZM 611</td>
<td>4.45</td>
<td>5.98</td>
</tr>
<tr>
<td>ZM 621</td>
<td>3.69</td>
<td>5.55</td>
</tr>
<tr>
<td>ZM 623</td>
<td>4.18</td>
<td>-</td>
</tr>
<tr>
<td>MSIRI 3B</td>
<td>3.07</td>
<td>4.53</td>
</tr>
<tr>
<td>MSIRI 3C</td>
<td>3.24</td>
<td>5.87</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td>0.18</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Grain type and other characteristics of MSIRI 3C

Since growers in Rodrigues have a strong preference for orange-yellow flint kernels, on one hand, and the CIMMYT drought-tolerant donor population was a white dent, attention was paid to kernel characteristics in the final stages of selection. After selection had been completed, the population of MSIRI 3C was observed to have the following undesirable kernel types: white kernels: 0.4%; ears with essentially yellow kernels: 2.9%; ears with essentially dent kernels: 0.7%. Although these are not of much consequence and are unlikely to deter the adoption of the variety, some further negative selection was practised in the first and second seed multiplications. The variety should now conform quite closely to the preference of Rodriguan growers.

The Development of a Drought-Tolerant Maize Variety for Rodrigues. N Govinden

MSIRI 3C has been observed during seed multiplication to have a large proportion of stay-green plants. At physiological maturity, such plants have several green leaves. They are valuable in countries such as Rodrigues where producers often use maize stover as fodder.

CONCLUSION

The project to develop an acceptable drought-tolerant variety for use in Rodrigues was completed after 5 years at the end of 2002 with the release of MSIRI 3C. Seeds were produced by MSIRI and distributed to about 60 growers in December 2003. More seeds were produced and distributed by the Agricultural Services in February 2004. Thus, many growers planted MSIRI 3C in the main planting season of 2004. The Agricultural Services reported good acceptance. Kernel type, in particular, was acceptable, and even stay-greenness was noted. However, the 2004 season was humid, and the reports of good maize yields and higher production, better than in the past decade, should not be attributed to the new variety. Its impact will only be observed under dry conditions. The Agricultural Services should therefore continue evaluating the variety.

The backcross breeding procedure used in the development MSIRI 3C should have conserved the Maize Streak Virus (MSV) resistance of MSIRI 3B. But this has not been demonstrated yet because the incidence of the disease is now too low in Rodrigues. An attempt will be made in 2005 under artificial MSV disease conditions. Seeds have also been sent to CIMMYT – Harare for multiplication and distribution to collaborators in the SADC maize network. They will evaluate the variety simultaneously for drought-tolerance and MSV-resistance.

ACKNOWLEDGEMENTS

Thanks are presented to the Food and Agricultural Research Council (FARC) for partial financial support. The collaboration of the Agricultural Services of Rodrigues in the evaluation of MSIRI 3C is acknowledged.

REFERENCES


THE IMPACT OF NATURAL POLLINATOR, *APIS MELLIFERA* LATREILLE ON ONION SEED PRODUCTION IN MAURITIUS

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¹Ministry of Agriculture Food Technology and Natural Resources  
²Agricultural Research and Extension Unit

ABSTRACT

About 330 hectares of land are under onion plantation every year and most of the seeds are imported from either South Africa or European Countries. Some growers produce seeds of “Local Red” variety and their yield varies from 600 to 800 kg per hectare. In this study, young broods of the honeybee, *Aphis mellifera linguistaca* (Italian) were allowed to forage on flowering onion plants and seed yield determined.

The study was conducted at four main onion growing sites, Crève Coeur, Plaine Sophie, Palmar and La Chaumière from July to October 2002. In each site, three types of treatments were set on “Local Red” variety. The first treatment (T1) consisted of caged onion plot with young broods of *A. mellifera*, the second (T2) was caged plot without *A. mellifera* and the third (T3) with uncaged plot.

In the first treatment, a beehive consisting of about 1500 young broods was introduced in caged plot at flowering stage for twelve weeks. Among the treatments, the seed yield in caged plot with *A. mellifera* was significantly highest and estimated to be about 1595.8 kg per hectare. The average yield in caged plot without *A. mellifera*, and uncaged one was 612.0 kg and 1337.8 kg per hectare respectively. There had been an increase in seed yield in caged plot with *A. mellifera* by 43.4 % at Palmar, 38.4 % at Crève Coeur, 19.3 % at Plaine Sophie and 60.4 % at La Chaumière. The results show that young broods of *A. mellifera* are potential pollinators of onion flowers. Beehives, when placed at the start of flowering of onion plants can assure maximum pollination and hence increase seed production.

Keywords: Onion, *Aphis mellifera*, seed production

INTRODUCTION

Onion (*Allium cepa*) is the most important condiment in the Mauritian diet with an average consumption of some 16500 tonnes annually. About 8000 tonnes are produced locally (CSO, 2001). In Mauritius, onion is produced from seeds that are either produced locally or imported from South Africa and Europe. The main local variety, known as “Local Red or oignon Mars”, is produced from seed-to-bulb in the first year and from bulb-to-seed in the second, whereas all hybrid varieties are imported and are grown from seed to bulb with a crop cycle of 5 to 6 months.

Seed availability has always been a major constraint in local production. Very often seeds are not available at required quantities and this hinders overall onion production. There are a few growers who produce seeds by traditional methods, with a yield of about 600 to 800 kg of seeds per hectare.

During the past two decades, research has been conducted to improve cultivars and standardize production technologies primarily for bulb production. So far, research has been focussed round agronomic aspects such as fertiliser requirements, spacing, time of planting, and disease and pest management. Research work is actually being carried out by the Agricultural Research Extension Unit (AREU) of the Ministry of Agriculture to perform artificial vernalisation for the improvement of production of seeds. Nevertheless, little emphasis is being laid on seed production technology.

Studies have been carried out worldwide on pollination of different food crops by the honeybee (*A. mellifera*), but very little has been done on onion. Benedex and Martinovich (1979-1980) worked on the structure and density of pollination insect population in onion seed fields in Hungary.
The impact of natural pollinator, *Apis mellifera* Latreille on onion seed production in Mauritius. MA Bhunoo and D Abeeluck

They found that the density of insect population increased as the number of flowers reaches full bloom whilst, at the same time, natural pollinators were insufficient. *Apis mellifera* was introduced at the edges of fields to increase the number of pollinators hence to increase seed production. Chang (1980) studied the flowering behaviour and pollination in onion in Taiwan. In the ex-USSR, Skrebtsov and Skrebtsova (1980) identified twenty-one (21) insect species on flowering onion crops in the Volgo Aklubinsk including pollinators but also predators of insects. *Apis mellifera* constituted 5 to 7% of all insects and more hives were thus introduced to improve pollination.

Southwick et al. (1981) have demonstrated that bee visitation rates increased in flower patches with increasing number of nectar bearing flowers, nectar volume and sugar concentration of nectar. Woyke (1982) carried out research on onion pollination, and good seed set and high seed yields were obtained on commercial onion fields at 2 sites in Poland provided with 6.7 and 2.0 colonies of *A. mellifera* per hectare respectively. In Poland, Wojtowski et al. (1982) have concluded that the commonest pollinator on carrot and onion fields was *A. mellifera*. In Mauritius (Abeeluck, D, pers. comm., 2000) had successfully induced pollination on melon grown under protected cultivation with young broods of *A. mellifera*.

This paper describes the first attempt at investigation on the effect of *A. mellifera* on onion seed formation and ultimately on seed yield.

**MATERIALS AND METHODS**

The study was carried out in four onion growing regions, namely Creve Coeur, Palmar, Plaine Sophie and La Chaumière (Table 1).

<table>
<thead>
<tr>
<th>Locality</th>
<th>Relief height Above sea level</th>
<th>Rainfall annually</th>
<th>Average Max. Temp.</th>
<th>Average Min. Temp.</th>
<th>Type of Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creève-Coeur</td>
<td>≤ 400 m</td>
<td>&lt;2500mm</td>
<td>23º - 25ºC</td>
<td>16º - 20ºC</td>
<td>Low land Humic/clay</td>
</tr>
<tr>
<td>Palmar</td>
<td>≈ 1-2m</td>
<td>≤ 1700mm</td>
<td>25º - 26ºC</td>
<td>18º - 21ºC</td>
<td>Sandy</td>
</tr>
<tr>
<td>Plaine Sophie</td>
<td>&lt; 500 m</td>
<td>≤ 3000mm</td>
<td>22º - 23ºC</td>
<td>15º - 18ºC</td>
<td>Latosolic brown prarie</td>
</tr>
<tr>
<td>La Chaumière</td>
<td>&lt;200m</td>
<td>≥ 900mm</td>
<td>25º - 26ºC</td>
<td>20º - 22ºC</td>
<td>Low land Humic/clay</td>
</tr>
</tbody>
</table>

**Setting onion beds**

At each site, land (0.125 hectare) was ploughed with a small power tiller to a depth of about 30 cm. Nine beds of 5 m² (1m by 5m long) were prepared. The beds were raised about 10 cm to provide good water drainage. Fertilisation was made as per recommendation.

Onion sets from the main cultivars; Local Red (March sets) produced from seedlings, were used. The sets were planted in drills on prepared beds, 2 cm deep in rows 10 cm apart at a rate of 2 kg per plot (5 m²). Planting was done during April 2002. After 8 days, the sets started to germinate. Irrigation, weed insect pest and disease control were effected as per standard practice.

**Treatment Allocation**

Three replicates of three consecutive planting beds (5m²) each were selected at random from the planting field in each selected site (Figure 1).

Complete Random Block Design was used in each locality. The allocation of the different treatments (T1, T2, T3) per respective Plot/Bed (A, B, C) was randomly distributed in each Replicate (R I, R II, R III) as shown in Figure 1.
The impact of natural pollinator, *Apis mellifera* Latreille on onion seed production in Mauritius. MA Bhunnoo and D Abeeluck

**Figure 1** Layout of treatments (T1, T2 and T3) per plot/bed (A, B, C) allocated in each region.

<table>
<thead>
<tr>
<th>Replicate I</th>
<th>Replicate II</th>
<th>Replicate III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot/Bed -A</td>
<td>Plot/Bed -B</td>
<td>Plot/ Bed -C</td>
</tr>
<tr>
<td>Cage without bees T3</td>
<td>Control without cage T2</td>
<td>Cage with bees T1</td>
</tr>
<tr>
<td>Plot/Bed -B</td>
<td>Plot/Bed -C</td>
<td>Plot/ Bed -A</td>
</tr>
<tr>
<td>Control without cage T2</td>
<td>Cage with bees T1</td>
<td>Cage without bees T3</td>
</tr>
<tr>
<td>Plot/Bed -C</td>
<td>Plot/Bed -A</td>
<td>Plot/Bed -B</td>
</tr>
<tr>
<td>Cage with bees T1</td>
<td>Cage without bees T3</td>
<td></td>
</tr>
</tbody>
</table>

**Caging**

Cages were constructed with wood and galvanised water pipe and covered with “Sarlon cloth”. One bed from each replicate was caged on the first day of flowering (July 2002) for 12 weeks when the flowers reached maturity and started drying up.

**Placement of Honeybees in caged plot**

In each replicate, a beehive consisting of about 1500 young broods was introduced in one of the caged plots at flowering stage for 12 weeks. Transfer of beehives was made at sunset. Hives were examined every week to decide to check whether supplement feeding (2 parts of sugar and 1 part water) was required to prevent bees from starving.

**Onion Seed Harvesting**

Seeds were harvested by the October 2002, when the umbels had some opened capsules, revealing the black, ripened seeds. Harvesting was done by cutting the flowering stalks 10 to 15 cm below the umbel. Harvested seed heads from each plot were placed into separated labelled paper bags and taken for drying.

**Drying, Threshing and Weighing of Seeds**

The heads were dried by forced air in boxes through the use of electrical fans at high speed and in some cases they were spread in a shallow layer on a clean surface (a nylon mate) and exposed to the sun. When the seeds were dried, threshing was done by hand and cleaned to remove chaff.

The dried seeds were then weighed with a metler balance (± 0.001g). Seed weight for each treatment, replicate and region was recorded separately.
The impact of natural pollinator, *Apis mellifera* Latreille on onion seed production in Mauritius. MA Bhumoo and D Abeluck

RESULTS

Yields of onion seeds were converted from gram per plot to kilogram per hectare. Results were henceforth presented as estimated yield of onion seeds in kg per hectare (Table 2).

Table 2  Estimated yield of onion seeds per treatment and per region converted and expressed in kg per hectare.

<table>
<thead>
<tr>
<th>Region/Location</th>
<th>Treatment</th>
<th>Replicate I</th>
<th>Replicate II</th>
<th>Replicate III</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cage with Bee (T1)</td>
<td>1408.86</td>
<td>1403.46</td>
<td>1404.56</td>
<td>1405.63</td>
</tr>
<tr>
<td></td>
<td>Cage Without bee (T2)</td>
<td>409.54</td>
<td>394.46</td>
<td>404.32</td>
<td>402.73</td>
</tr>
<tr>
<td></td>
<td>Without cage (T3)</td>
<td>1013.52</td>
<td>1019.96</td>
<td>1015.70</td>
<td>1016.39</td>
</tr>
<tr>
<td>Creve-Coeur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cage with bee (T1)</td>
<td>1586.52</td>
<td>1579.14</td>
<td>1584.04</td>
<td>1583.23</td>
</tr>
<tr>
<td></td>
<td>Cage Without bee (T2)</td>
<td>525.14</td>
<td>516.84</td>
<td>520.48</td>
<td>520.82</td>
</tr>
<tr>
<td></td>
<td>Without cage (T3)</td>
<td>1296.96</td>
<td>1270.56</td>
<td>1278.92</td>
<td>1282.15</td>
</tr>
<tr>
<td>Plaine Sophie</td>
<td>Cage with bee (T1)</td>
<td>1556.56</td>
<td>1564.32</td>
<td>1561.90</td>
<td>1560.93</td>
</tr>
<tr>
<td></td>
<td>Cage Without bee (T2)</td>
<td>481.76</td>
<td>456.40</td>
<td>471.74</td>
<td>469.97</td>
</tr>
<tr>
<td></td>
<td>Without cage (T3)</td>
<td>1068.38</td>
<td>1058.76</td>
<td>1060.18</td>
<td>1062.44</td>
</tr>
<tr>
<td>Palmar</td>
<td>Cage with bee (T1)</td>
<td>1378.54</td>
<td>1389.96</td>
<td>1381.74</td>
<td>1383.41</td>
</tr>
<tr>
<td></td>
<td>Cage Without bee (T2)</td>
<td>390.96</td>
<td>379.50</td>
<td>385.36</td>
<td>385.27</td>
</tr>
<tr>
<td></td>
<td>Without cage (T3)</td>
<td>858.46</td>
<td>870.32</td>
<td>865.06</td>
<td>864.61</td>
</tr>
<tr>
<td>La Chaumière</td>
<td>Cage with bee (T1)</td>
<td>1405.63</td>
<td>1403.46</td>
<td>1404.56</td>
<td>1405.63</td>
</tr>
<tr>
<td></td>
<td>Cage Without bee (T2)</td>
<td>402.73</td>
<td>394.46</td>
<td>404.32</td>
<td>402.73</td>
</tr>
<tr>
<td></td>
<td>Without cage (T3)</td>
<td>1013.52</td>
<td>1019.96</td>
<td>1015.70</td>
<td>1016.39</td>
</tr>
</tbody>
</table>

The estimated seed yield in treatment with *A. mellifera* (T1) was highly significant (P<0.001 & 0.005) in all the three replicates as well as in all regions.

The estimated seed yield per hectare from treatment T1, T2 and T3 was 1483.3, 444.72 and 1056.4 kg respectively (Table 3).

Table 3  Average Yield (in kg per hectare) of onion seeds in the four study sites.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Regions</th>
<th>Creve Coeur</th>
<th>Plaine Sophie</th>
<th>Palmar</th>
<th>La Chaumière</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Cage with bee</td>
<td></td>
<td>1405.62</td>
<td>1583.24</td>
<td>1560.92</td>
<td>1383.42</td>
<td>1483.3</td>
</tr>
<tr>
<td>B-Cage Without bee</td>
<td></td>
<td>402.78</td>
<td>520.82</td>
<td>469.96</td>
<td>385.28</td>
<td>444.72</td>
</tr>
<tr>
<td>C-Without cage</td>
<td></td>
<td>1016.4</td>
<td>1282.14</td>
<td>1062.44</td>
<td>864.62</td>
<td>1056.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>941.60</td>
<td>1128.74</td>
<td>1031.10</td>
<td>877.77</td>
<td>994.8</td>
</tr>
</tbody>
</table>

Overall estimated yield at the four study sites

The seed yield was highest at Plaine-Sophie (1128.74 kg/ha). A gradual decrease in yield was observed at Palmar (1031.1 kg/ha), Creve Coeur (941.6 kg/ha) and La Chaumière (877.77 kg/ha). The difference in seed yield can be related to factors such as, climatic conditions and soil at the study sites.

Differences between treatments varied with localities. At all localities, yield was comparatively highest in treatment with honeybees. Among sites, highest yield (1583.23 kg/ha) was registered at Plaine-Sophie. The estimated yields of onion seeds at Palmar, Creve-Coeur and La Chaumière was 1560.93, 1405.63 and 1383.41 kg/ha respectively.

Cost / Benefit Analysis

The estimate cost has been prepared based on the conditions in which this experiment was carried out. The yield was calculated per hectare. With 3 hives in one hectare of onion plantation, a grower can get an increase in seed yield of about 425 kg/ha worth Rs85000 and 36 litres of pure honey worth about Rs5400 with an annual net revenue of Rs56750.
CONCLUSION

The high yield in seeds in closed plots with *A. mellifera* was consistent over locations. This indicates that growers can increase their yield by placing honeybee hives in their onion fields when flowering starts, as Benedex and Martinovich (1979-1980) recommended.

Woyke (1982), found that good seed set and high seed yields were obtained on commercial onion fields at 2 sites in Poland provided with 7 or 2 honeybee (*A. mellifera*) colonies per hectare respectively.

In European countries (France, Poland and Hungary), the primary objective of honeybee keeping is for pollination of food crops. Robinson et al. (1989) stated that honeybees are assumed to contribute 80 % of insect pollination in USA. Nowadays there are associations of honeybee keepers nearly all over the world who rent hives to planters. The rental of honeybee colonies for commercial pollination is a viable component of the bee keeping industry in some developed countries. The importance of pollination to a regional bee-keeping industry has been documented in a regular annual survey in the northwest US (Burgett et al., 1996-1999). Commercial beekeepers in the region received over 60 % of their annual gross revenue from colony rentals in 1989 and 72 % in 1995; while in 1999 demand exceeded supply.

Honeybee was found to be an effective pollinator and a significant increase seed production was observed in plots with honeybees.

FUTURE WORK

The estimate of cost and benefit feasibility was worked out from results of the small plots of 5 m². It is now proposed to conduct further experimentation to validate the findings.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to onion growers for offering facilities to carry the experiments in their fields and to Dr S. Goburdhun, Associate Professor at the School of Agriculture, University of Mauritius, Dr Hassam Rojoa, Principal Research and Development Officer, Ministry of Agriculture, Food Technology and Natural Resources for their guidance during the study.

REFERENCES


The impact of natural pollinator, Apis mellifera Latreille on onion seed production in Mauritius. MA Bhunoo and D Abeluck


EFFECT OF WIND-PROTECTION ON AGRONOMIC PERFORMANCE OF BANANA CV. PETITE NAINE

B. Jhurree-Dussoruth

Agricultural Research & Extension Unit

ABSTRACT

Twenty-eight tissue-cultured (TC), drip-irrigated banana plants cv Petite Naine were grown within an artificial windbreak at Richelieu CRS (sub-humid zone) in Sept 2002 and their performance compared for two crop cycles with 28 others grown without wind-protection (control). In plant crop, the relative rapid vegetative development of the protected plants resulted in 3 weeks earliness in bunch emergence. The protected plants in the plant crop cycle were also significantly higher, wider and had more functional leaves both at flowering (19 v/s 15) and harvest (9 v/s 7) than the exposed ones. The significantly larger leaves and reduced leaf tearing in protected plants led to a significantly higher yield (58 t/ha v/s 43 t/ha) with 29.2 kg bunch of 10 hands and 189 fingers and better fruit quality. Furthermore, bunches of the exposed plants took 2 weeks extra to mature. In exposed plants of the first ratoon, except for the width of 3rd leaf, there was no significant difference in either plant characteristics, or yield parameters and duration of bunch development period. The non-significance was associated to the fact that strong wind affected the plants late in the cycle, i.e. 2 months prior to the harvest of 50% of the bunches. On the other hand, in plant crop, 2 cyclones caused severe leaf tearing early in the crop cycle. Therefore, the growth stage at which considerable leaf damage is caused appears to influence yield.

Keywords: Wind, leaf shredding, growth stage, flowering, yield, fruit damage

INTRODUCTION

Modern banana (Musa spp.) originated in the tropical zones of the South-East Asian region. However, commercial banana production has extended over the sub-tropical and Mediterranean climates (Robinson, 1996). The main commercial variety, Dwarf Cavendish, grown locally was probably introduced in Mauritius from China in 1837. Banana is one of the most locally appreciated fruit, by virtue of its taste, nutritive value, ease of availability, use in Hindu rituals and its hygienic nature.

In Mauritius, yield of the main variety has been very low (17-22 t/ha) whilst D. Cavendish can produce an average 29 kg bunch size (CIRAD-FLHOR, 1995) representing a yield of over 64 t/ha. In 2003 over 95% of the 519 ha under banana plantation (DAS, 2004) were located on mountainous slopes or grown under marginal conditions. Trials on agronomic aspects of banana, which started in 1997, and observations throughout the island indicated that water, wind and temperature were the three main factors influencing local banana production and productivity (AREU, 1998a, 1999, 2000, 2001). Moreover it was always noted that the leaf status of the plant directly influenced crop productivity. Local banana plantations are constantly exposed to high winds associated with cyclones and anticyclones. Wind can cause both direct physical damage by affecting the aerial parts (leaf tearing or whole plant blowdown) and indirect damage to crop yield through reduction in photosynthesis by torn leaves (Turner, 1998). This study aimed at evaluating the impact of exposure to wind on agronomic performance of banana variety Petite Naine (D.Cavendish).

MATERIALS AND METHOD

This study was carried out from 2002 to 2004 in a sub-humid zone at Richelieu Crop Research Centre (mean annual rainfall 600 mm, mean maximum temperature 30.0°C and mean minimum temperature 20.0°C). The soil is a low humic latosol (Richelieu Family) with a pH of 6.8-6.9. Tissue-cultured (TC) banana plants of var. Petite Naine (D. Cavendish) were planted on 18 September 2002 under drip irrigation and managed as a commercial crop by following recommended cultural practices (AREU,

1998b. Pre-harvest bunch management was limited to removal of the male bud while leaves rubbing against the banana bunches were left intact.

Twenty-eight TC plants were grown in 7 rows at spacing of 2.0 x 2.5m (2000 plants/ha) within a 4m high artificial windbreak (Sarlon cloth of porosity 58%). Twenty-eight others were grown in the open field (control). The TC plants were on average 46cm tall with collar girth 20.6cm and had 6.8 leaves. The design was a complete randomized one with a one-plant replicate. Comparisons between treatments and between plant cycles were made using t-tests assuming equal variances. The following parameters were recorded for plant crop (PC) and first ratoon (R1), selected 6 months after planting when PC was flowering.

**Occurrence of cyclones**

- Periods and gusts of cyclones

**Morphological and Phenological characteristics**

- Monthly leaf emission (in R1)
- Plant height and girth at 1m above ground level at flowering
- Total number of functional leaves at flowering and harvest
- Bunch emergence date and harvest date
- Length and width of the 3rd leaf at harvest

**Physical appearance of youngest fully emerged leaf (YFEL)**

The youngest fully emerged leaf was tagged at monthly interval and its physical appearance described at flowering and harvest using the following arbitrary indicators (no measurement was made): Intact lamina, large tear (tear approximately wider than 12 cm), small tears (tears less than 12cm wide) and senescence.

**Productivity and fruit quality**

- Number of hands and fingers per bunch, bunch weight
- Fruit characteristics (finger length and diameter of the middle finger from the outer whorl of the 2nd hand, peel and pulp weight, Brix, acidity)
- Bruising on fruit of each hand was scored using the following scale:
  - Score 0: Non-bruised
  - Score 1: Minor bruise (1-5% fruit peel affected)
  - Score 2: Significant bruise (5-20% fruit peel affected)
  - Score 3: >20% peel affected or damage incurred to pulp

All specks, maturity bronzing or any spot not caused directly or indirectly by wind were ignored.

**RESULTS**

**Occurrence of cyclones**

Cyclone Gerry classified as tropical cyclone with gusts of 90-120 kmh$^{-1}$ visited Mauritius on 13 February 2003 (MMS, 2003) and affected the PC during early reproductive phase in protected plants and late vegetative phase in the exposed ones (Table 1). Moderate tropical cyclone Manou (05-06 May 2003) also affected PC, but during the bunch development phase. Severe tropical storm Darius (01- 02 January 04) with gusts 105-108 kmh$^{-1}$ (MMS, 2004) was the only cyclone during the R1 and its damage was caused during the reproductive phase of the latter (Table 1).
Table 1  Crop stage at the passage of major cyclones in plant crop (PC) and first ratoon (R1)

<table>
<thead>
<tr>
<th>Crop cycle</th>
<th>Cyclone</th>
<th>Date</th>
<th>Stage of plant development</th>
<th>Major effect of cyclone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protected</td>
<td>Exposed</td>
</tr>
<tr>
<td>PC</td>
<td>Gerry</td>
<td>13 Feb 03</td>
<td>Early reproductive phase with -23% of plants bearing -54% to flower in fortnight. -90% to flower within 1 month</td>
<td>Late vegetative phase (60% to flower within month)</td>
</tr>
<tr>
<td></td>
<td>Manou</td>
<td>5–6 May 03</td>
<td>Over 52% plants reaching harvest stage within 1 month</td>
<td>No harvest within forthcoming month</td>
</tr>
<tr>
<td>R1</td>
<td>Darius</td>
<td>1–3 Jan 04</td>
<td>56% already flowered</td>
<td>61% already flowered</td>
</tr>
</tbody>
</table>

Vegetative development

Leaf production was relatively faster in summer than in winter. Protected plants in R1 produced an average of 33.6 leaves before flowering but this was not significant over the 31.1 leaves produced by exposed plants (Figure 1).

Figure 1: Average cumulative leaf production in first ratoon in protected and exposed plants

Plant characteristics at flowering and harvest

At flowering, protected plants of PC were significantly taller (181.5 v/s 159.1 cm), wider (59.6 v/s 56.7cm), had more leaves both at flowering (19.2 v/s 15.4) and harvest (9.3 v/s 7.2) and had a wider lamina of the 3rd leaf (84.1 cm v/s 76.7 cm) (Table 2). The exposed plants being relatively shorter their girth: height ratio was significantly larger. Plant height and girth, and the number of functional leaves of exposed plants were significantly improved from PC to R1. In both PC and R1, the 3rd leaf was significantly wider in protected plants. There was no significant difference between protected and non-protected plants in R1 for other plant parameters observed.

Physical appearance of YFEL

As over 50% flowering in PC (in both treatments) occurred after the passage of Gerry, 90-100% of all the youngest fully emerged leaf tagged at each monthly interval were finely shredded at time of bunch emergence. Protected plants that flowered before Gerry had on average 20% upper leaves with large tears. After the passage of Gerry, the vegetative plants produced large intact leaves but which became shredded over the following months at a faster rate in exposed plants. In R1, also at flowering most tagged leaves had fine tears. At harvest time, all tears were finely shredded and in exposed plants the tips of the strips were also dried.
Cycle length

Flowering in PC was uniform and was completed within one month. Protected plants flowered on average 5 months after planting (between 02 February to 25 March 03) while the exposed plants took 20 days extra (176 v/s 155 days) (Table 3). Harvest in PC was carried out on average 122 and 107 days after flowering (2 weeks difference) in control and protected plants respectively. In PC, harvest started in protected plants in end May 2003 and in one month’s time over 81% of bunches were already harvested. In exposed plants harvest started later but over 89% harvest was completed by end of July 2003.

Flowering period in R1 was more spread over time going from November 2003 to April 2004 in both protected and non-protected plants. By the end of December 2003 58% and 82% of plants had already flowered in protected and exposed plants respectively. Harvest in R1 occurred on average 92 - 93 days after flowering in both protected and exposed plants (Table 3). The interval between the floral emergences of the 2 cycles (E\textsubscript{PC}-E\textsubscript{R1}) was not significantly different between treatments (295 and 310 days).

Yield and fruit quality

In PC, the protected plants produced significantly more hands (10.1 v/s 9.8), more marketable fingers (189.4 v/s 178), heavier bunches (29.2 v/s 21.5 kg), larger fruits (35.5 v/s 33.7mm), longer fruits (21.3 v/s 19.5cm), and heavier fruits (average fruit weight 133.9 v/s 110.3g) than the non-protected ones (Table 4). Yield in protected and exposed plants was estimated to be 58 and 43 t/ha respectively. However in R1, there was no significant difference between protected and exposed plants for these parameters.

It was observed that the number of hands per bunch and the bunch weight were significantly improved in R1 for both exposed and protected plants. Brix, indicator of sweetness, was higher in exposed plants both in PC and R1. However wind-protection had no effect on acidity and overall Brix: acidity ratio in PC and R1.
Table 2  Comparison of average plant parameters and leaf status of exposed and wind-protected plants in plant crop (PC) and first ratoon (R1).

<table>
<thead>
<tr>
<th></th>
<th>Plant height (cm)</th>
<th>Plant girth (cm)</th>
<th>Girth/height ratio</th>
<th>Functional leaves at flowering</th>
<th>Functional leaves at harvest</th>
<th>Total leaves produced before flowering</th>
<th>Length of 3rd leaf (cm)</th>
<th>Width of 3rd leaf (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>R1</td>
<td>PC</td>
<td>R1</td>
<td>PC</td>
<td>R1</td>
<td>PC</td>
<td>R1</td>
</tr>
<tr>
<td>Exposed</td>
<td>159.1 (60.9)</td>
<td>182.3 (62.2)</td>
<td>56.7</td>
<td>60.9</td>
<td>0.36</td>
<td>0.34</td>
<td>15.4</td>
<td>14.8</td>
</tr>
<tr>
<td>Protected</td>
<td>181.5 (69.2)</td>
<td>191.3 (69.6)</td>
<td>59.6</td>
<td>60.2</td>
<td>0.33</td>
<td>0.33</td>
<td>19.2</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CIRAD/FLHOR</td>
<td>165-200</td>
<td>54-69</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3  Average duration of phenostages in exposed and protected plants in plant crop (PC) and first ratoon (R1)

<table>
<thead>
<tr>
<th></th>
<th>P-E (days)</th>
<th>E-H (days)</th>
<th>E&lt;sub&gt;pc&lt;/sub&gt; - E&lt;sub&gt;R1&lt;/sub&gt; (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>PC</td>
<td>R1</td>
</tr>
<tr>
<td>Exposed</td>
<td>176</td>
<td>122</td>
<td>93</td>
</tr>
<tr>
<td>Protected</td>
<td>155</td>
<td>107</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>*</td>
<td>n.s</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td>*</td>
<td>*</td>
<td>n.s</td>
</tr>
<tr>
<td>CIRAD/FLHOR</td>
<td>220</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

Table 4  Comparison of average yield parameters and fruit quality between exposed and protected plants PC and R1

<table>
<thead>
<tr>
<th>No. of hands</th>
<th>No of fingers</th>
<th>Wt of bunch (kg)</th>
<th>Diameter of middle finger (mm)</th>
<th>Length of middle finger (cm)</th>
<th>Fruit weight (g)</th>
<th>Brix</th>
<th>Acidity</th>
<th>Brix: acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>R1</td>
<td>PC</td>
<td>R1</td>
<td>PC</td>
<td>R1</td>
<td>PC</td>
<td>R1</td>
<td>PC</td>
</tr>
<tr>
<td>Exposed</td>
<td>9.8</td>
<td>10.7</td>
<td>178.4</td>
<td>187.6</td>
<td>21.5</td>
<td>29.4</td>
<td>33.7</td>
<td>36.5</td>
</tr>
<tr>
<td>Protected</td>
<td>10.1</td>
<td>10.9</td>
<td>189.4</td>
<td>197.8</td>
<td>29.2</td>
<td>31.6</td>
<td>35.5</td>
<td>36.6</td>
</tr>
<tr>
<td>P&lt;0.05</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
<td>*</td>
<td>n.s</td>
</tr>
</tbody>
</table>

CIRAD/FLHOR   | 8             | 145              | 29                             | 39                          | 18               | 200  |         |              |              |      |         |      |         |      |         |      |         |

P = Planting, E = Emergence date, H = Harvest date

Effect on fruit bruising

In both protected and exposed plants of PC and R1, low intensity bruising (Score 1) was the most common type of physical blemish on the peel at harvest, (over 28% of fingers per hand) (Table 5), while severe bruising going down to the pulp (Score 3) was very rare (less than 2%). In PC, fruit from exposed plants were significantly more bruised with intensity Score1 than protected ones. In R1, the number of fruit bruised (all intensities) was not significantly different between protected and non-protected plants.

Table 5 Average percentage of fruit bruising per hand in plant crop and ratoon 1

<table>
<thead>
<tr>
<th>Bruising score</th>
<th>Plant Crop</th>
<th>Ratoon 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>Exposed (%)</td>
<td>Protected (%)</td>
</tr>
<tr>
<td></td>
<td>56.0</td>
<td>65.4</td>
</tr>
<tr>
<td>Score 1</td>
<td>38.0</td>
<td>27.9</td>
</tr>
<tr>
<td>Score 2</td>
<td>5.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Score 3</td>
<td>0.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

DISCUSSION

Productivity in banana is governed by 'source' and 'sink' components of the banana system (Turner, 1998). Strong winds (gusts exceeding 60 km/h) is common in Mauritius, hence erection of a windbreak (natural or artificial) is recommended in a commercial banana plantation (AREU, 1998b). In this study, the tissue-cultured plants in both wind-protected and wind-exposed grew very fast during the first 4 months following planting as this coincided with summer. The rapid vegetative development (Figure 1) culminated in the plants flowering 45 to 65 days earlier (Table 3) than expected against its average performance (CIRAD-FLHOR, 1995). According to Robinson (1996), 10-15 functional leaves on plant at flowering is common for good yield and in this study both protected and exposed plants had at least 15 leaves. Moreover, since the larger leaves produced just prior to flowering can live over 150 days (Robinson, 1996) plant canopy at flowering was conducive to a good yield if no damage to the leaves occurred.

Trials conducted at AREU (AREU, 1998a, 1999, 2000, 2001) demonstrated indirectly that the leaf status of the plant (often influenced by wind) has a direct influence on banana yield. It has also been reported that any factor that causes irreversible damage to a leaf lamina has a negative effect on bunch growth (Parra et al., 2001). To achieve maximum bunch filling at least 9 leaves are needed at harvest (Robinson, 1996). In this study, the significantly more leaves present both at flowering and at harvest in protected plants of PC may have contributed to the significantly better performance of banana plants growing inside the 4m high artificial windbreak than plants exposed to wind. Moreover, since the width of 3rd leaf was significantly larger in protected plants, this could have improved the photosynthetic capacity of the plants. Galán Sáuco (2004) demonstrated that protection against wind by growing under greenhouse enables increase in fruit size and yield as it enables increase in leaf area. However, as early as 1958, Lorch (as reported by Turner 1998) demonstrated that the increase in leaf tearing reduces yield, mostly through reduction in rate of net photosynthesis. Furthermore, leaf tearing increases leaf folding and the stomata of torn leaves remain partially closed so that temperature of torn leaves remained similar (Turner, 1998) hence reducing gas exchange. Since no new leaf is produced after flowering in banana, any damage incurred to the existing canopy (source) implies a direct impact on photosynthesis and hence finger filling (sink) (Daniells and Foster, 2001; Parra et al. 2001).

Leaf tearing was more intense in exposed plants and the upper larger leaves were always more prone to production of finer tears (approximately tears less than 5cm wide), especially after the passage of strong winds. Eckstein (1994) demonstrated a 33% reduction in photosynthesis when leaves were torn into fine strips of 12mm width. In this trial, the leaves in PC were severely torn by cyclones Gerry and Manou and the tips of the shredded leaves also dried faster in the exposed plants.

Hence, in addition to the significant difference in the number of functional leaves at flowering and harvest, the relatively finer tears and their accelerated tip drying in exposed plants could probably account for their relatively lower crop performance and yield in PC. However, in R1 there was no significant difference in crop and yield parameters between the protected and the exposed plants although leaf tearing was more prevalent in control, especially after passage of Darius. This was associated to the phenologic growth stage of the plant at which severe leaf damage by cyclone was inflicted.

In PC, cyclone Gerry (Feb 03) caused severe leaf tearing when 23% of the protected plants were bearing and the exposed ones were during late vegetative stage, and also when 60% to 90% of the exposed and protected plants respectively were to flower within one month’s time (Table 1). Cyclone Manou (May 03) caused leaf damage when over 81% and 12% harvest was to be over in one month’s time in protected and exposed plants respectively. Hence, it is possible that during the 5.5 months from passage of Gerry (Feb 03) to end of harvest (end July 03) the relatively accelerated drying of tips of leaf strips in exposed plants may have led to a significant reduction in photosynthesis hence a significant reduction in crop yield parameters. On the other hand, in addition to the presence of more leaves, as pointed out before, the protected plants were also earlier in both bearing and harvest, and had tears that did not dry as fast as the plants directly exposed to the hot/dry environment, thus accounting for the crop performance in protected plants. Similarly, cyclone Darius during R1 affected the plants when over 50% flowering had taken place and when harvest was to start in 1 month time. Moreover, over 50% of harvest of R1 was over by end March 2004 in both exposed and protected plants. Hence, within the 3 months between passage of Darius and 50% harvest, the intensity of leaf tearing and leaf tip drying at that particular plant phenostage did not have enough time to lead to a significant reduction in yield in exposed plants. Furthermore, the number of functional leaves both at flowering and harvest were already similar in both protected and non-protected plants. This confirms the observations made by Parra et al. (2001) where fruit growth and development was less affected when wind damage occurred at advanced stages in the production cycle.

The micro-climate within the artificial windbreak also led to a significantly shorter bunch maturation period (E-H) in protected plants of PC compared to exposed plants (107 v/s 122 days). In R1, bunch developed throughout full summer (Oct 03-Feb 04) and harvest was carried out within only 92-93 days. Conversely, when bunch development in PC occurred mostly from Feb 03–Jul 03 (summer/winter) this could have induced a longer E-H, confirming that each phenostage is temperature dependent (Robinson, 1996).

Wind can also reduce fruit quality by enhancing leaf and dust abrasion (Robinson, 1996). In PC, although low intensity bruising was more prevalent among exposed plants, in R1 low intensity bruising was similarly prevalent in bunches from protected and exposed plants. Medium and severe bruising of fruits was less rarely encountered in both types of plants. Wind protection did not appear to significantly influence fruit physical quality.

CONCLUSION

This study also showed protection against wind improved yield more than quality. Severe leaf damage caused late in the cycle, i.e. at flowering or just near harvest had less time to produce significant reduction in yield. Moreover, in plants where leaf damage occurred before flowering and when combined with a relatively longer bunch filling period then the torn leaves caused significant reduction in yield. As the proportion and type of bruising was not significantly high in exposed plants, wind protection did not contribute in significantly reducing even the low intensity bruising. Windbreaks are relatively expensive and since the results of R1 showed that in a good year when leaf damage occurred only late in cycle no significant benefits were obtained from windbreaks, there is need to follow the other ratoon crops to prove as to whether setting up of windbreak is cost-effective.
REFERENCES

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EFFECT OF STATIC MAGNETIC FIELDS ON THE GROWTH AND YIELD OF BUTTERHEAD LETTUCE SEEDS (*LACTUCA SATIVA* VAR. SALINA).

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ABSTRACT

The effect of an imposed magnetic field on plant life is termed as magnetotropism. This phenomenon, encompassed in magnetophysics, has been increasingly the subject of a large number of investigations since the early effects in the 1960s. In this paper we report the effects of magnetic fields on the growth and yield of Butterhead lettuces (*Lactuca sativa* Var. Salina) whose seeds have been initially exposed to static magnetic fields of average intensity ranging from 40 to 8 mT. The seeds were exposed either 24 hours or 72 hours to north and south magnetic fields. The developed lettuces were cultivated under hydroponics conditions. Major increase in the growth of lettuces was observed from seeds that were treated with a south magnetic field for an exposure period of 24 hours. Under south field exposures, a significant increase [p < 0.05] in yield of 18.8 % and 12.9 % in fresh mass of lettuces was noted for the 24 hours and 72 hours exposed seeds respectively compared to control. The dry mass to fresh mass ratio was significantly increased by 9.5 % (24 hours) and 7.4 % (72 hours). For north field treatment, significant increases of 8.2 % in fresh mass of lettuces and 7.4 % in the dry mass to fresh mass ratio were observed (24 hours seeds exposure). However, no significant increase [p > 0.05] was obtained for the 72 hours exposure. In light of the data obtained, the effect of magnetic fields on growth and yield could be potentially exploited in economic plants.

Keywords: Static magnetic fields, exposure times, hydroponics, growth, yield.

INTRODUCTION

The study of the effects of magnetic fields on biological objects presents a great scientific and practical interest especially in the agricultural field. Studies were conducted for the first time by Savostin (1930) who observed 100 % increase in the rate of elongation of wheat seedlings under the influence of a magnetic field. Audus (1960) and Pittman (1965) noted a remarkable magnetotropic effect on root development and faster early growth in plants. In addition, Chauhan and Agarwal (1977) studied the effect of geomagnetic and external magnetic fields on the germination of seeds of Sonalika wheat (*Triticum Aestivum* L.) and found that the seeds (taking the embryonic root as reference) exposed to the south geomagnetic field and north field showed increased growth compared to those exposed to the geomagnetic north and south fields in parallel orientation. Krylov and Tarakanova (1960) reported that the seeds of corn and wheat with their embryonic roots oriented towards the south magnetic pole sprouted earlier than the seeds facing towards north magnetic pole. In our laboratory, a study using imposed magnetic fields was used to increase bioactive polyphenolic compounds. This investigation was the first of its kind to be conducted using cell cultures cultivated under well-monitored culture and environmental conditions (Aukhez et al., 2001). Furthermore, the effect of an imposed fixed magnetic field, with average field strength of 40 mT, was studied on both ripening and decaying processes of green tomatoes. The data showed that tomatoes that were affected by a south magnetic field ripened faster and decayed slower than those exposed to a north magnetic field (Beeharry et al., 2003) thereby highlighting the potential application of this technique to improve post harvest practices with regard to market demand. Modern techniques like biotechnology, organic cultures etc… have been developed and used to improve agricultural production. Among these new techniques, hydroponics systems seem to be a promising tool that is gaining popularity in Mauritius. Some of its advantages are: higher productivity per area, minimal use of water, low amount of fertilisers and pesticides usage and it offers...
Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (*Lactuca sativa* Var. Salina).

*D Poinapen et al.*

several harvest possibilities per annum due to the rapid plant growth (Kimura and Rodriguez-Amaya, 2003). Various methods like: modern greenhouses, new plant nutrient formulas and foliar feeding have been experimented to improve production rate in hydroponics system. In Mauritius, tomatoes, strawberries, and lettuces are currently hydroponically cultivated and there is a demand to expand this know-how to other plants of economic importance. The rationale to investigate the effects of magnetic fields on plants is to provide alternative means to optimise production and yield particularly for economic crops and useful pharmacological plants. In this regard, in this study, Butterhead lettuce seeds (*Lactuca sativa* Var. Salina) were exposed to static magnetic fields of average intensity ranging from 40 to 8 mT using different exposure periods. This paper reports the effects of magnetic fields on the growth and the yield of hydroponically grown lettuces.

**MATERIALS AND METHODS**

**Seeds Selection**

Butterhead lettuce seeds (*Lactuca sativa* Var. Salina) were used in this experiment. Seeds of almost the same sizes and masses were sorted out and were subjected to magnetic fields.

**Experimental Set-up**

Two permanent alnico magnets of size (17×2×1) cm were used to study the effects of a permanent magnetic field on Butterhead Lettuce seeds. The bar magnets were laid on a horizontal piece of tissue paper and a rectangular area of 0.5 x 2.0 cm$^2$ was drawn at both ends of the poles of the magnets (**Figure 1**) and the magnetic field intensity within the areas was made to vary by an average of 40 mT to 8 mT. Seeds of similar sizes were sorted out and were then placed with random orientation in the rectangular areas on each side of the poles of the bar magnet. The whole setting was placed in a carton box to ensure that the seeds would not be disturbed during magnetic field exposure. In this experiment, two different exposure times were considered.

**Figure 1** Experimental set-up used for exposure of seeds to magnetic fields.
Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (*Lactuca sativa* Var. Salina).

*D Poinapen et al.*

**Exposure time**

Two sets of experiments were conducted using two different exposure times. In the first one, the seeds were subjected to the magnetic fields for 24 hours labelled (1-D) while in the second one, they were exposed for 72 hours (3-D).

Experiment 1- After 24 hours, 1-D treated seeds were removed and were sown in a plastic tray containing subdivided small squares that were filled with pieces of cotton wool for hydroponics manipulation. The plastic tray was subdivided into three parts as shown in Figure 2. Ninety-nine seeds were used each time with 33 seeds exposed to the north pole, 33 others to the south pole and the remaining 33 kept as control. This experiment was repeated twice with a total of 198 seeds to provide statistical significance to the data obtained.

Experiment 2- After 72 hours, 3-D treated seeds were removed and the same procedures were followed as mentioned above. The experiment was repeated twice with a total of 198 seeds to provide statistical significance to the data obtained.

*Figure 2* Diagram representing the sowing of seeds in plastic tray

After germination has taken place, the trays were placed in a bigger tray containing the required nutritive solution for proper growth under hydroponics condition. After two weeks, the seedlings were transferred to the greenhouse and were placed in the hydroponics set-up.

**Measured Parameters**

Data were recorded at 3 different stages. At Stage 1 (nursery stage) the following plant parameters were measured: height of shoots, length of roots, length of primary leaves and width of primary leaves. At Stage 2, the dimensions of the lettuce leaves were recorded until the lettuces reached maturity and the parameters were: length of leave (Blade 1, Blade 2 and Blade 3) and width of leaves (Blade 1, Blade 2 and Blade 3). Finally, at Stage 3 (harvest) the fresh masses of the lettuces were measured. This was followed by dry mass estimation.

**Statistical Analysis**

The recorded data were analysed for significance of mean differences at 5 % level by 1-way ANOVA followed by Tukey’s HSD Post-test, using Prism™ v4.0 statistical software (GraphPad® Software; San-Diego, 2003).
RESULTS AND DISCUSSIONS

Stage 1: Nursery stage

The effect of magnetic fields on the growth of Butterhead lettuce seedlings at nursery stage is shown in Tables 1 and 2. Our data show that the magnitude of the measured parameters for lettuce seedlings (24 hours treated seeds) has increased. Compared to control, a significant increase \[p < 0.05\] was noted for south field treatment for the height of shoots (8.3 %), length of roots (21.6 %) and length of leaves (7.2 %). However, no significant increases were noted for North field treatment, except a 14.1 % \[p < 0.05\] increase for the length of roots compared to control.

Table 1: Variation of measured parameters for Butterhead lettuce seeds that have been initially exposed to magnetic fields for 24 hours (1-D)

<table>
<thead>
<tr>
<th></th>
<th>Height of shoots / cm</th>
<th>Length of roots / cm</th>
<th>Length of leaves / cm</th>
<th>Width of leaves / cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.05 ± 0.14</td>
<td>10.31 ± 0.55</td>
<td>7.81 ± 0.17</td>
<td>3.43 ± 0.07</td>
</tr>
<tr>
<td>North-field</td>
<td>8.12 ± 0.14</td>
<td>11.76 ± 0.46</td>
<td>7.84 ± 0.13</td>
<td>3.59 ± 0.07</td>
</tr>
<tr>
<td>South-field</td>
<td>8.72 ± 0.14</td>
<td>12.54 ± 0.46</td>
<td>8.37 ± 0.18</td>
<td>3.65 ± 0.07</td>
</tr>
</tbody>
</table>

Values represent mean ± SE * indicates significant difference wrt control at 5% level of significance.

For 72 hours treated seeds, the magnitude of the measured physical parameters was enhanced. Significant increase \[p < 0.05\] was observed under the influence of south field for the height of shoots (3.6 %), the length of roots (28.1 %) and the length (5.7 %) and width (7.1 %) of leaves when compared to control. The seedlings showed only a significant increase \[p < 0.05\] of 16 % in the length of the roots under North field treatment.

Table 2 Variation of measured parameters for Butterhead lettuce seeds that have been initially exposed to magnetic fields for 72 hours (3-D).

<table>
<thead>
<tr>
<th></th>
<th>Height of shoots / cm</th>
<th>Length of roots / cm</th>
<th>Length of leaves / cm</th>
<th>Width of leaves / cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.77 ± 0.10</td>
<td>9.32 ± 0.11</td>
<td>5.95 ± 0.06</td>
<td>2.38 ± 0.04</td>
</tr>
<tr>
<td>North-field</td>
<td>7.69 ± 0.09</td>
<td>10.81 ± 0.16</td>
<td>5.96 ± 0.08</td>
<td>2.46 ± 0.05</td>
</tr>
<tr>
<td>South-field</td>
<td>8.05 ± 0.07</td>
<td>11.94 ± 0.17</td>
<td>6.29 ± 0.12</td>
<td>2.55 ± 0.05</td>
</tr>
</tbody>
</table>

Values represent mean ± SE * indicates significant difference wrt control at 5% level of significance.

Our results are consistent with data obtained by previous investigators who reported enhancement in plant growth especially at early stages of plant development. It was indicated that magnetic treated seeds showed improvement in growth vigour, accelerated plant development more particularly in the early stages (Rokhinson et al., 1994). Other studies have also emphasised enhanced germination after exposing seeds (apple, apricot, wheat, sunflower, mungbean and tomato) to such treatment (Chao and Walker, 1967; Bhatnagar and Deb, 1977; Gusta et al., 1978; Phirke et al., 1990; Ting and Ho, 1972; Jae-Duk Moon and Hwa-Sook Chung, 2000; Takimoto et al., 2001; Aksenov et al., 2001).
Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (*Lactuca sativa* Var. *Salina*).

*D Poinapen et al.*

**Stage 2: Growth stage**

Table 3 and Table 4 show the length and width of the lettuce leaves. Both the length and width of the leaves have been increased under the influence of magnetic fields. For 24 hours south field treated seeds, the lettuces experienced a significant increase \[p < 0.05\] of 6% to 20% in length and width of the leaves compared to control. However, a lower increase \[p < 0.05\] of 4% to 10% was observed in length and width of leaves for north field treated lettuces compared to control cultures.

**Table 3** Mean variation of the length and the width of the leaves of lettuces whose seeds have been initially exposed to magnetic fields for 24 hours (1-D).

<table>
<thead>
<tr>
<th>Blade Number</th>
<th>Control</th>
<th>North-field</th>
<th>South-field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean length</td>
<td>Mean width</td>
<td>Mean length</td>
</tr>
<tr>
<td></td>
<td>of leaves</td>
<td>of leaves</td>
<td>of leaves</td>
</tr>
<tr>
<td></td>
<td>L/cm</td>
<td>B/cm</td>
<td>L/cm</td>
</tr>
<tr>
<td>1µ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>10.92 ±0.15</td>
<td>5.79 ± 0.12</td>
<td>11.38 ± 0.16</td>
</tr>
<tr>
<td>2</td>
<td>9.84 ± 0.15</td>
<td>6.13 ± 0.22</td>
<td>10.48* ± 0.13</td>
</tr>
<tr>
<td>2µ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>14.37 ±0.33</td>
<td>9.50 ± 0.11</td>
<td>14.93* ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>12.55 ±0.32</td>
<td>10.64 ± 0.33</td>
<td>13.80* ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>10.40 ±0.15</td>
<td>9.49 ± 0.29</td>
<td>10.94*±0.24</td>
</tr>
</tbody>
</table>

Values represent mean ± SE. * indicates significant difference wrt control at 5% level of significance.

**Table 4** Mean variation of the length and the width of the leaves of lettuces whose seeds have been initially exposed to magnetic fields for 72 hours (3-D).

<table>
<thead>
<tr>
<th>Blade Number</th>
<th>Control</th>
<th>North-field</th>
<th>South-field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean length</td>
<td>Mean width</td>
<td>Mean length</td>
</tr>
<tr>
<td></td>
<td>of leaves</td>
<td>of leaves</td>
<td>of leaves</td>
</tr>
<tr>
<td></td>
<td>L/cm</td>
<td>B/cm</td>
<td>L/cm</td>
</tr>
<tr>
<td>1µ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11.67 ± 0.09</td>
<td>6.70 ± 0.07</td>
<td>11.97 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>10.47 ±0.11</td>
<td>7.33 ± 0.08</td>
<td>10.60 ± 0.09</td>
</tr>
<tr>
<td>2µ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13.89 ±0.22</td>
<td>10.12 ± 0.13</td>
<td>14.61*±0.21</td>
</tr>
<tr>
<td>2</td>
<td>11.64 ±0.08</td>
<td>10.58 ± 0.21</td>
<td>12.72*±0.11</td>
</tr>
<tr>
<td>3</td>
<td>9.95 ± 0.11</td>
<td>9.90 ± 0.20</td>
<td>10.36±0.06</td>
</tr>
</tbody>
</table>

Values represent mean ± SE. * indicates significant difference wrt control at 5% level of significance.

Similarly, lettuces whose seeds have been magnetically exposed for 72 hours show significant increase in their leaf dimension for all blades \[p < 0.05\]. A significant increase of 3% to 14% in length and width of leaves was noted for south field exposed seeds (compared to control). For 72 hours north field treated seeds, leaves were 4% to 9% longer and wider in dimension compared to controls.

Our data are consistent with those made by Gubbel's (1982) who studied wheat, sunflower and pea seeds under the influence of magnetic fields with an exposure time of 10 seconds and showed that an early and more vigorous seedling growth was recorded. Piacentini et al., (2001) exposed cucumber *Cucumis sativus* L. etiolated seedlings to 50 Hz, 1-Gauss magnetic field 24 hours per day for two weeks in the dark and observed rapid growth and prolonged life of exposed seedlings compared to control. Similar studies also report better growth of plants (barley, mustard, and ‘Komatsuna’) that have been exposed to magnetic fields (Mericle et al., 1964; Freyman, 1980; Edminston 1972, Namba et al., 1995; Nisimura 1991).
Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (*Lactuca sativa* Var. Salina).
D Poinapen et al.

Stage 3: Harvest

At harvest, the fresh masses of the lettuces were measured and the dry mass to fresh mass ratio was computed. The results are shown in Figures 3 and 4. Data show that both the fresh masses of the lettuces and the dry mass to fresh mass ratio are enhanced under the influence of magnetic fields. For 24 hours treated seeds, significant increases \( p < 0.05 \) of 18.8 \% in the fresh mass and 9.5 \% in the dry mass to fresh mass ratio were obtained for south field treated lettuces compared to control. Under north field treatment, lettuces experienced significant increases \( p < 0.05 \) of 8.2 \% in the fresh mass and 7.1 \% in the dry mass to fresh mass ratio compared to control.

Figure 3a Average fresh mass of butterhead lettuce whose seeds have been initially exposed to magnetic fields for 24 hours (1-D).

\[
\begin{array}{|c|c|c|}
\hline
 & control & n-f & s-f \\
\hline
\text{Average fresh mass of lettuce/g} & \text{190} & \text{210} & \text{220} & \text{270} \\
\hline
\end{array}
\]

\* indicates significant difference wrt control at 5\% level of significance.

Figure 3b Variation of dry mass to fresh mass ratio of butterhead lettuces whose seeds have been initially exposed to magnetic fields for 24 hours (1-D).

\[
\begin{array}{|c|c|c|}
\hline
 & control & n-f & s-f \\
\hline
\text{Dry mass to Fresh mass ratio} & \text{0.041} & \text{0.042} & \text{0.044} \quad \text{**} \quad \text{0.047} \quad \text{**} \\
\hline
\end{array}
\]

\* indicates significant difference wrt control at 5\% level of significance.

In the case of 72 hours treated seeds, significant increases \( p < 0.05 \) of 12.9 \% in the fresh mass of lettuces and 7.4 \% in the dry mass to fresh mass ratio were observed for south field plants compared to control (Figure 4a, 4b). However, no significant increase was recorded for north field ones.

Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (*Lactuca sativa* Var. *Salina*).

*D Poinapen et al.*

**Figure 4a** Average fresh mass of butterhead lettuce whose seeds have been initially exposed to magnetic fields for 72 hours (3-D).

![Variation of Average Fresh Mass of BUTTERHEAD Lettuce](image)

* indicates significant difference wrt control at 5% level of significance.

**Figure 4b** Variation of dry mass to fresh mass ratio of butterhead lettuces whose seeds have been initially exposed to magnetic fields for 72 hours (3-D).

![Variation of Dry Mass to Fresh Mass Ratio of BUTTERHEAD Lettuce](image)

* indicates significant difference wrt control at 5% level of significance.

From our findings, the fresh mass of lettuces was increased after initial magnetic exposure of their seeds. Danilov et al. (1994) observed 28% to 51% increase in tomato (*Lycopersicon esculentum* Mill. Var. Monza) yield after their seeds had been initially exposed to magnetic fields. On the other hand, Tsuglenok, (1984) found an increase of 20% to 25% in the yield of buckwheat magnetically exposed seeds. Moreover, increase in yield has also been reported in buckwheat, barley, sunflower, cotton and lettuce seeds. (Zolyneak et al., 1972; Pittman, 1977; Gubbels, 1982; Phirke et al., 1994, 1996; Reina et al., 2001).
CONCLUSION

Our results clearly show that the growth of lettuces is influenced after treating their seeds with magnetic fields. More pronounced effects in the growth rate have been noted when the seeds were exposed to south magnetic fields. However, the 24 hours exposure period and the south field triggered a much higher increase in the growth and yield of lettuces. This is evident when fresh masses of south field lettuce treated seeds were compared with non-treated ones. These increases could have important economic implication and be beneficial especially for planters for increased yield and improved post harvest practices.

ACKNOWLEDGEMENTS

We are grateful to Mon Désert Alma Ltd. for providing us with the seeds and for granting us logistic facilities.

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*D Poinapen et al.*


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Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (*Lactuca sativa* Var. Salina). 
*D* Poinapen et al.

EARLY GROWTH AND PHYLLOCHRON OF EIGHT PALM SPECIES AT SIX SITES IN A TROPICAL ENVIRONMENT

N. Govinden and L. D’Espagnac

Mauritius Sugar Industry Research Institute

ABSTRACT

Field trials were planted at nine sites in Mauritius in 1999, 2000 and 2001 to evaluate the performance of eight species of palms for palm cabbage production. Growth was followed in six of these trials at regular intervals of twelve weeks. Non-destructive measurements were made on ten fully bordered plants per plot, in three replicates arranged in randomized complete blocks. The rates of development of the species in the nursery were quite different, being faster in the coconut (Cocos nucifera) and slower in the palmiste piquant (Acanthophoenix rubra). Although all the species established readily in the field and survived well under humid conditions, mortality under dry conditions was very variable, being highest in açai (Euterpe oleracea) and palmiste piquant and lowest in coconut. Plant height was measured from ground surface to the tip of the youngest fully expanded leaf. Increase in height followed a logistic pattern, typical of the growth of plants. Coconut was among the tallest species, at par at some sites with royal palm (Roystonea oleracea), pejibaye (Bactris gasipaes) and palmier de pâques (Veitchia montgomeryana). Palmiste piquant was the shortest species. Stem girth was measured at the base of the trunks. In all species the increase in girth was also described by logistic equations. Coconut had the largest girth at all times, and açai the smallest. The length and width of the leaflets of the species were different and, in most cases, increased in a logistic manner with age. At a given time, the different species had widely different numbers of green leaves. The coconut had more, and the royal palm fewer green leaves than the other species. In most species the number of green leaves increased with age and fluctuated in annual cycles. In all the species, cumulative total number of leaves produced since transplantation increased as a direct function of time. Thus, the rates of leaf production appeared to be constant. The reciprocal of this rate is known as the phyllochron. It varied quite significantly between sites and species. On average over all species, the palms produced a leaf every 48.5 days at Constance, at one extreme, compared to 64.5 days at Deep River, at the other. On average over sites, a leaf was produced in the pejibaye – the species with the smallest phyllochron - every 44.3 days, compared to every 76.7 days in the royal palm, the species with the largest phyllochron. However, when the actual number of leaves which emerged between sampling intervals was plotted against time, the rates of leaf emergence were observed not to be constant. They varied cyclical during the year. Fourier analysis of the data revealed large periodic fluctuations (semi-amplitudes) about the means amounting to 43% on average. The highest rates of leaf emergence and, therefore, lowest phyllochrons were observed in the period from late summer to mid-winter, and the lowest rates, six months later. This suggests a temperature effect with a time lag.

Keywords: Palmito, Palm cabbage, Stem girth, Palm height, Leaf number, Leaf emergence rate

INTRODUCTION

The palm heart industry of Mauritius is based on the cultivation of a single endemic palm species, the hurricane palm, Dictyosperma album var album. It has two shortcomings. Firstly, if grows slowly, taking between 5 and 7 years to produce a harvestable heart (Govinden, 2004a). Secondly, it is very susceptible to a destructive coleopterous insect pest, Brontispa limbata (Govinden, 2004b). The main palm producers reckon that there are prospects for the further development of the industry provided that at least one species without these two shortcomings be found. At their request, the Mauritius Sugar Industry Research Institute (MSIRI) has undertaken a project to evaluate new species for fast growth, adaptation to local conditions and acceptable palm heart quality. The project was terminated in 2004 and the best species are already being cultivated.
In a previous paper, we reported on one aspect of palm adaptation: their reaction to tropical cyclones (Govinden et al., 2003). In this one, we look at early growth. Fast growth is in fact more important than yield per se because, in Mauritius, palm hearts are not sold by weight, but by unit. Consequently, the main objective of the project was to identify species which grow fast, that is, attain the minimum acceptable heart size as fast as possible. In the third year (2001) trials, some species have still not reached the harvestable heart size, which is why yield is not reported here.

MATERIALS AND METHODS

Field trials were planted at 9 sites, 3 in 1999, 3 in 2000 and 3 in 2001, in different agro-ecological regions of Mauritius at latitude 20°S and longitude 57°E. Site conditions are presented in Table 1. Eight palm species were grown. In this paper, their names have been shortened for ease of reading as indicated. They comprised three endemics, the palmiste blanc de Maurice (*Dictyosperma album* var *album*), album for short, the palmiste blanc de l’Ile Ronde (*D album* var *conjugatum*), conjugatum for short and the palmiste piquant (*Acanthophoenix rubra*), rubra for short. Other species were coconut (*Cocos nucifera*), royal palm (*Roystonea oleracea*), royal for short, palmier de pâques (*Veitchia montgomeryana*), veitchia for short, açaí (*Euterpe oleracea*) and pejibaye (*Bactris gasipaes*).

Seeds were collected or imported and sown on beds or in trays. The seedlings were transferred to plastic pots and raised in nurseries as recommended (MSIRI, 2004). The seedlings were transplanted to the field when they reached about 30 cm in height.

At some sites the land was ploughed and at others it was not. The seedlings were placed in holes dug by hoes or in furrows prepared by tractors at a standard interrow spacing of 2 m and at three intra-row spacings of 1, 1.5 and 2 m. Plots consisted normally of 4 rows of 24 m, and the trials normally comprised 3 replicates. However, for lack of space there were 2 rows or 2 densities or 2 replicates at some sites. In this paper, density effects have not been considered. All results pertain to mean of densities.

The crop was managed as recommended (Govinden, 2004b). It was fertilized at planting and topdressed twice a year. Weeds were controlled manually and by means of herbicides. Only at one site – Bois Chéri – was it necessary to spray against *Brontispa limbata* regularly.

The development of the seedlings was observed in all the nurseries. Establishment one month after transplantation and survival after one year in the field were rated in all nine trials. Growth was followed at regular intervals of 12 weeks in six trials, two from each year (Table 1). Non-destructive measurements were made in each replicate on 10 fully-bordered plants per plot, except, of course, where there were only 2 rows.

Plant height was measured from ground surface to the tip of the youngest fully-expanded leaf. Stem girth was measured at the base of the trunks. The length and largest width of the longest leaflet were measured on the youngest fully-expanded leaf. The number of fully-expanded green leaves was counted. By dabbling them with paint each time, a record was obtained of the leaves which emerged and expanded between successive sampling dates and, hence, of the total number of leaves formed since transplantation.

Number of leaves was used to compute the phyllochron. To recall that phyllochron is the time interval between the emergence of two successive leaves. It is particularly useful to describe the rate of emergence of leaves in species such as cereals, banana and palms, which have a single growing point and no branches. For crops grown in temperate climates, it is common to use thermal time (as degree days) instead of chronological time (days) because growth ceases when temperature drops to below a minimum (Kirby, 1995).
Early growth and phyllochron of eight palm species at six sites in a tropical environment. *N Govinden and L D Espagnac*

Table 1  Site conditions of trials to evaluate the performance of palms species for palmito production

<table>
<thead>
<tr>
<th>Site</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date planted</td>
<td>Deep River</td>
<td>Sans Souci</td>
<td>Bois Chéri</td>
</tr>
<tr>
<td>9.02.99</td>
<td>24.02.99</td>
<td>2.07.99</td>
<td>17.04.00</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>110</td>
<td>280</td>
<td>480</td>
</tr>
<tr>
<td>Mean air temperature (°C)</td>
<td>22.9</td>
<td>21.5</td>
<td>20.1</td>
</tr>
<tr>
<td>Mean annual rainfall (mm)</td>
<td>2500*</td>
<td>3700</td>
<td>3800</td>
</tr>
<tr>
<td>Climatic zone</td>
<td>Superhumid</td>
<td>Superhumid</td>
<td>Superhumid</td>
</tr>
<tr>
<td>Average plot size (m²)</td>
<td>160</td>
<td>160</td>
<td>80</td>
</tr>
<tr>
<td>No of densities</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>No of replicates</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Growth measurement</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Supplementary watering after transplantation

Early growth and phyllochron of eight palm species at six sites in a tropical environment. N Govinden and L D’Espaignac

This correction is not necessary for crops grown in tropical climates such as Mauritius because minimum temperatures are never inferior to the base temperature. The mean number of leaves was first obtained from the sample of ten plants. The cumulative number of leaves was then regressed against time, and the phyllochron was obtained from the reciprocal of the tangent of the resultant straight lines. In this standard method, the phyllochron is assumed to be constant and the result is an average phyllochron over the time period considered.

In a second method, the rate of leaf emergence was first calculated as the difference between the number of leaves at successive sampling dates divided by the time interval (12 weeks). The phyllochron is the reciprocal of this rate of leaf emergence. It was plotted against time in order to follow its evolution.

RESULTS AND DISCUSSION

Rate of development in the nursery, and establishment and survival in the field

The rates of development of the seedlings in the nursery after potting varied widely, from very slow in palmiste piquant to very fast in coconut (Table 2). Since the rates of development appear to be proportional to seed size, palmiste piquant having the smallest and coconut the largest seed, the differences can be attributed to differences in the energy reserves of the seeds. The time that the species have to be kept in the nursery before attaining transplantable size varied from a minimum of about one month in the case of coconut, to about 6 months in the case of palmiste blanc de Maurice and pejibaye and almost 2 years in the case of palmiste piquant. These differences have to be borne in mind when planning plantations.

Table 2 Rating of palm species for seedling development rate in the nursery, establishment and survival in the field

<table>
<thead>
<tr>
<th>Species</th>
<th>Development Rate</th>
<th>Establishment Wet</th>
<th>Establishment Dry</th>
<th>Survival Wet</th>
<th>Survival Dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmiste blanc de Maurice</td>
<td>Moderate</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Palmiste blanc de l'île Ronde</td>
<td>Slow to moderate</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Very good</td>
<td></td>
</tr>
<tr>
<td>Palmiste piquant</td>
<td>Very slow</td>
<td>Good</td>
<td>Good</td>
<td>Very poor</td>
<td></td>
</tr>
<tr>
<td>Coconut</td>
<td>Very fast</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
<td></td>
</tr>
<tr>
<td>Royal palm</td>
<td>Slow</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Fair</td>
<td></td>
</tr>
<tr>
<td>Palmier de pâques</td>
<td>Fast</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Açai</td>
<td>Moderate</td>
<td>Good</td>
<td>Good</td>
<td>Very poor</td>
<td></td>
</tr>
<tr>
<td>Pejibaye</td>
<td>Moderate</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Poor</td>
<td></td>
</tr>
</tbody>
</table>

Establishment of the seedlings after transplantation was defined as survival after one month. It depends essentially on the ability of existing roots to absorb sufficient moisture to keep the leaves and the growing point from drying out. New roots do not develop until much later. Under normal humid conditions, establishment was good (<10% mortality) to excellent (<5% mortality) in all species. The species with the lowest establishment rates - palmiste piquant and açai - were also the smallest at transplantation, but a cause and effect relationship may not be implied.

Survival was rated one year after transplantation, that is, when the rainy season returned. The seedlings died mainly during the first dry season from October to December. As defined here, survival depends on the ability of existing and new roots to penetrate into the soil to tap moisture present down in the soil profile during the dry season. At the more humid sites, all the species survived well to very well, but at the drier sites, some species survived very well, e.g. coconut, whereas others suffered from high mortality e.g palmiste piquant and açai. The last two species appear to require shade for proper establishment as has been reported for açai in Brazil (Clement et al., 1993) and observed with palmiste piquant in Réunion. Since all the trials were in full sun, the results indicate that the other six species do not need shade for establishment.
Growth in size

The growth of plants or plant organs may be defined as the increase in size, volume or weight with time. In small annual crops such as potato or bean, samples can be taken at intervals and weighed after oven-drying. Such a procedure is obviously not applicable to tree crops such as palms. We chose the non-destructive measurement of plant height and stem girth to estimate whole-plant growth, and of leaflet length and width to estimate the growth of organs.

Plant height of all the species at all the sites followed the typical logistic pattern of growth (Figures 1a-1f). A sample of the logistic equations is presented in table 3 to show the excellent fits (R^2) of the model. This pattern is characterized by a slow start during which plant height did not increase or increased very slowly. It was followed by a progressively rapid increase lasting for 84 weeks or more depending on the species. The rate of this grand period of growth differed between the species, with coconut having the fastest rate of growth in height at most sites and palmiste piquant the lowest rate. In the final phase of growth, the rate of increase in height slowed down. The time when this phase started differed between sites. It seems that the duration of the grand period of growth depends not so much on plant age as on plant height, the rate of growth slowing down in most species when they reached about 5 m tall. While the differences in plant height are useful to quantify growth and estimate the yield and crop cycle of a given species at the different sites, they are not so useful to compare species. This is because some species such as the palmier de pâques have tall but thin stems whereas others such as the palmiste blanc de l’île Ronde have short and thick ones.

The girth of the stems at the base also followed the typical logistic pattern of growth (Figures 2a-2f), and the model gave excellent fits in most of the species and sites (Table 3). In most species the first phase of slow growth lasted at least 36 weeks as at Bois Chéri (Figure 2b) and at Britannia (Figure 2c). As was the case with plant height, the duration of the grand period of growth seemed not to be age-dependent, but rather size-dependent, and to be quite different in the different species. In açaí, for instance, the rate of increase in girth slowed down when the girth reached about 30 cm, in contrast to such species as royal palm and coconut, where it did not slow down until girth reached about 80 cm. The differences in girth are likely to influence yield. Species with a small girth at the base cannot logically be expected to have a large crown shaft, and hence, a large edible heart. But the reverse may not be true. In species such as the royal palm, the stem is conical in shape and the girth of the crown shaft is distinctly smaller than that of the base. In contrast, species such as the pejibaye have cylindrical trunks whose girth vary little from the base to the crown shaft.

The growth in leaflet length also followed the expected logistic pattern (Table 3). Only one set of results from each year is presented (Figures 3a, 3c and 3e). Coconut always had the longest leaflet, and açaí and palmiste piquant the shortest.

Likewise, leaflet widths of species in only one trial from each year are presented (Figures 3b, 3d and 3f). Palmier de pâques always had the widest leaflet, and royal palm one of the narrowest. A few instances of reduction in leaflet width can be seen particularly well in palmier de pâques. They probably represent an effect of cyclone Dina which occurred at 133 weeks at Bois Chéri (Figure 3b), and at 84 weeks at Belle Vue (Figure 3d) as was reported previously (Govinden et al., 2003).
Figure 1 Growth in height of eight palm species at six sites
Early growth and phyllochron of eight palm species at six sites in a tropical environment. N Govinden and L D Espagnac

Figure 2 Growth in stem girth of eight palm species at six sites

(a) Deep River

(b) Bois Chéri

(c) Britannia

(d) Belle Vue

(e) Constance

(f) Savannah
Figure 3 Growth in leaflet length (3a, 3c, 3e-left) and leaflet width (3b, 3d, 3f-right) of eight palm species at three sites.
Table 3  Coefficients of some logistic equations to describe whole-plant and leaf growth in eight palm species at selected sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Species</th>
<th>Equation: $y=a/(1+be^{-cx})$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>-----</td>
<td>-----</td>
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</tr>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td></td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>Belle Vue</td>
<td>Açai</td>
<td>369</td>
<td>15.1</td>
<td>0.02</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
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<td>Album</td>
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<td>11.8</td>
<td>0.03</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coconut</td>
<td>417</td>
<td>9.7</td>
<td>0.02</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conjugatum</td>
<td>247</td>
<td>12.9</td>
<td>0.22</td>
<td>0.99</td>
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</tr>
<tr>
<td></td>
<td>Pejibaye</td>
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<td>13.7</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Royal</td>
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<td>0.02</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rubra</td>
<td>179</td>
<td>17.3</td>
<td>0.02</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Veitchia</td>
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<td>10.3</td>
<td>0.02</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Stem girth (cm)</td>
<td></td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>Britannia</td>
<td>Açai</td>
<td>79</td>
<td>9.7</td>
<td>0.07</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Album</td>
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<td>31.0</td>
<td>0.02</td>
<td>0.99</td>
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<tr>
<td></td>
<td>Coconut</td>
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<td>21.3</td>
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<tr>
<td></td>
<td>Conjugatum</td>
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<td></td>
<td>Pejibaye</td>
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<td>15.8</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Royal</td>
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<td>74.7</td>
<td>0.05</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Veitchia</td>
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<td>Leaflet length (cm)</td>
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<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>Constance</td>
<td>Açai</td>
<td>52</td>
<td>4.5</td>
<td>0.02</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Album</td>
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<td>4.0</td>
<td>0.04</td>
<td>0.95</td>
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<td>Coconut</td>
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<td>6.4</td>
<td>0.08</td>
<td>0.95</td>
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<td></td>
<td>Conjugatum</td>
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<td>3.4</td>
<td>0.03</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pejibaye</td>
<td>44</td>
<td>12.0</td>
<td>0.14</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rubra</td>
<td>32</td>
<td>3.2</td>
<td>0.03</td>
<td>0.91</td>
<td></td>
</tr>
</tbody>
</table>

Leaf emergence and phyllochron

The number of green leaves present at any time is the resultant of three determinants of leaf demography: leaf emergence and senescence, which have opposite effects, and leaf duration. Since we followed only leaf emergence, it is not possible to attribute the effects to their underlying causes.

At a given time, the different species had widely different numbers of green leaves (Figures 4a-4f). The coconut and the palmiste blanc de Maurice had more, and the royal palm and the palmiste piquant had fewer green leaves. At all the sites, the differences in green leaf numbers between the extreme species were in the order of 100 percent. Only at Britannia (Figure 4c) was there a clear trend in all species for green leaf number to increase with age. At the other sites, the trend was present in some species and absent in others. For instance, at Bois Chéri (Figure 4b), the number of green leaves in palmiste blanc de l’île Ronde increased from about 4 ½ to about 7 ½ in the measurement period of about 3 ½ years, whereas the number of green leaves in royal palm remained constant around 4.

The typical logistic increase was observed in a few species at a few sites, e.g palmiste blanc de l’île Ronde at Bois Chéri (Figure 4b) and Britannia (Figure 4c), whereas in other species and sites, the logistics growth pattern was masked by short-term changes. At most sites and in most species, cyclic fluctuations were observed in green leaf numbers, for instance, in all species at Belle Vue (Figure 4d) and Constance (Figure 4e). At the different sites, the number of green leaves reached a maximum at slightly different periods of the year and were minimum about six months later.

The species had different cumulative total number of leaves (Figures 5a-5f). At all sites, royal palm had the lowest total number of leaves at all times, but the species with the highest total number of leaves were not the same across sites. Palmiste blanc de l’île Ronde had the highest number at one site only – Belle Vue (Figure 5d) – also the driest, which may indicate a specific adaptation. At the other five sites, the palmiste blanc de Maurice had the highest number for the first part of the measurement period but it was overtaken by the pejibaye in the second or third year at three sites.
Figure 4 Growth in number of green leaves of eight palm species at six sites

(a) Deep River

(b) Bois Cheri

(c) Britannia

(d) Belle Vue

(e) Constance

(f) Savannah

Early growth and phyllochron of eight palm species at six sites in a tropical environment. N Govinden and L D'Espagnac
Figure 5 Cumulative total number of leaves of eight palm species at six sites

(a) Deep River

(b) Bois Cheri

(c) Britannia

(d) Belle Vue

(e) Constance

(f) Savannah


227
Excellent fits ($r^2$) were obtained in all species and sites when the total number of leaves was regressed linearly against time. The tangent with the x-axis gives the rate of leaf emergence (number of leaves per week), and the reciprocal of this rate (week/leaf) is the phyllochron. The phyllochrons and their standard deviation in days estimated in this way are presented in Table 4 together with the fits ($r^2$) of the linear regressions. The phyllochrons varied with sites and species and, on average, the standard deviation amounted to about 4%. The analysis of variance of phyllochron performed on the assumption of homogeneous variance revealed that the species by site interaction was not significant. Means of species and of sites are presented in Table 5. Site means were significantly different. On average over species, a palm produced a leaf every 48 ½ days at Constance compared to 64 ½ days at Bois Chéri. The difference in these two extremes amounts to 33%. The Bois Chéri site is undoubtedly marginal for most crops due to cooler temperatures in winter, excessive rainfall in summer and shallow infertile soil. In contrast, the soil at Constance is very good, the temperature is one of the highest, but the rainfall is rather modest. On average over sites, a leaf was produced in pejibaye every 44 ⅓ days compared to 76 ⅔ days in royal palm. The difference between these two extremes amounts to 73%. Therefore, it may safely be concluded that the palm species have widely different rates of leaf emergence and average phyllochrons.

Table 4  Phyllochron (days ± standard deviation) and coefficient of determination (R2) of linear regressions of eight palm species at six sites

<table>
<thead>
<tr>
<th>Palm species</th>
<th>Site</th>
<th>Phyllochron (Days ± S.D)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Album</td>
<td>Deep River</td>
<td>47.4±1.18</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>56.1±0.91</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>51.7±1.32</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>52.7±1.04</td>
<td>0.994</td>
</tr>
<tr>
<td></td>
<td>Constance</td>
<td>43.3±0.74</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>48.3±2.28</td>
<td>0.985</td>
</tr>
<tr>
<td>Conjugatum</td>
<td>Deep River</td>
<td>48.8±1.43</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>61.6±1.54</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>45.9±0.78</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>48.5±0.78</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>Constance</td>
<td>44.3±0.84</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>44.2±1.59</td>
<td>0.991</td>
</tr>
<tr>
<td>Rubra</td>
<td>Britannia</td>
<td>52.6±1.38</td>
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<tr>
<td></td>
<td>Belle Vue</td>
<td>72.1±3.56</td>
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</tr>
<tr>
<td></td>
<td>Constance</td>
<td>61.8±3.78</td>
<td>0.964</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>52.3±0.78</td>
<td>0.899</td>
</tr>
<tr>
<td>Coconut</td>
<td>Deep River</td>
<td>65.9±3.30</td>
<td>0.988</td>
</tr>
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</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>59.1±2.04</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>59.8±3.03</td>
<td>0.975</td>
</tr>
<tr>
<td></td>
<td>Constance</td>
<td>47.3±1.97</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>61.5±4.33</td>
<td>0.990</td>
</tr>
<tr>
<td>Royal palm</td>
<td>Deep River</td>
<td>92.5±11.27</td>
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<tr>
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<td>Bois Chéri</td>
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<td>Britannia</td>
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<td>Constance</td>
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<tr>
<td></td>
<td>Savannah</td>
<td>56.9±3.09</td>
<td>0.988</td>
</tr>
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<td>Veitchia</td>
<td>Bois Chéri</td>
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<tr>
<td></td>
<td>Constance</td>
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<td>0.996</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>41.6±1.71</td>
<td>0.988</td>
</tr>
<tr>
<td>Açai</td>
<td>Deep River</td>
<td>62.4±4.87</td>
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</tr>
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<td>Britannia</td>
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<td>Belle Vue</td>
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<td>0.966</td>
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<tr>
<td></td>
<td>Constance</td>
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</tr>
<tr>
<td></td>
<td>Savannah</td>
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</table>
Early growth and phyllochron of eight palm species at six sites in a tropical environment. N Govinden and L D’Espagnac

Table 5  Phyllochron (days) of eight palm species at six sites obtained from linear regression

<table>
<thead>
<tr>
<th>Species</th>
<th>Deep River</th>
<th>Bois Chéri</th>
<th>Britannia</th>
<th>Belle Vue</th>
<th>Constance</th>
<th>Savannah</th>
<th>Mean</th>
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<td>52</td>
<td>53</td>
<td>43</td>
<td>48</td>
<td>49.8</td>
</tr>
<tr>
<td>Conjugatum</td>
<td>49</td>
<td>62</td>
<td>46</td>
<td>49</td>
<td>44</td>
<td>44</td>
<td>49.0</td>
</tr>
<tr>
<td>Rubra</td>
<td>(68)</td>
<td>(71)</td>
<td>53</td>
<td>72</td>
<td>62</td>
<td>52</td>
<td>(63.0)</td>
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<td>Coconut</td>
<td>66</td>
<td>72</td>
<td>59</td>
<td>60</td>
<td>47</td>
<td>62</td>
<td>61.0</td>
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<tr>
<td>Royal palm</td>
<td>93</td>
<td>90</td>
<td>75</td>
<td>87</td>
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<td>56</td>
<td>46</td>
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<td>Açai</td>
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<td>50</td>
<td>61</td>
<td>52</td>
<td>(60)</td>
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<td><strong>53.8</strong></td>
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<td><strong>56.5</strong></td>
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</tbody>
</table>

During each 12-week sampling interval the number of leaves which emerged varied within very narrow limits of 1 to 2 leaves. Consequently, the regression of the cumulative total number of leaves against time is bound to give straight lines with good fits and, hence, constant rates of leaf emergence and constant phyllochrons. A selection of the plots of the actual number of leaves which emerged during each sampling interval (Figures 6a-6f) shows that far from being constant, the rates of leaf emergence and the phyllochrons varied in a cyclic manner. Fourier analysis of the data gave slightly different average phyllochrons with larger standard deviations than the standard method (Table 6). The coefficients of determination ($r^2$) were much smaller and often not significant, indicating that the Fourier equations explained only a part, sometimes a quite small part, of the variation. Only in a few instances were the phyllochrons estimated by the Fourier analysis similar to those obtained by the standard linear regression method. One reason for the poor fit of the Fourier model is that it consists of a single equation to describe the change over the years. In many instances, the rates of leaf emergence changed with time or age of the crop, perhaps for inherent reasons or because of changes in climatic determinants.

A unique feature of the Fourier model is that it provides a measure, known as the semi-amplitude, of the magnitude of the periodic fluctuation about the mean. The semi-amplitudes were always quite large and amounted, on average, to 43% of the means (Table 6). In contrast, in the linear regression method, the rates of leaf emergence and the phyllochrons were assumed to be constant. We demonstrate here that this assumption does not hold.

The Fourier analysis shows that, at a given site, the different species had peak rates of leaf emergence at almost the same time after planting and, therefore, at the same period of year (Table 7). This points to a common underlying cause. However, there were differences between sites, with the peaks occurring as early as late February at Britannia and as late as early June at Belle Vue.

The rate of leaf emergence is one of the determinants, together with the rate of leaf senescence and with leaf duration, of green leaf number. The period of year when they peaked can be compared in cases where the species had distinct maximum green leaf numbers (Figures 4a-4f) and where the Fourier model was significant (Table 6). The results of twenty such comparisons (Table 7) show that there was an average time lag of 10 weeks between the peaks in rates of leaf emergence and in green leaf numbers.


229
Figure 6  Rate of leaf appearance of palm species at different sites

(a) Album - Deep River

(b) Album - Bois Cheri

(c) Conjugatum - Britannia

(d) Conjugatum - Belle Vue

(e) Veitchia - Constance

(f) Veitchia - Savannah


230
Table 6: Phyllochron (days ± standard deviation), semi-amplitude (days) and coefficient of determination (R²) obtained by Fourier analysis of eight palm species at six sites

<table>
<thead>
<tr>
<th>Palm Species</th>
<th>Sites</th>
<th>Phyllochron (Days ± S.D)</th>
<th>Semi-amplitude (days)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Album</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deep River</td>
<td>47.5±2.49</td>
<td>18.0</td>
<td>0.81**</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>56.9±3.09</td>
<td>22.7</td>
<td>0.69***</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>51.6±3.67</td>
<td>21.3</td>
<td>0.59**</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>53.3±3.55</td>
<td>25.5</td>
<td>0.68***</td>
</tr>
<tr>
<td></td>
<td>Constance</td>
<td>41.8±2.78</td>
<td>12.2</td>
<td>0.59*</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>46.9±6.35</td>
<td>22.0</td>
<td>0.53 NS</td>
</tr>
<tr>
<td><strong>Conjugatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deep River</td>
<td>49.6±3.19</td>
<td>17.2</td>
<td>0.65*</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>64.7±7.35</td>
<td>25.1</td>
<td>0.32 NS</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>46.0±1.96</td>
<td>16.9</td>
<td>0.78***</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>47.8±2.46</td>
<td>18.3</td>
<td>0.71**</td>
</tr>
<tr>
<td></td>
<td>Constance</td>
<td>42.9±2.82</td>
<td>14.9</td>
<td>0.84*</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>43.3±3.84</td>
<td>15.0</td>
<td>0.61(*)</td>
</tr>
<tr>
<td><strong>Rubra</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>57.3±9.08</td>
<td>1.2</td>
<td>0.00 NS</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>75.9±15.92</td>
<td>46.1</td>
<td>0.22 NS</td>
</tr>
<tr>
<td></td>
<td>Constance</td>
<td>57.0±16.93</td>
<td>16.2</td>
<td>0.05 NS</td>
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<tr>
<td></td>
<td>Savannah</td>
<td>52.4±2.81</td>
<td>8.4</td>
<td>0.42 NS</td>
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<tr>
<td><strong>Coconut</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deep River</td>
<td>63.9±3.01</td>
<td>24.4</td>
<td>0.93*</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>74.2±4.96</td>
<td>33.0</td>
<td>0.65**</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>60.5±5.65</td>
<td>25.6</td>
<td>0.55*</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>58.7±10.86</td>
<td>41.3</td>
<td>0.47(*)</td>
</tr>
<tr>
<td></td>
<td>Con stance</td>
<td>46.8±3.43</td>
<td>13.1</td>
<td>0.67*</td>
</tr>
<tr>
<td><strong>Royal palm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deep River</td>
<td>88.7±11.50</td>
<td>62.9</td>
<td>0.90*</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>93.6±6.13</td>
<td>61.3</td>
<td>0.85***</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>73.5±7.29</td>
<td>21.6</td>
<td>0.31 NS</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>90.8±14.89</td>
<td>49.5</td>
<td>0.40 NS</td>
</tr>
<tr>
<td></td>
<td>Con stance</td>
<td>53.3±7.39</td>
<td>22.7</td>
<td>0.56 NS</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>57.1±3.72</td>
<td>19.6</td>
<td>0.81 NS</td>
</tr>
<tr>
<td><strong>Veitchia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>54.1±5.11</td>
<td>29.3</td>
<td>0.64**</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>54.0±6.44</td>
<td>23.3</td>
<td>0.47*</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>60.0±8.13</td>
<td>37.6</td>
<td>0.47*</td>
</tr>
<tr>
<td></td>
<td>Con stance</td>
<td>47.6±4.15</td>
<td>11.3</td>
<td>0.47 NS</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>41.8±4.24</td>
<td>17.5</td>
<td>0.65(*)</td>
</tr>
<tr>
<td><strong>Açai</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deep River</td>
<td>71.0±18.26</td>
<td>45.4</td>
<td>0.26 NS</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>74.3±8.93</td>
<td>44.3</td>
<td>0.46*</td>
</tr>
<tr>
<td></td>
<td>Britannia</td>
<td>54.7±5.29</td>
<td>17.1</td>
<td>0.27 NS</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>62.3±7.52</td>
<td>19.4</td>
<td>0.22 NS</td>
</tr>
<tr>
<td></td>
<td>Con stance</td>
<td>50.4±3.72</td>
<td>20.3</td>
<td>0.61*</td>
</tr>
<tr>
<td><strong>Pejibaye</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deep River</td>
<td>54.0±16.13</td>
<td>42.8</td>
<td>0.63 NS</td>
</tr>
<tr>
<td></td>
<td>Bois Chéri</td>
<td>46.6±6.85</td>
<td>19.6</td>
<td>0.35 NS</td>
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<tr>
<td></td>
<td>Britannia</td>
<td>41.2±6.61</td>
<td>18.7</td>
<td>0.45 NS</td>
</tr>
<tr>
<td></td>
<td>Belle Vue</td>
<td>52.7±5.04</td>
<td>27.8</td>
<td>0.38 (*)</td>
</tr>
<tr>
<td></td>
<td>Con stance</td>
<td>36.2±0.52</td>
<td>6.6</td>
<td>0.98*</td>
</tr>
<tr>
<td></td>
<td>Savannah</td>
<td>38.3±4.73</td>
<td>14.7</td>
<td>0.51 NS</td>
</tr>
</tbody>
</table>

MAS 2005. Food and Agricultural Research Council, Réduit, Mauritius. 231
Early growth and phyllochon of eight palm species at six sites in a tropical environment. N Govinden and L D’Espagnac

Table 7  Time after transplanting (weeks) and period of year to peak rate of leaf emergence and peak number of green leaves in some palm species at six sites

<table>
<thead>
<tr>
<th>Table 7</th>
<th>Time after transplanting (weeks) and period of year to peak rate of leaf emergence and peak number of green leaves in some palm species at six sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Peak Rate of Leaf emergence</strong></td>
</tr>
<tr>
<td></td>
<td>Weeks</td>
</tr>
<tr>
<td>Deep River</td>
<td>Album</td>
</tr>
<tr>
<td></td>
<td>Conjugatum</td>
</tr>
<tr>
<td></td>
<td>Coconut</td>
</tr>
<tr>
<td>Bois Chéri</td>
<td>Album</td>
</tr>
<tr>
<td></td>
<td>Conjugatum</td>
</tr>
<tr>
<td></td>
<td>Coconut</td>
</tr>
<tr>
<td></td>
<td>Veitchia</td>
</tr>
<tr>
<td>Britannia</td>
<td>Conjugatum</td>
</tr>
<tr>
<td></td>
<td>Coconut</td>
</tr>
<tr>
<td></td>
<td>Veitchia</td>
</tr>
<tr>
<td>Belle Vue</td>
<td>Album</td>
</tr>
<tr>
<td></td>
<td>Conjugatum</td>
</tr>
<tr>
<td></td>
<td>Veitchia</td>
</tr>
<tr>
<td>Constance</td>
<td>Album</td>
</tr>
<tr>
<td></td>
<td>Conjugatum</td>
</tr>
<tr>
<td></td>
<td>Coconut</td>
</tr>
<tr>
<td></td>
<td>Açaí</td>
</tr>
<tr>
<td></td>
<td>Pejibaye</td>
</tr>
<tr>
<td>Savannah</td>
<td>Conjugatum</td>
</tr>
<tr>
<td></td>
<td>Veitchia</td>
</tr>
<tr>
<td>Average (all species and sites)</td>
<td>76</td>
</tr>
</tbody>
</table>

The periodic fluctuations in green leaf numbers and in the rates of leaf emergence could be caused by fluctuations in climate. Three climatic factors change in annual cycles, all in the same direction: rainfall, temperature and incoming radiation, the latter two being functions of daylength. Increase in temperature has the well-known effect of accelerating the rates of plant processes from cell division to rate of growth. Daylength affects phenology rather than growth. However, Mauritius is so small that all sites have maximum daylengths and temperatures at the same periods. Consequently, neither temperature or day length alone nor the two together could cause the site differences. We hypothesize that temperature is the primary cause of differences in the periods of maximum rates of leaf emergence, and that the effect is modulated by rainfall. This would explain why peak leaf emergence rates was delayed at Constance with lower rainfall and occurred at Belle Vue, the driest site, much later than at the other sites. Temperature and rainfall data are being collected to validate this hypothesis.

CONCLUSION

The rates of development in the nursery of the different palm species were found to be very different. This has a direct bearing on the cost of production of the seedlings. By the time that palmiste piquant was ready to be transplanted, coconut, for instance, may be almost ready for harvest. Although similar large differences between species were found in rates of growth in height, girth, leaf size, leaf emergence and leaf numbers, they may have much less direct effects on yield or crop cycle. This is because yield of palm hearts has several components. Height is less important than girth because some tall species such as veitchia have very thin stems and therefore, small palm hearts. In contrast, species with a thick girth, such as coconut and royal palm, have larger hearts even when they are still short. Consequently, the two species which combined rapid increase in plant height with large stem girth – coconut and royal palm – may be expected to reach a harvestable size earlier than species which, in contrast, were short and thin, such as açaí and palmiste piquant.

Early growth and phyllochron of eight palm species at six sites in a tropical environment. N Govinden and L D’Espagnac

Although leaf growth rate is one of the main underlying causes of differences in growth of plant height and stem girth, it is not the only one. In the first years of growth, some species may allocate much of the photosynthate produced by the leaves to the roots at the expense of the aerial parts, so that the growth of their harvestable product is slower inspite of their fast leaf growth. The difference in some of the growth parameters measured in this study may not be sufficient to explain the differences in yield or crop cycle of the species, but they will be especially useful to understand the differences between growth at the different sites. Without this understanding, it will be impossible to extrapolate the results obtained in this study to all the zones where the different interesting palm species may be cultivated.

ACKNOWLEDGEMENTS

Thanks are presented to members of the Groupement Palmiste for partial financial support and for land, labour and other resources for the trials. We are grateful to Dr C Soopramanien, Chairman of the project coordination committee and to the other members of the committee for their support and interest. We acknowledge the help of Mrs C Rammawaz and P Nemdharry for the preparation of growth curves, of Ms N Demba for the calculation of phyllochron and of Mr K Rummun for the calculation of the logistic growth equations.

REFERENCES


PHENOLOGICAL, FRUIT AND CHEMOTAXONOMIC CHARACTERIZATION OF LITCHI CULTIVARS IN MAURITIUS: PRELIMINARY FINDINGS.

M. Madhou\textsuperscript{1,2}, T. Bahorun\textsuperscript{2} and N. Ramburn\textsuperscript{1}

\textsuperscript{1}Agricultural Research and Extension Unit
\textsuperscript{2}Department of Biosciences, Faculty of Science, University of Mauritius

ABSTRACT

Phenological, fruit and chemotaxonomic characterization of litchi cultivars in Mauritius was initiated in 2001. The study was conducted in three localities at different altitudes, namely, Labourdonnais (50m), Réduit (250m) and Paillotte (350m). The evaluated cultivars were the locally grown cultivars Tai So, Calcuttia Late and Hong Kong and the imported cultivars Yook Ho Pow, Hei Ye, Brewster, Bengal and Bosworth3. There are indications that for the most commonly-grown cultivar Tai So, flowering was induced when temperature dropped below 20\degree C for 8 to 11 weeks. Depending on variety and site, flowering extended from end of June to end of July. Fruit set was from end of August to first week of October, while harvest started from mid November to beginning of January. Flowering was earliest at the cooler sites but fruit set and harvest were hastened by warmer temperatures. Early maturity was achieved with cultivars with low chilling requirements, grown on warm sites. Among the cultivars characterised, Yook Ho Pow was the most superior with early maturity, large fruit size and high % of chicken-tongued seeds. Moreover, the Tai So at Labourdonnais has shown a significantly high % of chicken-tongued seeds that needs further investigation to determine whether it is site specific or genetic. As polyphenolic constituents are increasingly being considered as markers of choice in plant systematics, polyphenolic profiles of leaf extracts were used to evaluate genetic variability between cultivars and within the Tai So cv. The flavonoid and proanthocyanidin profiles of mature leaves as revealed by thin layer chromatography showed slight qualitative but considerable quantitative variation between cultivars. It is still to be determined whether the cause of this variation is genetic or climatic. The Flavan-3-ol concentration of the Tai So clone from Labourdonnais was significantly lower than that of the Tai So clone from other sites. High Pressure Liquid Chromatography (HPLC) technique is being used for a comprehensive analysis of the polyphenolic profiles of leaf extracts for chemotaxonomic study.

Keywords: Litchi, Phenology, Flowering, Fruit characterization, Chemotaxonomy and Polyphenols.

INTRODUCTION

Introduced in Mauritius in 1763 (Rouillard and Guého, 1999), litchi (\textit{Litchi chinensis} Sonn.,) is a crop which is gaining economic importance in Mauritius with annual export to Europe reaching up to 250 tonnes during good climatic years. Desirable characteristics of the fruit to increase its market share are bright red colour, large size, high % of chicken-tongued seeds, high pulp:seed ratio, high brix and early maturity (end of October/beginning of November).

There is no reported local study to explain the good or poor performance of the locally established (Tai So, Calcuttia Late and Hong Kong) and the imported cultivars from Australia and China in the 90’s (Bosworth 3, Kwai May Pink, Bengal, Gee Kee, Brewster, Wai Chee, Jensen, Casino, Fay Si Shu, Heong Lai, Yook Ho Pow, Salathiel, Haak Yip, Huai Zhi, Yuan Zhi and Hei Ye). Several glasshouse studies have shown that floral initiation takes place after temperatures drop below 20\degree C for a few weeks (Menzel, 1983; Menzel and Simpson, 1988; Batten and McConchie, 1995). All the reported experiments were glasshouse studies with constant temperature for specific durations. Useful information related to litchi flowering can be generated if these parameters are tested under field conditions locally with cultivars grown in Mauritius. Such information would contribute to selecting sites for successful flowering and for matching the right cultivars to specific sites according to their temperature requirements as considerable cultivar difference in response to temperature has been reported (Menzel et al., 1989). Moreover, since a high percentage of chicken-tongued seeds is a desirable characteristic in litchi cultivars, it is crucial to investigate the factors influencing this trait.
So far the nomenclature of cultivars in Mauritius, especially the Tai So has been based on morphological characters of the tree, leaves and fruits although the origin of many of the very old trees in backyards is unknown (Julien, 1970). Yet, there is a lot of confusion in the naming of litchi cultivars. It is usual for one cultivar to have many names and different cultivars to have the same name (FAO, 1989; Aradhya et al., 1995). Therefore a proper characterisation is essential for optimum germplasm management and establishment of appropriate breeding programmes whenever required. Chemotaxonomic markers can be of great interest to fulfil this objective. Chemotaxonomic markers, such as polyphenolics, more particularly flavonoids have already shown their value as reliable elements to establish relationships within and among various plant taxa (Williams et al., 1983; Webb and Harborne, 1991; Petrovic et al., 1999; Lai Fang et al., 2000). In fact polyphenolics in litchi pericarp have successfully been used to differentiate 11 litchi cultivars from Thailand, China and Reunion Island (Spranger et al., 1998). These molecules are markers of choice because of their structural variability, widespread distribution, stability, ease and speed of identification (Harborne, 1967). Biochemical characterisation based on phenolic markers is hence a tool that can be used to relate the morphological and phenological characteristics of litchi clones to their genetic variability.

To understand the factors influencing flowering, early maturity and good fruit quality under local conditions, a study has been initiated to (a) determine the temperature pattern influencing floral initiation, fruit set and fruit development among different cultivars on different sites in field-grown litchi trees (b) characterise the fruits of the local & introduced cultivars and (c) evaluate the genetic variability among local and imported cultivars and within the Tai So cultivar based on their polyphenolic chemistry.

**MATERIALS AND METHODS**

Nine cultivars growing on three sites were studied during 2001, 2003 and 2004 (Table 1). All trees under study were fertilised and irrigated as recommended (Bahorun et al., 1998).

<table>
<thead>
<tr>
<th>Site</th>
<th>Cultivar</th>
<th>Number of trees observed</th>
<th>Date planted</th>
<th>Source/Origin of the planting material</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labourdonnais (alt 50m; Normal Min and Max Temp 16.1°C/31.1°C; Normal Rainfall: 1361mm)</td>
<td>Tai So</td>
<td>4</td>
<td>1991</td>
<td>Labourdonnais</td>
<td>Observations made in 2004 only</td>
</tr>
<tr>
<td></td>
<td>Yook Ho Pow</td>
<td>1</td>
<td>1998</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td>Réduit (alt 250m; Normal Min and Max Temp 15.3°C/ 28.2°C; Normal Rainfall: 1509mm)</td>
<td>Tai So</td>
<td>4</td>
<td>1991</td>
<td>Barkly ES</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hei Ye</td>
<td>1</td>
<td>1991</td>
<td>China</td>
<td>Did not set fruits in 2004</td>
</tr>
<tr>
<td></td>
<td>Brewster</td>
<td>1</td>
<td>1991</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bengal</td>
<td>1</td>
<td>1991</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bosworth 3 (B3)</td>
<td>2</td>
<td>1991</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yook Ho Pow</td>
<td>1</td>
<td>1998</td>
<td>Australia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Huai Zhi</td>
<td>1</td>
<td>1991</td>
<td>China</td>
<td></td>
</tr>
<tr>
<td>Paillotte (alt 350m; Normal Min and Max Temp 15.2°C/ 27.7°C; Normal Rainfall: 2039mm)</td>
<td>Tai So</td>
<td>2 to 6</td>
<td>1987</td>
<td>Barkly Experimental Station</td>
<td>Did not flower in 2004</td>
</tr>
<tr>
<td></td>
<td>Hong Kong</td>
<td>1</td>
<td>1987</td>
<td>Barkly ES</td>
<td>Observations made in 2004 only</td>
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<tr>
<td></td>
<td>Calcutta Late</td>
<td>1</td>
<td>1987</td>
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</tr>
<tr>
<td></td>
<td>B3</td>
<td>1</td>
<td>1997</td>
<td>Australia</td>
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</table>
Phenological observations

Observations were made on ten tagged terminals uniformly distributed and positioned at eye level on each tree. The timings for flower panicle emergence (2-3 cm long panicle), fruit set (visible fruitlet) and harvest were recorded. The diameter of one fruit per panicle was also monitored at weekly interval from the time of fruit set until harvest.

Climatic data

Daily minimum and maximum temperatures were obtained from the closest meteorological stations during the study period. The date at which the temperature started to fall consistently below the critical temperature of 20°C was recorded and the number of weeks from this temperature drop to start of flowering noted. The daily duration of temperatures above the critical temperature of 20°C during the preflowering period was also read from thermogrammes available at the Mauritius Meteorological Services.

Fruit Characterisation

At harvest, 50 fruits were randomly collected from each tree and characterised according to skin colour (as per Royal Horticultural Society Colour chart), texture (prickly or smooth), shape (round or oval), size, weight, °brix, % of chicken-tongued seeds and pulp:seed ratio.

Chemotaxonomic Characterisation

Extraction

50g of mature leaves were extracted first with acetone/water (70/30 v/v) (300 ml) and with pure methanol (2 X 300 ml). Filtrates were concentrated in vacuo at 37°C and the resulting aqueous extract was washed with dichloromethane to remove lipids and chlorophylls before being freeze-dried. The residue was then taken up in absolute methanol (1g FW/5ml).

Leaf extracts were examined by one-dimensional thin-layer chromatography on silica gel plates (Merck). Proanthocyanidins were analysed after migration in toluene-acetone-formic acid (3:3:1, v/v/v) (Lea et al., 1979) and visualized by vanillin-HCl spray reagent. Flavonoids were separated in ethyl acetate-formic acid-water (8:1:1, v/v/v) and revealed by 1% 2-aminoethyldiphenyl borate solution in methanol followed by 5% poly-(ethylene glycol) 4000 in absolute ethanol at 365 nm (Lamaison and Carnet, 1990).
RESULTS & DISCUSSION

Phenological Observations

Figure 1 Phenological observations (extreme values) made on the three sites during the three years

Tai So, which was the only cultivar present on the three sites, behaved in a similar pattern during the three years of observation. Panicle emergence was earliest at Paillotte (end of June/beginning of July), followed by Réduit (first to second week of July) and last at Labourdonnais (second to third week of July) (Figure 1). Fruit set was earliest at Labourdonnais (end of August/beginning of September), followed by Réduit one to two weeks later and last at Paillotte (last two weeks of September). Similarly, fruit maturity occurred first at Labourdonnais (mid November to first week of December), followed by Réduit (first to second week of December) and last at Paillotte (second to third week of December). Similar behaviour of panicle visibility, fruit set and fruit maturity were observed for Yook Ho Pow in 2004.

Differences were also observed among the studied cultivars. The timing of flowering as well as duration of flowering and time taken for fruit development and ripening influenced the harvest date of the cultivars. The cultivars which flowered late include Bengal, Calcutta Late, Hei Ye and Hong Kong. Menzel et al., (1989) observed that different cultivars responded differently in their temperature requirements prior to flowering. Hence late flowering in certain cultivars could be attributed to their longer chilling period. Such cultivars may be more adapted to the cooler sites.

The harvest time of the cultivars was also influenced by duration of flowering. At Réduit, it was found that although Brewster flowered at the same time as Tai So, the flowering season of the former was 2 weeks longer and it was consequently harvested later in the season. The time taken from fruit set to harvest also differed for different cultivars. Though Hei Ye and Brewster set fruits at the same time in

Réduit, fruit growth and ripening were faster for the latter. Irrespective of site similar duration of flowering was observed for Yook Ho Pow and Tai So, yet the duration of fruit growth and ripening was 2 to 3 weeks longer for the latter within sites and hence it was harvested later (**Figure 1**). Depending on their harvest date, the cultivars could be classified as follows:

- **Very Early (Yook Ho Pow):** Flowered early, set fruit early and were also harvested very early (Beginning of November to Late November depending on the site).

- **Early to Mid (Tai So):** Flowered early, set fruit early but harvested over a longer span extending from November to mid December.

- **Mid to Late (B3 and Brewster):** Flowered early but set fruit late and harvested only in the second to third week of December.

- **Late (Bengal, Calcuttia Late, Hei Ye and Hong Kong):** Flowered late, set fruit late and harvested in mid December extending to January.

The seasonality of the cultivars depend on environmental conditions. Tai So is considered to be an early to mid cultivar in Southern Queensland (Campbell and Campbell, 2001) and Israel (Goren et al, 2001). However B3 was reported to be an early cultivar in Southern Queensland but is a very late cultivar in Israel. Our study indicated that it behaved like a mid to Late cultivar locally. These findings show that maturity time depends on both the cultivar as well as the environment prevailing on the location. Hence it is important to collect more field data on more locations to validate the seasonality of locally grown cultivars.

**Temperature Effect on Flowering**

Flowering for Tai So was associated to the pattern of temperature drop below 20°C. **Figure 2**, representative of the three sites, showed that panicle visibility followed the drop in temperature below 20°C. It was also observed that different climatic conditions prevailing during different years influenced the timing of flowering, an earlier drop in temperature led to earlier flowering within sites (**Figure 2**).

**Figure 2** Mean Daily Temperature (5 day average) at Paillotte during the three study years

Trend is representative for the sites.
Note: PV: Panicle is visible.
The trend was similar for panicle emergence (of Tai So) at the three sites. The time taken from the consistent temperature drop to the start of flowering for Tai So ranged from 8 to 10 weeks at Labourdonnais and from 8 to 11 weeks at Paillotte and Reduit as shown in Table 2.

Table 2 Number of weeks taken from temperature drop to visible panicles

<table>
<thead>
<tr>
<th>Site</th>
<th>Paillotte</th>
<th>Réduit</th>
<th>Labourdonnais</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of consistent temperature drop (below 20°C)</td>
<td>15 Apr</td>
<td>18 May</td>
<td>19 April</td>
</tr>
<tr>
<td>Number of weeks to panicle emergence</td>
<td>10-11</td>
<td>8-9</td>
<td>10-11</td>
</tr>
</tbody>
</table>

The earliest flowering for Tai So at Paillotte was associated to the pattern of temperature drop below 20°C. Table 2 shows that the drop in temperature below 20°C was earliest at Paillotte, followed by Réduit and Labourdonnais.

In a set of studies conducted on a range of cultivars including Tai So, Menzel and Simpson (1988) found that panicles emerged after 6-8 weeks at 15/10°C and 8-10 weeks at 20/15°C. However these were glasshouse studies where the temperatures were constant i.e. 12 hrs for day and night temperatures respectively. Although our study was conducted in the field where the temperature fluctuated, the time taken for panicle emergence following exposure to low temperatures was not very far from those found in these glasshouse studies.

Even if critical temperatures of 15°C (in subtropical areas) and 20°C (in tropical areas) have been established for floral initiation in litchi (Menzel, 2001), limited data are available on the effect of supracritical temperature duration on flowering. The only study reported was conducted by Menzel and Simpson (1995) on the cultivar Wai Chee. They found that flowering was best after ten weeks at 15°C, while periods of 8 hours or more above 20°C per 24 hours were detrimental. The number of hours exceeding the critical temperature of 20°C in this study is tabulated in Table 3.

Table 3 Mean Daily Number of hours exceeding 200°C during pre-flowering period

<table>
<thead>
<tr>
<th>Number of weeks before panicle emergence</th>
<th>Daily number of hours above 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paillotte</td>
</tr>
<tr>
<td></td>
<td>2001</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: Data was averaged over weekly periods. Data was not available for Paillotte and for Labourdonnais in 2003.

Observations showed that the litchi trees were exposed to temperatures exceeding 20°C for 8 to 19 hours at Labourdonnais and 3 to 23 hours at Paillotte which in both cases exceeded the critical daily period of 8 hours mentioned by the previous authors. This finding could possibly be attributed to the tolerance of Tai So to higher temperatures (low chill) compared to Wai Chee or to other local environmental parameters.
Phenological, fruit and chemotaxonomic characterization of litchi cultivars in Mauritius: preliminary findings.

**M Madhou et al.**

**Temperature Effect on Fruit Set and Maturity**

The maximum temperatures in Labourdonnais and Réduit were found to be 2°C - 4°C and 1°C higher than in Paillotte respectively (**Figure 3**) from the time of panicle emergence to harvest.

**Figure 3** Mean daily maximum temperature (5 day average) for 2001 during panicle development and fruit growth

![Daily Mean Maximum Temperature](chart)

Research has shown that warmer temperatures increased the rate of panicle development and flower development as well as shortened the duration of anthesis in a range of cultivars including Tai So (Menzel and Simpson, 1991). Hence it is likely that in this study early fruit set and maturity were positively influenced by warmer temperatures. This is supported by the fruit growth observations on Tai So on the three sites (**Figure 4**).

**Figure 4** Fruit growth (Tai So) on the three sites in 2001.

![Average fruit diameter](chart)

Though flowering was initiated first in Paillotte followed by Réduit and Labourdonnais respectively, fruit growth was higher on warmer sites and harvest took place in the reverse order. This observation is similar to observations made both locally (Ramburn, 1997) and in Australia where in cooler subtropical areas such as Nambour (Lat 270S) early cultivars like Tai So develop panicles early (in May) but in warmer tropical areas like Cairns (Lat 170S) panicles appear only in July, but harvest is done first in Cairns (November) before Nambour (December) (Menzel and Simpson, 1994). These temperature responses are therefore important factors to be determined when identifying locations for early maturity.
Phenological, fruit and chemotaxonomic characterization of litchi cultivars in Mauritius: preliminary findings.
M Madhou et al.

Fruit characterisation

Most cultivars had oval fruits except B3, Hei Ye, Hong Kong and Huai Zhi which had round fruits. Fruit texture at harvest was prickly except for Hong Kong which was smooth to prickly. The outer skin colour was generally deep red (RHS Code: 46A-46B), except for B3 and Yook Ho Pow which were yellow green, Yook Ho Pow being more on the green side (RHS Code: 149A) and B3 being more yellow (RHS Code: 150B). Fruit characteristics are tabulated in Table 4.

Table 4: Fruit Characteristics of studied cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Average Fruit Weight (g)</th>
<th>Chicken-tongued seeds (%)</th>
<th>Pulp: seed ratio</th>
<th>°Brix</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Length</td>
</tr>
<tr>
<td>Tai So/Labourdonnais</td>
<td>19 ± 0.5</td>
<td>55 ± 17.6</td>
<td>9 ± 6.6</td>
<td>18 ± 1.5</td>
<td>33 ± 1.5</td>
</tr>
<tr>
<td>Tai So/Reduit</td>
<td>19 ± 0.4</td>
<td>14 ± 10.4</td>
<td>6 ± 1.5</td>
<td>18 ± 1.1</td>
<td>33 ± 2.2</td>
</tr>
<tr>
<td>Tai So/Paillotte</td>
<td>19 ± 0.6</td>
<td>28 ± 16.8</td>
<td>7 ± 1.5</td>
<td>18 ± 2.1</td>
<td>34 ± 1.5</td>
</tr>
<tr>
<td>Yook Ho Pow</td>
<td>21 ± 1.9</td>
<td>81 ± 8.9</td>
<td>12 ± 2.4</td>
<td>20 ± 0.8</td>
<td>35 ± 1.1</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>18 ± 0.5</td>
<td>0</td>
<td>6 ± 1.2</td>
<td>18 ± 0.9</td>
<td>29 ± 0.9</td>
</tr>
<tr>
<td>Hei Ye</td>
<td>16 ± 1.6</td>
<td>0</td>
<td>5 ± 1.5</td>
<td>15 ± 0.7</td>
<td>27 ± 2.8</td>
</tr>
<tr>
<td>Bengali</td>
<td>20 ± 4.3</td>
<td>3 ± 2.3</td>
<td>3 ± 0.8</td>
<td>17 ± 0.6</td>
<td>36 ± 2.1</td>
</tr>
<tr>
<td>B3</td>
<td>17 ± 2.6</td>
<td>43 ± 46.3</td>
<td>8.7 ± 4.2</td>
<td>15 ± 1.0</td>
<td>30 ± 1.4</td>
</tr>
<tr>
<td>Brewster</td>
<td>17 ± 2.1</td>
<td>10 ± 9.2</td>
<td>11 ± 6.4</td>
<td>14 ± 0.5</td>
<td>32 ± 1.1</td>
</tr>
<tr>
<td>Calcuttia Late</td>
<td>20 ± 1.9</td>
<td>5 ± 8.1</td>
<td>4 ± 1.3</td>
<td>17 ± 3.2</td>
<td>36 ± 0.5</td>
</tr>
<tr>
<td>Huai Zhi</td>
<td>16 ± 1.2</td>
<td>0</td>
<td>5 ± 1.2</td>
<td>18 ± 1.2</td>
<td>31 ± 1.5</td>
</tr>
</tbody>
</table>

Yook Ho Pow, Bengal, Calcuttia Late and Tai So were large in size (length and width attaining 30 mm or more). Hong Kong, Huai Zhi, Hei Ye and B3 were smaller. These findings agree with Goren et al. (2001) who found that Hei Ye and B3 tend to be small in Israel.

Yook Ho Pow and Tai So recorded very good fruit quality with high average weights, high chicken-tongue seed percentages, high pulp to seed ratio and high brix. Yook Ho Pow is hence a newly introduced cultivar of commercial interest. Apart from the mentioned quality characteristics it also matured early as discussed earlier. However its green colour might not be appealing to consumers. The Tai So studied at Labourdonnais had a higher % of chicken-tongued seeds than the Tai So of the other two sites. This difference could be either due to biotic or/and abiotic factors of the site or to a genetic difference. The trees observed at Labourdonnais originated from parent clones which existed on the site while the Tai So of Reduit and Paillotte were from a selected clone which is propagated at Barkly ES (North-Coombes and Julien, 1945). These two clones should be studied simultaneously on the three sites in order to determine whether the high % of chicken-tongued seeds is site dependent or genetic.

Chemotaxonomic characterisation

Chemotaxonomic markers, such as polyphenolics, more particularly flavonoids have already shown their value as reliable elements to establish relationships within and among various plant taxa (Williams et al., 1983; Webb and Harborne, 1991; Petrovic et al., 1999; Lai Fang et al., 2000). Thin Layer Chromatography was used in first instance to investigate the flavonoid and flavan-3-ol profiles of the studied cultivars. TLC analysis of leaf flavonoids is shown in Table 5.
It was found that flavonoids A, B, D, F, G, I, J and L were present in almost all the studied cultivars, except for a few cases. However flavonoid K was not very common among cultivars, it was present only in Yook Ho Pow, Yuan Zhi and Bengal and present in trace amounts in two out of the eight Tai So cultivars (Table 5).

Quantitative variation was recorded between the Tai So cultivars. Compounds A and B were present in all Tai So cultivars except for 1 cultivar in Réduit where they were found in trace amounts. Compound C was present in all Tai So cultivars but prominent only in cultivars from Labourdonnais and Paillotte. Compound D was also present in all cultivars but only trace amounts were detected in both cultivars from Réduit and one cultivar from Paillotte. The only qualitative difference was noted for flavonoid E, which was present only in 2, clones from Paillotte and Réduit respectively and for M which was detected in trace amounts in only three cultivars from Réduit and Paillotte respectively. Flavan-3-ol profiles of the studied cultivars are shown in Table 6.

Table 5 Thin layer chromatography data showing the distribution of leaf flavonoids in litchi cultivars
[system Ethyl Acetate/Formic acid, 8:1:1(v/v/v); Detection: DPBAE, 360nm]

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Flavonoids Compounds Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Tai So/L</td>
<td>+</td>
</tr>
<tr>
<td>Tai So/R</td>
<td>tr</td>
</tr>
<tr>
<td>Tai So/P</td>
<td>++</td>
</tr>
<tr>
<td>YHP/R</td>
<td>+</td>
</tr>
<tr>
<td>HY/R</td>
<td>++</td>
</tr>
<tr>
<td>HZ/R</td>
<td>tr</td>
</tr>
<tr>
<td>HKG/P</td>
<td>+</td>
</tr>
<tr>
<td>CL/P</td>
<td>tr</td>
</tr>
<tr>
<td>BENG/P</td>
<td>+</td>
</tr>
</tbody>
</table>


Estimated from the areas and colour intensity of the colour spots

Table 6 TLC data showing the distribution of leaf flavan-3-ol derivatives in the studied litchi cultivars
[system Toluene/Acetone/Formic acid, 3:3:1, (v/v/v); Detection: Vanillin/HCL, visible light]

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Proanthocyanidin oligomers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RF 0.1</td>
</tr>
<tr>
<td>Tai So/L</td>
<td>tr</td>
</tr>
<tr>
<td>Tai So/R</td>
<td>tr</td>
</tr>
<tr>
<td>Tai So/P</td>
<td>tr</td>
</tr>
<tr>
<td>YHP/R</td>
<td>tr</td>
</tr>
<tr>
<td>HY/R</td>
<td>tr</td>
</tr>
<tr>
<td>HZ/R</td>
<td>+</td>
</tr>
<tr>
<td>HKG/P</td>
<td>+</td>
</tr>
<tr>
<td>CL/P</td>
<td>+</td>
</tr>
<tr>
<td>BENG/P</td>
<td>+</td>
</tr>
</tbody>
</table>


Estimated from the areas and colour intensity of the colour spots
Very low levels (almost negligible) of flavan-3-ols were recorded in Tai So cultivars from Labourdonnais.

Common derivatives to all cultivars were the oligomers (Rf 0.1 and 0.3), catechins and the dimers with Rf between 0.4 and 0.5. However quantitative variation was recorded for these constituents. One of the Tai So tree in Paillotte recorded only trace amounts of these derivatives while they were prominent in one Tai So tree from Réduit and in Yook Ho Pow (except for catechins) (Table 6). The only qualitative variation recorded was for the oligomer Rf 0.2 which was absent in one Tai So tree from Réduit, Huai Zhi and Bengal.

The qualitative and quantitative variations in flavonoid and flavan-3-ol distribution of different cultivars were attributed to their genetic variability as many studies have shown that such differences are reflective of inter specific or intra specific variation in a number of plants (Spranger et al., 1998; Bohm et al., 1999; Santos and Salatino, 2000; Williams et al., 2000, Amaral et al., 2001; Lai Fang et al., 2001a, 2001b; Bahorun et al., 2003; Chang and Jeon, 2004; Mimura et al., 2004).

However quantitative variation was also recorded within Tai So cultivars. Only trace amounts of flavan-3-ols were detected in the four Tai So trees from Labourdonnais while higher concentrations were detected in other studied Tai So trees. The cause of this variation is still being investigated. It could be attributed to climatic factors as research work worldwide has shown that environmental factors such as temperature, solar radiation, altitude and rainfall can influence the polyphenolic levels of plants (Parks et al., 1972; Van Braderode and Van Kooten., 1983; Bahorun et al., 2003; Alonso-Amelo et al., 2004; Chang and Jeon, 2004; Mimura et al., 2004).

However it should be noted that the fruit quality of the Tai So cultivars from Labourdonnais was also different from the other sites in terms of percentage of chicken-tongue seeds (Table 4) and secondly the trees from Labourdonnais originated from a common source in the North as opposed to the other Tai So trees which were propagated from Barkly Experimental Station. So there also exists the possibility of genetic variation between these two sources but it is recommended to conduct further research (use of isozyme analysis and DNA fingerprinting) to confirm this.

CONCLUSION

Preliminary observations indicate that floral panicle of the most commonly grown cultivar Tai So emerged after temperatures dropped below 20°C for a period of 8 to 11 weeks. During that period, trees were exposed to temperatures exceeding the critical temperature of 20°C for 3 to 23 hours for every 24 hours. Continuous temperature reading devices have to be installed in the orchards to get more precise data on the influence of temperature on floral initiation of litchi. Under local conditions, early maturity would be associated with a low chilling cultivar planted in a warm region. In this respect late flowering cultivars requiring longer chilling periods could be evaluated on-farm on cooler sites where litchi is presently not productive.

During the period of study, the newly introduced cultivar Yook Ho Pow showed some interesting characteristics and performed even better than Tai So in terms of maturity and fruit quality. Data collected over this study period also indicated that the Tai So clone from Labourdonnais recorded higher percentages of “chicken-tongue” seeds than the Tai So on other sites. Moreover, thin layer chromatography showed that the flavan-3-ol concentration of that Tai So clone was lower than for the Tai So from the other sites. This observation is currently being confirmed by HPLC analysis of hydrolysed leaf extracts. More detailed studies of the genetic make up of selected clones (by DNA analysis) as well as more studies relating climatic conditions to fruit quality are required before attributing this better performance of Tai So in the North to genetic variation or to climatic conditions.
ACKNOWLEDGEMENTS

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Phenological, fruit and chemotaxonomic characterization of litchi cultivars in Mauritius: preliminary findings.
M Madhou et al.


AGROFORESTRY - A POTENTIAL SYSTEM TO POVERTY ALLEVIATION: THE CASE OF CALLIANDRA CALOTHYRSUS ON THE SLOPES OF MOUNT KILIMANJARO, TANZANIA.

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ABSTRACT

Lack of adequate and high quality animal feed is becoming a major problem on the slopes of Mount Kilimanjaro, leading to low livestock productivity. Dissemination of quality fodder such as Calliandra and Leucaena trichandra has been carried out in Kilema, Marangu and Mamba wards since 1999 by the Selian Agricultural Research Institute to improve the nutrition and productivity of animals. An informal survey was conducted in May 2003 to capture the farmers’ dissemination processes, experiences and opinions, management practices and biomass yield. It was found that farmers are well aware of the good qualities of Calliandra and are eager to grow more. It was also noted that Calliandra and Trichandra grow well in the Upland zone due to high rains and zero-grazing system, whereas in the Intermediate zone supplemental irrigation could improve its performance. It has however failed in the Lowland zone due to low rainfall and a free-grazing system. Those farmers with more than 100 calliandra and/or trichandra trees and have started feeding their dairy animals reported an increment of up to two litres per animal per day. They also reported that Calliandra and Trichandra are very palatable to livestock, reduce the cost of purchasing the commercial cakes by 50%, and saves time for looking for fodder. Despite the fact these fodder are very useful in the surveyed villages, the majority of farmers do not have enough plants to realize its full potential. Unavailability of seeds and seedlings is a major limitation to the expansion of Calliandra and Trichandra growing. Other limitations that featured very strongly are pests and diseases, especially in the Intermediate zone. Joint efforts by different partners are therefore needed to train farmers in pest and disease management, seed harvesting and processing, and dissemination so as to reach more farmers.

Keywords: Calliandra calothyrsus, fodder, poverty, income, Kilimanjaro, Tanzania

INTRODUCTION

The high population density and continuing land fragmentation in the highlands of mount Kilimanjaro have reduced the average land holdings to 1.7 acres with a range from 0.5 to 3 acres (Fernandes et al., 1984), Lyamchai et al., 1998; Lyimo et al., 1999). Free grazing is no longer possible and even pasture establishment is quite limited. The main fodder resources available are banana-based diets (banana pseudo stems and leaves), indigenous fodder trees, grasses collected from the lowlands, roadsides and riversides. In the dry season, animals are fed on crop residues such as maize stover and bean haulms. Most of these feeds are normally low in quality and digestibility. In general, the livestock diets lack protein and mineral sources which can only be obtained by supplementing the diets with commercial concentrates. Unfortunately, most farmers cannot afford to buy the commercial concentrates due to low availability and high prices. All these problems have caused the average number of animals per household to drop from 15 in the sixties to 7 in the late nineties on average (Lyamchai et al., 1998, Lyimo et al., 1999), and resulted in low animal productivity. Farmers realize few benefits from livestock keeping, as the animals’ production potential is not fully exploited. Such low productivity in livestock has contributed to poverty for the majority of farmers.

It is against this background that different exotic, high-value fodder species were introduced in 1999 in Kilema, Mamba and Marangu wards by the Selian Agricultural Research Institute (SARI) in collaboration with AFRENA-ECA. Fodder species introduced included Calliandra calothyrsus (calliandra), Sesbania sesban, Leucaena trichandra (trichandra) and Flemingia macrophylla, and Napier grass. The species were introduced primarily to alleviate fodder shortages, provide higher quality fodder, and increase milk production.
Activities under AFRENA-ECA stopped in the same year, in 1999, due to lack of funding. However, a visit to the Marangu sites in September 2002 showed that farmers had continued growing, utilizing and sharing Calliandra and Trichandra with other neighbouring farmers. Those who were feeding Calliandra and/or Trichandra to milking cows noted an increase in quality and quantity of milk, and therefore went on looking after the few trees they had. Others experimented in nursery establishment to add more trees. Surprisingly, the other fodder materials that were introduced together with Calliandra and Trichandra did not get such a big attention from the farmers. The farmers’ choice of Calliandra and Trichandra coincides with research results that imported fodder species such as *Leucaena trichandra*, *Morus alba* and *Desmodium intortum* have shown promise but by far the most successful and popular with small-scale dairy farmers has been Calliandra (New Agriculturalist, 2003).

Calliandra is a small, leguminous tree native to the humid and sub humid regions of Central America and Mexico (Palmer et al, 1991) and grows well in a wide range of ecological conditions. The tree grows quite fast and produces quality fodder, fuelwood, stakes, poles and bee forage as well as in soil fertility improvement, erosion control and serving as an ornamental tree (Roothaert et al., 1998; Wambungu 2002, New Agriculturalist 2003). The benefits are realized within the first year after planting and can continue for more than 10 years. Trichandra also has similar qualities and characteristics. Calliandra and Trichandra have been found to be rich in protein thus appropriate for improving the nutrition of animals and consequently the quality and quantity of milk production. Researchers and farmers in Central Kenya have found that high-protein fodder shrubs such as Calliandra can solve many problems - and boost income by 10% (New Agriculturalist, 2003). Franzel et al., (2003) found that Calliandra and Trichandra fodder shrubs are an attractive alternative to the expensive protein concentrates that farmers feed their cows and goats. The leaves can be used either as a substitute for dairy meals or as a supplement to it; in both cases farmers claimed Calliandra to increase milk production by 88%.

Based on what transpired from the September 2002 visit, it was proposed and agreed to conduct an informal survey on Calliandra dissemination to explore the processes, farmers’ experiences and opinions, as well as biomass yield, planting and feeding patterns. Other factors examined included competition with other crops, demand from neighbours or friends, advantages and problems associated with Calliandra, and its utilization in conjunction with other forages such as Napier grass and local species. This report documents and synthesizes the data collected, and gives some recommendations. A summary on the post-survey activities and their impact on scaling up Calliandra and Trichandra are given.

**METHODOLOGY**

An informal survey using a checklist was conducted from 10th to 12th February and 5th to 8th May 2003 in Kilema and Marangu wards where a total of 44 farmers were interviewed. The extension staff selected the interviewed farmers ensuring that they are composed of:

- farmers who had interacted with researchers in 1999, planted some Calliandra and/or Trichandra trees, and were involved in a farmer-to-farmer visit to Kenya in July 1999,
- farmers who had interacted with researchers in 1999 and planted Calliandra and/or Trichandra, but did not visit Kenya,
- farmers who had not interacted directly with researchers, but through their fellow farmers had either planted, heard about or become interested in Calliandra and/or Trichandra.

Group interviews were held with category (i) farmers whereas the rest of the farmers were individually visited and interviewed at their homesteads.

**RESULTS**

The informal survey was conducted in May 2003 in Masaera, Marawe-Kyura, Mshiri, Ashira, Rauya and Makuyuni villages. Forty two (42) farmers were interviewed 18 out of which were female. Each interviewed farmer has an average of 2 to 3 cows and 3 to 4 goats. 67 % and 23 % of these households have improved cows and goats respectively. Such a high percentage of improved animals especially cows indicates that framers are aware of their better productivity than the indigenous types. Calliandra established well above 1000 m.a.s.l contour line and was attributed to high moisture availability, zero grazing system and highly interested farmers. Common niches where Calliandra and Trichandra were
planted are along the walkways, farm boundary and around the homesteads for the majority of the farmers.

The interviewed farmers are endowed with a large variety of indigenous fodder trees such as Persea americana, Mangifera spp, Cussonia holistii, Dracaena steudeneri, ‘Maringiri’, Bridelia micrantha, Commiphora zimmermanni, Eriobotrya japonica, banana pseudo stems and leaves. These indigenous fodder are mixed with calliandra and trichandra when feeding. The survey showed that the majority of farmers (67%) got 5 to 20 seedlings. The rest got between 100 and 250 seedlings.

**Table 1** Numbers of Calliandra seedlings farmers initially received and numbers they currently have (42 farmers)

<table>
<thead>
<tr>
<th>Numbers of trees</th>
<th>Farmers' initial plantings</th>
<th>Farmers current status (2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Numbers of farmers</td>
<td></td>
</tr>
<tr>
<td>1-10</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>11-20</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>21-50</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>51-100</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>101-300</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>301-500</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Above 501</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>New farmers</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Note: Most farmers received trees for the first time in 1999 from SARI, a few received in 2000, 2001, or 2002 from their fellow farmers.

**Table 1** depicts that there has been a gradual increase in the number of plants per farmer since 1999. Despite researchers absence, farmers went ahead with taking care of the introduced fodder and feeding their animals. Having fed their animals, they reported increased milk quantity by 1 to 2 L per animal per day and also increased butter content as they get higher ratings of their milk at milk collecting centers. Similar results were reported by farmers in Babati district (Lyamchai et al., 2003). 44% of the interviewed farmers mentioned Calliandra and Trichandra to have reduced the use of commercial concentrates by 50% and reduced time for looking for fodder as more fodder is available around the homestead. As a result, farmers increased the number of trees (**Table 1**) and management. For instance manure that is normally applied to high value crops namely banana, coffee and vegetables is now applied to Calliandra and Trichandra (**Figure 1**).

**Figure 1** Manure is normally applied to high value crops like coffee, banana and vegetables but currently it is also applied to Calliandra and Trichandra.

43% of the farmers had attempted to increase the population of Calliandra and Trichandra in their farms especially those who went to Kenya in 1999 for a farmer-to-farmer visit and saw how beneficial these fodders were to the Kenyan farmers. It was also noted that some farmers had started collecting the seeds (**Table 2**), shared with neighbours and friends and tried their own nurseries.
Table 2 Amount of Calliandra seeds collected

<table>
<thead>
<tr>
<th>Name</th>
<th>Village</th>
<th>Amount collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mrs Jovita Shayo</td>
<td>Marawe-Kyura</td>
<td>4 Litre container full</td>
</tr>
<tr>
<td>Mr Raymond Mnenei</td>
<td>Ashira</td>
<td>¾ kg</td>
</tr>
<tr>
<td>Edward Kwimbere</td>
<td>Marawe-Kyura</td>
<td>Very small (about 1 handfull)</td>
</tr>
<tr>
<td>Mrs Kalebi Mosha</td>
<td>Mshiri</td>
<td>¼ kg</td>
</tr>
<tr>
<td>Mr Frank Shayo</td>
<td>Mshiri</td>
<td>½ kg</td>
</tr>
</tbody>
</table>

Few farmers tried feeding Calliandra to local chickens and goats and realized increased growth rates in goats and more yellow egg yolks. They also tried several strategies to fight against pest and diseases on Calliandra and Trichandra such as a mixture of cow urine and dung against scales, wood ash and Tithonia solution against ants, scales and mites; and Tephrosia against moles. There were also some negative experience with Calliandra such as blotting of animals to the extent of killing when fed on Calliandra alone, not good for staking as it is too weak and it is easily attacked by scales, ants, mites and moles. Some other comments by farmers are shown in Box 1.

Box 1 Some quotes from farmers pertaining to the advantages and disadvantages of Calliandra

Calliandra advantages

Fausta Lazaro:
- “It saves costs because when there is no money to buy commercial feeds, Calliandra can do”
- “the soil underneath the plant turns black and the roots of Calliandra, hold the soil; not easily moved downslope by runoff”.
- Regarding the palatability she said “The animals like the fodder very much; they eat almost every part of it”.

Sabila Pual Shayo: “Hopefully Calliandra is going to cut down the costs I used to incur on buying maize bran very soon”.

Mrs Samweli Kimei: “I have also noted that Calliandra is a good fire wood as it catches fire even if it is not dries enough. It also sprout quite quickly making it available even in dry season when other forages are in scarce”

Mrs Glory Matowo: “Calliandra sprouts well even in dry season and it is very palatable to animals because, in a mixture with other fodder, animals do rush, select and eat Calliandra first”.

Raymond Mneney: “Given the stories I have heard and the little experience I am having on Calliandra, I have noted that I do not have enough of it. I am ready to uproot other unwanted trees, to create some space for Calliandra”.

Disadvantages of Calliandra

Victoria Desideri: “The major disadvantage I have experienced on Calliandra is that it is easily attacked by scales and ants (sisimizi)” she said. Also from Mrs Grace Nyange: “scale infestation is our biggest worry; scales can wipe out all the plants in a month”

Mr Voice Abisai Mtui: “The only disadvantage I have noted with Calliandra is that it is easily attacked by ‘Kisoori’ (Fusarium)”

Penda Laouo and Ruaichi: It is too much liked by goats. It is too hard to raise when you have baby goats roaming around. It is just too much liked by goats.
CONCLUSION AND WAY FORWARD

There is high potential for Calliandra and Trichandra in the area above 1000 m.a.s.l due to high moisture availability, zero grazing system and highly interested farmers. However, more species diversification is needed to minimize the risks of pest and diseases and improve animal nutrition. Economic analysis is also required to quantify the benefits of these shrubs to farmers and environment.

The upland and intermediate zones are endowed with a large variety of indigenous fodder trees and shrubs but very few of them have been studied and documented. It is therefore important to study them to understand their best management, propagation, nutritive values and utilization in conjunction with the exotic ones.

Scales, ants, mites and moles easily affect Calliandra especially during the dry season. Farmers have effectively used several integrated pest management strategies to control the pests and diseases. These strategies, however, need to be verified, documented and disseminated for a wider use.

Those farmers who participated in farmers exchange visits were highly motivated and are currently among those with more than 500 plants. They are also good dissemination agents within and outside their villages. Selecting more enthusiastic farmers and train them on seed harvesting and processing, management and utilization would accelerate the promotion of the intended fodder trees.

There is still a wide variation amongst farmers in the number of trees, management and utilization of the new fodder trees. Continuation of the training, sensitization and distribution of seeds was recommended and several approaches have been employed to disseminate Calliandra and Trichandra in the targeted villages after the survey. These include public meetings and trainings, placement of a fulltime extension staff in the target villages, use of farmer disseminators, use of extension materials and establishment of Calliandra database. Each strategy has had different effect as follows:

Sensitisation meeting and trainings
A series of sensitisation meetings and trainings to disseminate and empower the farmers with the skills on Calliandra management and utility have been conducted since May 2003. About 1,900 farmers have attended such meetings and trainings but only about 10% of them adopted the technology. Meetings and seminars usually attract a large number of farmers but it does not guarantee the take-up of messages and trainings.

Farmer disseminators
Some few excelling farmers who had been a source of information and planting materials since 2000 to other farmers were encouraged to establish nurseries so as to sale seedlings to the neighbouring farmers and villages. This approach had a strong convincing power as new farmers learn by seeing and hearing from the ‘horse’s mouth’ as depicted by Frank Shayo who commented “once people, mostly women, see Calliandra along my walkway and around the house they becomes curious to know it since it is a new plant in the area. I always take time explaining to them its uses, advantages and my experience. As soon as they hear that this particular tree increases milk production and reduces the costs of using maize bran and commercial concentrates, they immediately request for it’.

Full time extension staff
In March 2004, a local extension agent was contracted to interact with farmers frequently, provide guidance and solutions whenever necessary through door-to-door visits. Occasionally, research staff visits the sites for monitoring, evaluation and technical backup. This has been a very effective approach because it ensures frequent training, reminding, lessening from farmers and provides solutions to problems as soon as they occur. The approach was also used to disseminate seeds after a physical assessment of the farmer’s field. Within ten months we have raised the number of Calliandra and/or Trichandra adopters from 59 to 750 since March 2004.

Use of Extension materials
Available extension materials on Calliandra and other improved fodder trees were collected and used to train farmers. Their effectiveness in enhancing farmers’ adoption has not yet been studied.
Agroforestry - a potential system to poverty alleviation: The case of Calliandra calothyrsus on the slopes of mount Kilimanjaro, Tanzania. CJ Lyamchai and M Kingamkono

Establishment of database

A calliandra database was established where all farmers dealing with Calliandra and/or Trichandra are recorded. The database is used as a monitoring tool. Currently the database has 365 farmers with full-grown Calliandra and/or Trichandra 50% of which have more than 100 plants each. About 150 new farmers have been supplied with seeds and they established nurseries. The seedlings will be ready for transplanting in the coming (2005) long rainfall season.

Partnerships

Given the large number of farmers demanding for these fodders, the dissemination of Calliandra and/or Trichandra has been conducted in partnership with different organizations and institutions. These include Himo Environmental Management Trust Fund (HEM), Kilimanjaro Environmental Development Agency (KEDA), Heifer Project International –Tanzania, ICRAF, community based organizations (Kilimo Hai Kirua Vunjo), churches, schools and the extension system. Common interest, same geographical coverage and related activities strengthened the partnership between Selian A.R.I (SARI) and the partners. While KEDA and HEM contributed their experience in community mobilization in Kilimanjaro, SARI and ICRAF provided scientific knowledge and physical resources (seeds, transport and fund). Since we all had a common goal of increasing productivity in Moshi Rural district, implementation was relatively easy and cost effective. A good example is KEDA accepting to commit one staff to be fully engaged with interacting with farmers in promoting the fodder trees. On one hand this has significantly reduced the number of costly trips from SARI to the study area and on the other hand it has significantly increased the number of adopters thirteen times within 10 months. Furthermore, SARI did not have to forge partnership with HPI because KEDA had already established a strong relationship and were already distributing dairy goats and cattle in the study area.

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MAS 2005. Food and Agricultural Research Council, Réduit, Mauritius. 254
CHARACTERISATION OF THE CREOLE CATTLE IN MAURITIUS

Regis LAM SHEUNG YUEN

Agricultural Research and Extension Unit

ABSTRACT

The Creole cattle, termed as a local cattle, is a medium sized Bos taurus animal which has been in Mauritius for over two centuries and has adapted very well to the sub-tropical conditions over the years. It is currently facing extinction with very little work having been done on it. The study was carried out in two phases. In phase 1 a survey was conducted over the whole island for a period of 3 ½ months to collect data on morphological characteristics. A total of 273 adult animals, 14 males and 259 females, were identified based on their phenotypes and characterised. In phase 2, data on productive and reproductive parameters were extracted from records kept at Curepipe Livestock Research Station (a state owned farm) for the period 1999 to 2003 and analysed. The average lactation length was 255 ± 74 days with an average daily milk yield of 8.6 ± 1.0 litres/day. The peak yield occurred before 30 days of lactation. The pre-weaning (0 –90 days) average daily gain (ADG) was 551 and 524 g/d for male and female calves respectively while the post weaning ADG (up to 1 year of age) was 511 and 425 g/d for males and females respectively. The age at first calving was 946 ± 85 days. The overall conception rate for heifers was 67%. The calving interval was 432 ± 100 days.

Keywords: Characterisation, Creole cattle, phenotype, productive and reproductive parameters.

INTRODUCTION

The Creole breed of cattle also known as the “Vache Creole” is a medium sized Bos taurus animal and is characterized by its white colour, an absence of hump and its polledness. It falls best under the dual-purpose category of cattle being used for milk production while the males are fattened for beef production.

The origin of this breed remains a mystery and up to now nobody has been able to trace back with certainty from where these cattle originate. However there is general consensus that this breed has its origin in North Europe and most probably came through France if not from France. According to Bennie (1956), it is possible that this breed was introduced in the eighteenth century although the first introductions of cattle date back to 1511.

Up to the 70’s, the Creole cattle was the predominant breed kept in Mauritius (Table 1) and constituted over 75 % of the total population. (Livestock Statistics, 1966).
Table 1 Distribution of adult Creole Cattle across the island (1964)

<table>
<thead>
<tr>
<th>District</th>
<th>Number of males Creole</th>
<th>Number of males All breeds</th>
<th>Number of females Creole</th>
<th>Number of females All breeds</th>
<th>Total number of head Creole</th>
<th>Total number of head All breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port Louis</td>
<td>210</td>
<td>245</td>
<td>1054</td>
<td>1252</td>
<td>1264</td>
<td>1497</td>
</tr>
<tr>
<td>Pamplemousses</td>
<td>807</td>
<td>1232</td>
<td>3123</td>
<td>4857</td>
<td>3930</td>
<td>6089</td>
</tr>
<tr>
<td>Riviere du Rempart</td>
<td>915</td>
<td>1447</td>
<td>2895</td>
<td>4118</td>
<td>3810</td>
<td>5565</td>
</tr>
<tr>
<td>Flacq</td>
<td>1872</td>
<td>2088</td>
<td>6699</td>
<td>7323</td>
<td>8571</td>
<td>9411</td>
</tr>
<tr>
<td>Moka</td>
<td>393</td>
<td>409</td>
<td>2839</td>
<td>3062</td>
<td>3232</td>
<td>3471</td>
</tr>
<tr>
<td>Plaines Wilhems</td>
<td>531</td>
<td>686</td>
<td>3379</td>
<td>4553</td>
<td>3910</td>
<td>5239</td>
</tr>
<tr>
<td>Grand Port</td>
<td>510</td>
<td>715</td>
<td>2226</td>
<td>3132</td>
<td>2736</td>
<td>3847</td>
</tr>
<tr>
<td>Savanne</td>
<td>318</td>
<td>476</td>
<td>1175</td>
<td>1907</td>
<td>1493</td>
<td>2383</td>
</tr>
<tr>
<td>Black River</td>
<td>1206</td>
<td>1522</td>
<td>2180</td>
<td>2944</td>
<td>3386</td>
<td>4466</td>
</tr>
<tr>
<td>Overall</td>
<td>6762</td>
<td>8820</td>
<td>25570</td>
<td>33148</td>
<td>32332</td>
<td>41968</td>
</tr>
</tbody>
</table>


Production Environments

Housing system

Historically Creole animals were found predominantly in the smallholder sector where they were usually kept in small numbers ranging from 1 to 5 animals as a side activity. Initially they were being kept in tie-stalls in dark straw/thatch roofed sheds but with time the rearing conditions improved and the sheds were made of wood and iron sheets. Nowadays the animals that remain are reared in buildings made mostly of bricks/iron sheets or bricks/concrete with more consideration being given to aeration and light penetration. However, cattle rearing still remains a secondary activity of the family.

Feeding

The vast majority of animals has always been reared on the cut and carry system with sugar cane tops being the main fodder type during the five to six months (June to November) of the cane harvest season and mixed fodder for the rest of the year. A list of assorted fodder and grasses, vegetable crop residues, twigs, shrubs, creepers and tree foliage used for feeding cattle during the intercrop season has been compiled by Boodoo et al., (1988 a).

In the past, the use of concentrates was very limited but with time their utilization has become more widespread although many farmers who have adopted this practice do so only at certain specific times such as during late pregnancy and/or for a few months after calving. However it is to be noted that on government owned production units or research stations the inputs are substantially higher. Commercial concentrates like cowfeed and other reconstituted feeds, oil cakes (e.g. cottonseed cake and soyabean meal) as well as mineral supplements are used at all times.

Reproduction

In the past, natural service was being practised with the use of communal bulls but in the 1960’s following an extension campaign by the Veterinary Services, Artificial Insemination became the accepted method of reproduction by farmers (Sibartie, 1988), with natural service still being practised on large farms. Unfortunately this led to pure Creole cattle being crossed with the Friesian breed and resulted in animals with varying levels of crossing over time.

Health

In Mauritius there is no major disease affecting the cattle industry. The main health problem facing this sector is mastitis although most cases are sub-clinical rather than clinical ones (FAO, 1974). One survey showed that 72% of the animals had mastitis in one or more of their mammary glands (FAO, 1974).
The biting flies (*Stomoxys nigrina* and *S. calcitrans*) are the most important pests affecting cattle in Mauritius and they are found mostly on the Central area of the island where it is much more humid than the coastal areas.

**MATERIALS AND METHODS**

Since there is no recording system which would enable the identification of pure Creole animals, for the purpose of this exercise all adult animals that were phenotypically close to the pure Creole cattle were used. Thus all animals that had the typical white/creamish body coat colour were classified as suitable for the exercise. These animals were identified during the Farm Animal Genetic Resources Census and a data sheet filled for each animal of 2 years of age or older. A total of 252 animals from the small farmers and 21 from Government owned stations were characterized. Measurements were either taken within a range or as exact readings.

The productive and reproductive parameters were calculated from records of animals kept at Curepipe Livestock Research Station, which is a state owned farm. Most of these records stretched over the period 1999 to 2002.

**RESULTS AND DISCUSSION**

**Sex ratio**

Number of females: 259  
Number of males: 14

The small number of males as compared to the females is explained by the system of production and reproduction governing cattle rearing in Mauritius. Reproduction in the cattle sector is based mostly on use of artificial insemination and therefore most of the males are usually fattened and sold for slaughter after reaching 2 years of age.

**Location of the animals**

As at 1st October 2001, the phenotypically pure adult Creole animals were distributed as follows over the island (Table 2).

**Table 2** Distribution of adult Creole animals across the island (2001)

<table>
<thead>
<tr>
<th>District</th>
<th>Number of males Creole</th>
<th>Number of females Creole</th>
<th>Total number of head</th>
<th>Number of males All breeds</th>
<th>Number of females All breeds</th>
<th>Total number of head All breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port Louis</td>
<td>Nil</td>
<td>31</td>
<td>0</td>
<td>Nil</td>
<td>136</td>
<td>167</td>
</tr>
<tr>
<td>Pamplemousses</td>
<td>1</td>
<td>61</td>
<td>40</td>
<td>443</td>
<td>41</td>
<td>504</td>
</tr>
<tr>
<td>Riviere du Rempart</td>
<td>6</td>
<td>196</td>
<td>51</td>
<td>489</td>
<td>57</td>
<td>685</td>
</tr>
<tr>
<td>Flacq</td>
<td>1</td>
<td>112</td>
<td>29</td>
<td>808</td>
<td>30</td>
<td>920</td>
</tr>
<tr>
<td>Moka</td>
<td>1</td>
<td>35</td>
<td>58</td>
<td>469</td>
<td>59</td>
<td>504</td>
</tr>
<tr>
<td>Plaines Wilhems</td>
<td>3</td>
<td>25</td>
<td>47</td>
<td>137</td>
<td>50</td>
<td>162</td>
</tr>
<tr>
<td>Grand Port</td>
<td>2</td>
<td>52</td>
<td>23</td>
<td>206</td>
<td>25</td>
<td>258</td>
</tr>
<tr>
<td>Savanne</td>
<td>Nil</td>
<td>22</td>
<td>7</td>
<td>209</td>
<td>7</td>
<td>231</td>
</tr>
<tr>
<td>Black River</td>
<td>Nil</td>
<td>63</td>
<td>4</td>
<td>287</td>
<td>4</td>
<td>350</td>
</tr>
<tr>
<td>Overall</td>
<td>14</td>
<td>597</td>
<td>259</td>
<td>3184</td>
<td>273</td>
<td>3781</td>
</tr>
</tbody>
</table>

This shows that there has been a drastic decrease (> 90 %) in the number of Creole cattle over the island between 1964 (Table 1) and 2001.
Physical characteristics

Creole used in the study showed various minor characteristics of crosses but were considered pure. All Creole used had an off white/creamish colour and short, straight and glossy hair type. Pure Creole also had white to pink ears, muzzle and eyelids with no horns, off white hooves and ears that are laterally oriented. Creole with some crossing have horns, ears with black or brown colours, muzzles and eyelids with black, brown or grey colour variations (Figure 1).

Figure 1 Creole cattle showing various minor characteristics of crosses but are nevertheless considered as pure

The colour of the hoof could be off-white, black, brown or grey. The dewlap is generally small in size with variations that show absent or medium dewlap. The navel flap in most animals is small or absent.
Production characteristics

The information on reproductive and productive parameters based on actual records on station is given in Table 3.

Table 3 Production characteristics of Creole Cattle

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characteristics</th>
<th>No. of Records</th>
<th>Average ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average lactation length (days)</td>
<td>32</td>
<td>255 ± 62</td>
</tr>
<tr>
<td>2</td>
<td>Average daily yield (litres/day)</td>
<td>32</td>
<td>8.6 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>Average milk production per lactation (litres)</td>
<td>32</td>
<td>2206 ± 650</td>
</tr>
<tr>
<td>4</td>
<td>Birth weight of males (kg)</td>
<td>19</td>
<td>33.8 ± 4.6a</td>
</tr>
<tr>
<td>5</td>
<td>Birth weight of females (kg)</td>
<td>28</td>
<td>30.8 ± 3.3b</td>
</tr>
<tr>
<td>6</td>
<td>Weaning weight of males at 90 days (kg)</td>
<td>14</td>
<td>82.1 ± 8.5a</td>
</tr>
<tr>
<td>7</td>
<td>Weaning weight of females at 90 days (kg)</td>
<td>20</td>
<td>77.5 ± 6.3a</td>
</tr>
<tr>
<td>8</td>
<td>Pre-wean average daily gain of males (g/day)</td>
<td>15</td>
<td>551 ± 91a</td>
</tr>
<tr>
<td>9</td>
<td>Pre-wean average daily gain of females (g/day)</td>
<td>20</td>
<td>524 ± 73a</td>
</tr>
<tr>
<td>10</td>
<td>Post-wean average daily gain of males (g/day)</td>
<td>9</td>
<td>511 ± 87a</td>
</tr>
<tr>
<td>11</td>
<td>Post-wean average daily gain of females (g/day)</td>
<td>17</td>
<td>425 ± 78b</td>
</tr>
</tbody>
</table>

Means with same superscripts indicate no significant difference (p<0.05) between the sexes

Lactation

The daily records of individual milk production for 32 lactations for Creole cows at Curepipe Livestock Research Station were analysed. The curve for a typical lactation is shown in Figure 2. The peak yield for the lactation occurs before 30 days which is in contrast to previous studies where it is reported that the peak occurred during the second month of lactation. (Boodoo et al., 1988b, Boodoo, 1989).

Figure 2 Lactation curve for Creole cattle
The average lactation length was 255 ± 74 days. It is much lower than the quoted figures of 301 days or 10 months lactation length (Boodoo et al., 1988b; Boodoo, 1989; Milliken, 1968) and 308 days for large breeders (FAO, 1974). In contrast Bennie (1956) reported a lactation length of 201 days at the smallholder level but at the same time pointed out that this figure could be increased drastically by improved nutrition.

The average daily yield for the 255 days was recorded to be 8.6 ± 1 litres/day, which is in accordance with the reported figures of 8.3 and 9.6 liters per cow in the two localities of the island (Boodoo et al., 1988b). The average milk produced from these 32 lactations was 2206 ± 650 litres, which is also comparable with the reported figure of 2342 kg milk per lactation (FAO, 1974) but lower than the 2788 ± 232 litres reported by Boodoo, 1989.

Liveweight and Average Daily Gain

Weaning weight
The mean weaning weights of Creole calves at 90 days are given in Table 4. These calves each received 375 litres (bucket fed) of milk and 47.5 kg of calf starter concentrate (16-18% crude protein) during the 90 day period, fodder being ad-libitum.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average weaning weight (kg)</td>
<td>82.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD (kg)</td>
<td>8.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Minimum (kg)</td>
<td>69</td>
<td>60</td>
</tr>
<tr>
<td>Maximum (kg)</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>Count</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>

Same superscripts indicate no significant difference (p ≤ 0.05) between the sexes

Pre-weaning Average Daily Gain
The means of average daily gains (ADG) for male and female Creole calves from birth to weaning at 90 days are given in Table 5. These results are much higher than the reported figure of 417 g/d (both sexes together) under comparable pre-weaning treatments (FAO, 1974).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ADG pre-weaning (g/d)</td>
<td>551&lt;sup&gt;a&lt;/sup&gt;</td>
<td>524&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE (g/d)</td>
<td>91</td>
<td>73.2</td>
</tr>
<tr>
<td>Minimum (g/d)</td>
<td>422</td>
<td>311</td>
</tr>
<tr>
<td>Maximum (g/d)</td>
<td>717</td>
<td>678</td>
</tr>
<tr>
<td>Count</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

Same superscripts indicate no significant difference (p ≤ 0.05) between the sexes
Post-weaning Average Daily Gain

The means of average daily Gains (ADG) for male and female Creole animals after weaning up to 1 year of age are given in Table 6.

Table 6  Post-weaning ADG up to 1 year of age

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ADG post-weaning (g/d)</td>
<td>511&lt;sup&gt;a&lt;/sup&gt;</td>
<td>425&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE (g/d)</td>
<td>87</td>
<td>78</td>
</tr>
<tr>
<td>Minimum (g/d)</td>
<td>397</td>
<td>323</td>
</tr>
<tr>
<td>Maximum (g/d)</td>
<td>646</td>
<td>563</td>
</tr>
<tr>
<td>Count</td>
<td>9</td>
<td>17</td>
</tr>
</tbody>
</table>

Different superscripts indicate that there is a significant difference (p ≤ 0.05) between the sexes.

Reproductive parameters

The information on reproductive parameters based on actual records on station is given in Table 7.

Table 7  Reproduction characteristics of Creole cattle at Curepipe Livestock Research Station.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Characteristics</th>
<th>Records</th>
<th>Average ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weight at 1&lt;sup&gt;st&lt;/sup&gt; service (kg)</td>
<td>25</td>
<td>325 ± 41</td>
</tr>
<tr>
<td>2</td>
<td>Weight at 1&lt;sup&gt;st&lt;/sup&gt; conception (kg)</td>
<td>18</td>
<td>330 ± 45</td>
</tr>
<tr>
<td>3</td>
<td>Weight after 1&lt;sup&gt;st&lt;/sup&gt; calving (kg)</td>
<td>15</td>
<td>407 ± 24</td>
</tr>
<tr>
<td>4</td>
<td>Age at 1&lt;sup&gt;st&lt;/sup&gt; service (days)</td>
<td>22</td>
<td>663 ± 118</td>
</tr>
<tr>
<td>5</td>
<td>Age at 1&lt;sup&gt;st&lt;/sup&gt; conception (days)</td>
<td>16</td>
<td>664 ± 87</td>
</tr>
<tr>
<td>6</td>
<td>Age at 1&lt;sup&gt;st&lt;/sup&gt; calving (days)</td>
<td>16</td>
<td>946 ± 85</td>
</tr>
<tr>
<td>7</td>
<td>Number of service to conception</td>
<td>19</td>
<td>1.5 ± 0.8</td>
</tr>
<tr>
<td>8</td>
<td>Calving interval (days)</td>
<td>40</td>
<td>432 ± 100</td>
</tr>
<tr>
<td>9</td>
<td>Gestation length</td>
<td>17</td>
<td>278 ± 6</td>
</tr>
</tbody>
</table>

The age at first calving has been found to be 946 days or 31.5 months compared with 32.5 months in the field (Bennie, 1956). Bennie also reported a calving interval of 434 days at the Government owned Station of Palmar and this is similar to the average of 432 days found here.

The figure of 1.5 services per conception is much lower than the 2.9 found by (Bennie, 1956) for a government herd.

CONCLUSION

Along with a decrease in the livestock population, the Creole breed is gradually disappearing from the national herd. It is unfortunate that there has been very little work done in the past when the population size of this breed was much bigger. Nevertheless, it is quite clear from the above results that this breed has got interesting potentials both in terms of production and reproduction. However, a good selection and breeding programme is necessary in order to reverse the recent decreasing trend. In the short term, more work on the Creole cattle is essential and a solid conservation programme with all the different stakeholders should be envisaged if this breed is to be saved from extinction.
REFERENCES


AN ECONOMIC ANALYSIS OF MEDIUM AND SMALL DAIRY FARMS IN MAURITIUS

P. Toolsee, G. Saraye, R. Ramnauth, R. Fakim and A. Boodoo

Agricultural Research and Extension Unit

ABSTRACT

The objective of the study was to assess the economics of dairy farming with reference to the yearly farm income and the cost of producing a litre of fresh milk in medium (≥4 cows) and small dairy farms (4 cows) in Mauritius. Primary data were collected at the beginning of the study in each individual farm (August 2000). Data on production, expenses and returns were recorded on a monthly basis for each farm over a period of 12 months. The ‘net farm income’ method was used to calculate the farm income and, the ‘cost per equivalent income’ method was used to calculate the cost of production of a litre of milk. The net farm income (period of 12 months) ranged from Rs 19,238 to Rs 107,062 in small farms, and in medium farms, it ranged from Rs 112,545 to Rs 280,598. The cost of producing a litre of milk was Rs 5 and Rs 6 in medium and small farms respectively. Factors affecting the net farm income and the cost of producing a litre of milk are discussed.

Keywords: Economic analysis, medium farms, net farm income, non-cash revenue, unpaid labour and expenses.

INTRODUCTION

The smallholder dairy farms produce about 90% of the annual fresh milk, which amounts to around 5.5 million litres, around 5% of the national consumption. There are presently 2100 farmers owning about 6500 head of dairy cattle (Livestock Extension Division, AREU, 2004). The smallholder dairy producers, commonly known as cowkeepers, have preserved many of their traditional practices of husbandry and management. Cowkeeping constitutes a part-time activity and it is the women who are responsible for the day to day running of the unit.

The last decade witnessed the emergence of the so-called medium size dairy farms owning 4 to 20 cows. These farmers have been looking at dairying as an economic activity and they are looking forward to modernize their activities. About 75 medium size farms are known to be operating islandwide (Livestock Extension Division, AREU, 2004). Little information is available on the economics of production on the small and medium size dairy farms. This study was therefore undertaken to collect on farm data pertaining to revenue and expenses on both types of farms and make an economic analysis.

OBJECTIVE

The objective of the study was to calculate the net farm income and the cost of production of a litre of milk on the small and medium size farms.

METHODOLOGY

Regions

The study was conducted in the Centre (St-Pierre, Nouvelle Decouverte), East (Bon Accueil, Lallmatie) and Northern (Triolet) regions of the island. 24 medium size and 42 small farms were monitored. Data collection lasted from August 2000 till March 2002. Small farms were defined as those having less than 4 cows and medium farms those having 4 or more cows. The number of farms and cows that participated in the study is shown in Table 1.

Table 1  Number of farms surveyed per region

<table>
<thead>
<tr>
<th>Regions</th>
<th>North</th>
<th>Centre</th>
<th>East</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of</td>
<td>Number of</td>
<td>Number of</td>
<td>Number of</td>
</tr>
<tr>
<td></td>
<td>farms</td>
<td>cows</td>
<td>farms</td>
<td>cows</td>
</tr>
<tr>
<td>Medium</td>
<td>8</td>
<td>48</td>
<td>9</td>
<td>45</td>
</tr>
<tr>
<td>Small</td>
<td>15</td>
<td>32</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

Data collection

Each farm was visited twice weekly over a period of 12 months. The following data were recorded:

Milk yield: the amount of milk sold in the morning and afternoon as reported by the farmers.
All expenses related to the farm (concentrate bought at the Feed Sale Centers, transport cost, fuel, electricity, water, interest on loan, lease on land etc).
All income related to the farm (sale of calves, culls, manure etc).
Any change in the inventory of animals on the farms (during the 12 months period).

On certain farms data were collected for less than 12 months and in others for more than 12 months. All the data were then standardized over a period of 12 months.

Data analysis

A model was developed in Microsoft Excel program for data analysis. The following assumptions were made:

- Mean value of cow at Rs 30000
- Mean value of heifer at Rs 15000
- Estimated cost of labour needed to take care of one cow per year at Rs 15000
- Useful life of machinery 10 years
- Useful life of building 20 years
- Selling price of milk at farm gate at Rs 9.00 (guaranteed price offered by the Agricultural Marketing Board)

Net farm income (NFI)

It was observed that in both the small and medium size farming operations, the farm family provided all or most of the labour resource. Furthermore, unless the operation was organized as a corporation, family members were usually not compensated on a set wage basis but they withdrew money as needed to meet living and other personal expenses. Net farm income (NFI) is a measure of how a dairy farm’s business is faring and provides key information about the results of operating activities.
An economic analysis of medium and small dairy farms in Mauritius. P Toolsee et al.

over a period of time (usually one year). The net income per operation shows the amount that is available to meet family expenses. For the dairy business to be competitive its NFI should, in most years, considerably exceed the amount needed to meet family living. Calculating NFI requires data for 12 months with regards to revenues, expenses, change in inventory and depreciation.

In brief, NFI is the total income minus the total expenses minus the depreciation on building and machinery. It takes into account all cash and non-cash income and expenses and also the sale of assets that are part of the customary operations of the business. However, it does not consider a charge for family or operator labour, management or a return to owner’s equity capital (Aylew et al., 2003, Frank, 2001).

**Income**

Total farm income is calculated by summing the cash income and non-cash income.

**Cash income**

Cash income is the income derived from sale of milk, sale of calves, culls (cows), manure or any other transactions that involve receipt of money.

**Non-cash income**

Livestock is a necessary asset in the production function of a livestock enterprise. This asset falls into the same classification as the inventory change, however, it is a non-current asset and it is usually considered as “non-cash” income. To calculate non-cash income, the number of livestock in each class (heifer, cow) at the beginning and the end of the accounting year is multiplied by their assumed values. Then the ending value is subtracted from the beginning value. This equals the non-cash income.

**Expenses**

Total expenses were calculated by summing cash expenses, depreciation (on building and machinery) and non-cash expenses.

**Cash expenses**

This is the sum of expenses incurred on farm for buying of concentrate, transport cost, fuel, electricity and water, lease on land, interest on loans and any other transaction involving payment of money. The amount of interest paid was computed only for the period under study on any loan taken from a financial institution.

**Depreciation**

This is the decrease in monetary value of assets used in production, e.g. machinery and building. The straight-line method of depreciation was used to calculate the value of depreciation.

**Non-cash expenses**

This includes the changes in prepaid expenses and accounts payable.

**Cost of production of a litre of milk**

The cost of production per unit is the total cost associated with production divided by the number of units produced. For calculating the cost of producing a litre of milk, the value of unpaid labour needs to be included in the total farm expenses (Bernet et al., 2000). The estimated value of Rs 15000 was used for unpaid labour required to take care of one cow per year. In this paper two methods of calculating cost of producing a litre of milk are illustrated, mainly cost per equivalent income and cost per unit sold (Bailey, 1999).
Cost per equivalent income

In the cost per equivalent income method a divisor called the equivalent unit is calculated first. This was obtained by dividing the total farm income (for the 12 months period) by the average price of the milk. Summing all the expenses on the dairy farm (including value for unpaid labour) and dividing by the equivalent unit gives the cost of production of a litre of milk. The cost per equivalent income method is more relevant to calculate the cost of a litre of milk as dairy farms are joint product enterprises, i.e. they have multiple sources of income from milk (the major produce), manure and sale of animals as secondary products.

Cost per unit sold

In the cost per unit sold method, to calculate the cost of a litre of milk all expenses (including the value for unpaid labour) is divided by the number of litres of milk sold (major produce). Value of secondary products is not included in this method.

RESULTS AND DISCUSSION

Table 2 shows the net farm income against the type of farms.

<table>
<thead>
<tr>
<th>Type of Farms</th>
<th>Cows</th>
<th>Mean (Rs)</th>
<th>Range (Rs)</th>
<th>Type inventory change</th>
<th>Inventory change (Rs)</th>
<th>NFI (Rs) - Range (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDIUM FARMERS</td>
<td>6.0 - 6.9</td>
<td>190679</td>
<td>135762 - 280598</td>
<td>+ve</td>
<td>15000 - 90000</td>
<td>133729 - 245616 (3)</td>
</tr>
<tr>
<td></td>
<td>5.0 - 5.9</td>
<td>178617</td>
<td>133729 - 245616</td>
<td>-ve</td>
<td>85000</td>
<td>135762 - 187170 (4)</td>
</tr>
<tr>
<td></td>
<td>4.0 - 4.9</td>
<td>139382</td>
<td>112545 - 192712</td>
<td>-ve</td>
<td>185000 - 72000</td>
<td>112545 - 112752 (3)</td>
</tr>
<tr>
<td></td>
<td>3.0 - 3.9</td>
<td>89085</td>
<td>73984 - 107062</td>
<td>-ve</td>
<td>15000 - 75000</td>
<td>73984 - 107064 (3)</td>
</tr>
<tr>
<td></td>
<td>2.0 - 2.9</td>
<td>54722</td>
<td>36936 - 75611</td>
<td>-ve</td>
<td>15000 - 45000</td>
<td>38442 - 70966 (7)</td>
</tr>
<tr>
<td></td>
<td>1.0 - 1.9</td>
<td>25494</td>
<td>19238 - 36550</td>
<td>-ve</td>
<td>15000 - 60000</td>
<td>19,238 - 36550 (8)</td>
</tr>
<tr>
<td>SMALL FARMERS</td>
<td>6.0 - 6.9</td>
<td>112545</td>
<td>84804 - 99172 (3)</td>
<td>+ve</td>
<td>15000 - 45000</td>
<td>139382</td>
</tr>
<tr>
<td></td>
<td>5.0 - 5.9</td>
<td>135762</td>
<td>135729 - 245616 (3)</td>
<td>+ve</td>
<td>13000 - 111000</td>
<td>13000 - 111000</td>
</tr>
<tr>
<td></td>
<td>4.0 - 4.9</td>
<td>112545</td>
<td>112545 - 112752 (3)</td>
<td>-ve</td>
<td>185000 - 72000</td>
<td>3000 - 106000</td>
</tr>
<tr>
<td></td>
<td>3.0 - 3.9</td>
<td>73984</td>
<td>73984 - 107064 (3)</td>
<td>-ve</td>
<td>15000 - 75000</td>
<td>73984 - 107064</td>
</tr>
<tr>
<td></td>
<td>2.0 - 2.9</td>
<td>36936</td>
<td>38442 - 70966 (7)</td>
<td>-ve</td>
<td>15000 - 45000</td>
<td>38442 - 70966</td>
</tr>
<tr>
<td></td>
<td>1.0 - 1.9</td>
<td>19238</td>
<td>19,238 - 36550 (8)</td>
<td>-ve</td>
<td>15000 - 60000</td>
<td>19,238 - 36550</td>
</tr>
</tbody>
</table>

* number of farms in parenthesis
** -ve- decrease in livestock inventory value, 0- no change in livestock inventory
+ve- increase in livestock inventory

An economic analysis of medium and small dairy farms in Mauritius. P Toolsee et al.

**Figure 1** shows the net farm income against the number of cows present.

![Net farm income vs number of cows](image)

The net farm income ranged from Rs19238 to Rs 107062 for farms having 1 to 3.9 cows while for farms having 4 to 6.9 cows the net farm income ranged from Rs 112545 to Rs 280598. The value of net farm income is affected mainly by changes in inventory (increase or decrease in number of animals) and number of litres of milk sold on a yearly basis. Farms with 4 or more cows had a much higher net farm income and the relationship between NFI and number of cows was high ($R^2=0.875$).

**Table 3** shows the cost of producing a litre of milk using the ‘cost per equivalent income’ method and the ‘cost per unit sold’ method.

<table>
<thead>
<tr>
<th>Number of cows</th>
<th>Cost per equivalent income (Rs/Litre)</th>
<th>Cost per unit sold (Rs/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Small Farms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 - 1.9</td>
<td>6.28 ± 0.77</td>
<td>4.78 - 7.62</td>
</tr>
<tr>
<td>2.0 - 2.9</td>
<td>5.75 ± 1.06</td>
<td>4.06 - 7.83</td>
</tr>
<tr>
<td>3.0 - 3.9</td>
<td>6.10 ± 0.38</td>
<td>5.63 - 6.56</td>
</tr>
<tr>
<td>Medium Farms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0 - 4.9</td>
<td>5.41 ± 0.76</td>
<td>4.41 - 6.54</td>
</tr>
<tr>
<td>5.0 - 5.9</td>
<td>4.71 ± 0.48</td>
<td>4.24 - 5.21</td>
</tr>
<tr>
<td>6.0 - 6.9</td>
<td>5.13 ± 0.64</td>
<td>4.29 - 6.09</td>
</tr>
<tr>
<td>8.5</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>9.3</td>
<td>4.72</td>
<td></td>
</tr>
</tbody>
</table>

The average cost of producing a litre of milk using the ‘cost per equivalent income’ method for small and medium farms was Rs 6 and Rs 5 respectively and it was Rs 9.95 and Rs 8.75 respectively when using the ‘cost per unit sold’ method.
Figure 2 shows the costs of a litre of milk for all farms in the study and the red horizontal line represents the milk price (Rs 9 per litre) that has been used in the analysis.

**Figure 2** Cost of production of a litre versus number of cows (using cost per equivalent income method)

All the farms below the horizontal line (guaranteed selling price of milk at Rs 9) are having a cost of production less than the selling price. 3 out of 24 medium farms were producing a litre of milk in the cost range of Rs 7 to Rs 8, while the other farms had a cost of production between Rs 5 to Rs 7. This means all medium farms had an absolute profit margin and the dairy enterprise is rewarding.

**CONCLUSION**

The ‘net farm income’ increases with an increase in the number of cows kept on the farm. The average net farm income for a farm with four cows was Rs 120000. On a monthly basis it represents Rs 10000, which is higher than the monthly salary (about Rs 6000) for a manual grade worker in the non-farming sector.

Using the ‘cost per equivalent income’ method the data show that all farms were producing milk at a cost less than the guaranteed selling price of Rs 9.00 per litre. These farms were profitable at the assigned opportunity cost values for labour. It is to be noted that this method of economic analysis applies to farms that are currently operating and it will not apply to new farms entering the dairy business.

**REFERENCES**


AGRICULTURAL DIVERSIFICATION UNDER CHANGING LAND USE: MODELLING THE RIVIERE DES ANGUILES CATCHMENT

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2Department of Physics, University of Mauritius, Mauritius

ABSTRACT

Although sugarcane is considered a soil conservation crop, little is known of the current erosion rates on the island and the effect of changing crop types on sediment yields. In this study, modelling of the potential soil loss in the Riviere Des Anguilles catchment (RDAC) (with diverse landscape profiles and land use patterns) was undertaken to obtain an understanding of the extent to which different land use types affect soil erosion. The Revised Universal Soil Loss Equation (RUSLE) erosion model was applied to RDAC within a GIS framework. Our results show that the rates are generally highest on steep slopes (>20%) with high annual rainfall along the river and in the elevated part of the RDAC. Predicted soil loss results indicate a strong inverse relationship with vegetation cover, varying from low soil loss values (1 to 4 t.ha\(^{-1}.yr\(^{-1}\)) for infrequently disturbed land use types to moderate to high values (up to 80 t.ha\(^{-1}.yr\(^{-1}\)) for frequently disturbed land use types. Results also show that land use changes will have a considerable influence on soil erosion with mean soil loss for RDAC doubling under pineapple, and quadrupling under vegetables. Such crops should be confined to low slope angles supported by soil management practices.

Keywords: Agriculture, Land Use, RUSLE, GIS, Erosion, Agricultural Practices

INTRODUCTION

Approximately 50% of the total land surface is under sugarcane cultivation (including areas that have slopes of 30% or more) that constitutes 87% of the cultivable land. Apart from sugar other forms of agriculture are present but on a relatively small scale including tea, vegetables, tobacco, and fruits such as pineapple, banana, and litchi, mango and papaya. Soils previously classified by Arlidge and Wong You Cheong (1975) as unfit for agriculture is now being bulldozed de-rocked and irrigated. One of the most important types of land degradation in Mauritius occurs by physical loss of topsoil (Atawoo and Heerasing, 1997). Although sugarcane is considered a soil conserving crop soil erosion under sugarcane can be prevalent on highly erodible soils and steep slopes, with the concomitant effect of lowering yields. Pressure on sugarcane production is further compounded by an increasing shortage of labour coupled with rising production costs and lower sugar prices. Due to economic constraints, sugarcane cultivation is seriously being considered for diversification into other agricultural systems (Mauritius Sugar Syndicate, 2001). In addition, to attain a certain degree of self-sufficiency in food production, the government has emphasized the need for promotion of agricultural diversification and an apparently flourishing export market for exotic agricultural and horticultural products reinforces this demand. It is postulated that a diversification of sugarcane fields into other agricultural systems will place increasing strain on land resources leading to further soil degradation.

In order to select appropriate conservation measures and land management strategies, the identification and quantification of erosion sources is necessary. Prediction technology used for estimation of soil loss is regarded as a suitable tool in depicting the nature of the factors governing erosion (Morgan, 1995). Empirical soil erosion models continue to play an important role in soil conservation planning (Liu et al., 2000), and to assess the distribution and extent of erosion in catchment areas. Numerous qualitative studies on soil erosion on catchment scale have been undertaken (Smith et al., 2000). However, there remains a general lack of information under tropical conditions. Furthermore, little is known regarding the potential rate of soil loss under current conditions or for crop diversification on Mauritius. In this context the main objective of the study is to estimate the average annual soil loss due to water erosion under current conditions and to predict the outcome in terms of soil loss under...
future land use diversification. A specific catchment (Riviere Des Anguilles) on the southern side of the island is analyzed for this purpose.

MATERIALS AND METHODS

The Rivière Des Anguilles Catchment (RDAC) has a length of 14.9 km and a catchment area of 32.6 km² (Figure 1). This catchment was chosen due to its diversity in landscape profiles, and particularly for its well differentiated and diverse land use pattern. Similar to most southern catchments of Mauritius, RDAC runs from sea level to an elevation of about 650m a.s.l. with variable slopes gradients (Ordinance Survey, 1991). The lower plains of the catchment falls within the humid zone with an average annual rainfall between 1500 mm and 2000 mm. In contrast, the upper catchment is superhumid with over 3000 mm of rainfall p.a. The RDAC appears as a representative study area in which the soil erosion rates of important land use types can be assessed and, due to the different crop types currently in the catchment, the impact in terms of soil erosion under future diversification of agricultural systems.

Figure 1 Location Rivière Des Anguilles catchment (RDAC)

Empirical models predict soil loss under a wide range of conditions. The most widely applied soil loss models for rainfall erosion is the Universal Soil Loss Equation (USLE), an improved version of which is the Revised USLE (RUSLE) (Renard et al., 1994). The RUSLE, utilised in this study, is an erosion model designed to predict the long-term average annual soil loss carried by runoff from specific field slopes in specified cropping and management systems. It is one of the most technically advanced and widely applicable (Lane et al., 1992). Theoretical evaluations and sensitivity analysis performed on the RUSLE show the advantage of the inherent flexibility and dynamic structure of the RUSLE. Studies have demonstrated that the RUSLE is capable of adequately modelling soil loss under different land use, despite being applied to conditions beyond its database (Smith et al., 2000; Wang et al., 2000). In the RDAC, these conditions include mountainous terrain and non-agricultural conditions of the upper catchment area. RUSLE in particular, is capable of adequately modelling soil loss in a wide range of conditions, including tropical regions. The flexibility of the RUSLE model has proved to be advantageous for application on a catchment scale (Smith et al., 2000), and for simulating a series of “what if” scenarios. Hence, soil erosion rates, of current and future conditions, of different cropping
Agricultural diversification under changing land use: Modelling the Riviere Des Anguilles catchment. JJ Le Roux et al.

systems can be estimated and compared (Renard et al., 1994). The model groups influences on the erosion process into five categories including climate, soil profile, relief, vegetation and land use, and land management practices.

The RUSLE equation is (Renard et al., 1994):

\[ A = R \times K \times LS \times C \times P \]

where \( A \) is the spatial average soil loss; \( R \) is the rainfall runoff erosivity factor; \( K \) is the soil erodibility factor; \( LS \) is the slope length and steepness factors; \( C \) is the cover management factor; and \( P \) is the support practice factor.

Empirical models can also be used in a spatial context by means of a geographical information system (GIS) (Cochrane and Flanagan, 1999). Using soil loss models in a GIS environment enables the production of soil erosion hazard maps on a catchment scale and allows for the classification and spatial visualization of erosion potential. The GIS-based application used in the study is referred to as SEAGIS (Soil Erosion Assessment using GIS), developed by DHI, (1999). The application is developed as an ArcView GIS extension and requires Spatial Analyst. With the support of GIS techniques, several “what if” scenarios can be analysed and assist during the planning of suitable crop diversification. GIS techniques were integrated with RUSLE to group the many influences of the erosion process into the five categories mentioned above. Once the stochastic distribution of each parameter was determined, the spatial distribution and data of each soil erosion factor were digitised into a GIS (Arcview 3.2) as themes. From this, soil erosion factor maps were produced. These factor maps could then be treated as variables in the algebraic calculations for RUSLE; the product of each factor value gave the expected soil loss in t.ha\(^{-1}\).yr\(^{-1}\).

A summary of parameter value derivations is given below:

**Rainfall erosivity (R)**

In the absence of long-term rainfall intensity data for Mauritius, average monthly rainfall values were used to calculate the R factor. Long term, monthly rainfall data ranging from 1960 to 1990 were obtained from several weather stations on Mauritius (Mauritius Meteorological Services, 2000). In this study, the R factor values for seven rainfall zones, provided by Proag (1995), were estimated using the modified Fournier’s Index developed by the FAO (Arnoldus, 1980):

\[ R = 0.0302 \times (RI)^{1.9} \]

where \( RI = \sum (MR)^{2}/AR \), MR is monthly rainfall in mm, and AR is annual rainfall in mm.

**Soil erodibility (K)**

A soil map of Mauritius at a scale of 1:100000 (Parish and Feilafe, 1965) was used to identify the different soil types within the catchment. Soil properties were determined from a total of 37 soil samples taken from each of the 5 soil families, from different sites with uniform topography and land use (Le Roux, 2004). Sampling and analysis procedures were done according to standard methods described by Goudie et al. (1990).

**Slope parameters (LS)**

The data source for the LS factor was a topographical map (Ordinance Survey, 1991) of Mauritius at a scale of 1: 25000 showing contours at 10m intervals. Slope gradients were calculated using digital terrain modelling routines in Arcview 3.2.

**Cover parameters (C)**

For modelling purposes, the catchment was subdivided frequently disturbed land use types (sugarcane, intercropped sugarcane, and a vegetable stand) and infrequently disturbed land use types (banana plantations, tea plantations, scrub, forested land and urban areas). When assessing the annual crop
cover effect on erosion, RUSLE considers the type of crop and its growth stages. To relate the canopy effect to seasonal rainfall erosivity distribution, the year is divided up into various crop stages: (1) the harvest - soil preparation – planting stage; (2) the first growth stage; (3) the second growth stage; and (4) the mature growth stage. The cover factor was calculated by weighing the growth stage cover factors according to the relative erosivity of the respective growth stages, and then summed to produce the average annual C factor. The combination of information from these variables includes residue cover, canopy cover, canopy height, surface roughness, below-ground biomass, prior cropping, soil moisture and time. Most of the C factor values for the RDAC were derived from fieldwork (Le Roux, 2004) assisted by knowledge of how crops change with time by means of other sources (McPhee and Smithen, 1984). In order to predict potential soil erosion rates under potential land uses, soil losses from future land cover scenarios were studied using RUSLE. Three cropping systems that are relevant to the immediate and near future agricultural development opportunities in Mauritius are forested land, vegetables, and pineapple. Since land of the RDAC is either suitable or at least conditionally suitable for other food crops (Arlidge and Wong You Cheong, 1975; Jhoty et al., 2001), the three potential crop systems were each assumed to cover the whole catchment (3032 ha), except for existing urban areas (349 ha), and were simulated according to RDAC conditions.

**Support practice factor (P)**

Observations on the most common support practices for the RDAC (contouring and buffer strips) were made during fieldwork and supplemented by other sources of literature (MSIRI, 1997; 2000).

**RESULTS AND DISCUSSIONS**

**Soil loss under current agricultural practices**

Due to the extensive number of input parameters, only the end products of all the input data and erosion factors given above are presented here as the accompanying soil erosion prediction maps and graphs. Average annual soil losses (in t ha⁻¹) as predicted by the RUSLE are shown in **Figure 2**.

**Figure 2** Mean soil loss predicted by the RUSLE under current conditions in the RDAC

---

Very high soil loss values of more than 80 t ha\(^{-1}\) yr\(^{-1}\) are attained under the vegetable stand; moderate values (between 13 to 20 t ha\(^{-1}\) yr\(^{-1}\)) under intercropped cane; very low (less than 2 t ha\(^{-1}\) yr\(^{-1}\)) to low (10 t ha\(^{-1}\) yr\(^{-1}\)) under sugarcane; very low (4 t ha\(^{-1}\) yr\(^{-1}\)) to moderate (16 t ha\(^{-1}\) yr\(^{-1}\)) for banana plantations; very low (less than 1 t ha\(^{-1}\) yr\(^{-1}\)) to high (41 t ha\(^{-1}\) yr\(^{-1}\)) for tea plantations; and low rates (less than 10 t ha\(^{-1}\) yr\(^{-1}\)) for natural vegetation, including scrub and forested areas. Despite its smaller size (32.6 km\(^2\)), the RDAC show similar soil loss totals (4229 t yr\(^{-1}\) predicted by RUSLE) compared to the Hawaiian catchments (Calhoun and Fletcher, 1999), since most of the RDAC is under extensive cultivation and has a higher soil loss rate (11 t ha\(^{-1}\) yr\(^{-1}\) predicted by RUSLE).

Different patterns in soil loss values between land use types are a direct outcome of the different influences of the soil erosion factors on erosion. For example, although the R values (2139 MJ ha\(^{-1}\) mm hr\(^{-1}\)) of the upper catchment are the highest, the effects of high plant cover results in very low soil loss values (0 – 5 t ha\(^{-1}\) yr\(^{-1}\)). Potential soil loss results seem to indicate a strong inverse relationship with vegetation cover. Sugarcane provides a dense cover within less than two months after regrowth or planting. In addition, soil under ratoon sugarcane is tilled on average every seven years, when new cane is planted. Between those seven years, the cane stubbles are left intact during harvest. As a result the mean soil loss values computed by the RUSLE for sugarcane are very low (1.5 t ha\(^{-1}\) yr\(^{-1}\)). These values correspond well with the soil loss values (0.2 – 5 t ha\(^{-1}\) yr\(^{-1}\)) under sugarcane obtained by the MSIRI (2000), using a rainfall simulator. Trash and cane stubbles are known to protect the soil and reduce erosion by 10–50% (Yang, 1995).

Land management, particularly the infrequency of disturbance, can mainly account for low soil loss values as predicted by the RUSLE for natural vegetation, tea and banana plantations. Soils under these land use systems are infrequently disturbed and consequently have long since been consolidated and appear relatively resistant to erosion. In contrast, newly tilled soil will be easily detached compared to a consolidated soil and cropping systems such as intercropped cane, with less cover and frequently disturbed soil, give rise to moderate predicted soil loss. Disturbance contributes to the high erosion rates (>80 t ha\(^{-1}\) yr\(^{-1}\)) under vegetables since vegetables have a short 3–5 months cycle and at least two crops are planted each year.

**Soil loss under future land use**

Soil loss values computed for potential cropping systems are displayed on soil erosion prediction maps (Figure 3) and graphs for comparative purposes (Figure 4). The average annual soil losses (in t ha\(^{-1}\)) for the catchment under vegetables, pineapple and forest predicted by the RUSLE are shown in Figure 3. Mean soil losses from the RUSLE model are 42 t ha\(^{-1}\) yr\(^{-1}\), 20 t ha\(^{-1}\) yr\(^{-1}\), and 0.2 t ha\(^{-1}\) yr\(^{-1}\) under vegetables, pineapple, and forest, respectively. For all three alternative cropping systems, most of the erosion is predicted in the upper catchment area and adjacent to the river. Total soil losses of 127798 t, 60370 t and 508 t are predicted under vegetables, pineapple and forest, respectively (Figure 4). When compared to current conditions (4229 t), the mean soil loss for the catchment will double under pineapple (increase by 100%), and quadruple under vegetables (increase by 300%).

The generally high soil loss rates with a mean of 42 t ha\(^{-1}\) yr\(^{-1}\) under vegetables can be ascribed to poor vegetation cover, ineffective conservation practices and high rainfall. Worst cases scenarios occur on land units with slopes (>20%), high rainfall (>2400 mm) and poor cover (<30%). Therefore, the highest rates of soil loss are predicted on steep slopes with erodible soils of the upper catchment area and along adjacent to the river.
**Figure 3** Mean soil loss in t.ha⁻¹.yr⁻¹ predicted by the RUSLE under future land use (vegetables, pineapple and forest)

**Figure 4** Mean and total soil loss predicted by the RUSLE under current (left) and future (right) cropping systems in the RDAC
Proposed pineapple plantations appear to be associated with moderate (12 to 25 t ha\(^{-1}\) yr\(^{-1}\)) to extremely high (>150 t ha\(^{-1}\) yr\(^{-1}\)) erosion rates with a mean of 20 t ha\(^{-1}\) yr\(^{-1}\). Mean and maximum soil loss values differ significantly due to the interactive effects of soil erosion factors. It is anticipated that soil loss will be very high during the introductory and early stages of pineapple development. Appropriate erosion control measures will be needed in order to minimise long-term erosion problems under pineapple and vegetable. Cover management together with heavy support practices may provide protection against erosion (McPhee and Smithen, 1984). Soil loss could decrease with time as support practices take effect. Therefore, planning will need to carefully consider the balance between the probability of long-term erosion damage and the maintenance needed to ensure the viability of pineapple plantations. Results show that pineapple will not be viable in the upper catchment area and steep slopes of the valley and should be confined to nearly level (0-2%) to gently undulating (2-4%) slopes of the lower catchment area.

Predictions indicate that no appreciable erosion damage (<4 t ha\(^{-1}\) yr\(^{-1}\)) will occur in the RDAC under commercial forestry. In Mauritius, selective logging provides continuous cover. The dense cover of ground vegetation and tree litter on the surface leads to very low rates of erosion. In addition, the presence of a root mat provides protection against drip from the canopy. After the establishment of all the inputs necessary for agroforestry, the system can be highly cost effective. However, periodic damage from cyclonic winds is a limiting factor to the accrued benefits of forestry (Ministry of Agriculture and Natural Resources, 1999). Nevertheless, it still remains a vital land use on account of the protection it affords to the upper catchment area. Therefore, natural as well as commercial forests in the upper catchment area and along the steep valley slopes should remain undisturbed.

CONCLUSIONS AND RECOMMENDATIONS

The Revised Universal Soil Loss Equation (RUSLE) was used in conjunction with a GIS to compile soil erosion prediction maps of the Riviere Des Anguilles catchment (RDAC) in southern Mauritius. In addition, the study attempted to predict soil erosion under future crop cover in the catchment. Using the RUSLE in a GIS, it was possible to estimate the soil loss for different future scenarios, given information on the mean and variability in vegetation parameters. Mean annual soil loss for the current situation in the RDAC is estimated at approximately 11 t ha\(^{-1}\) yr\(^{-1}\) by RUSLE. A detailed study of the results indicates that decreasing rates of soil loss for each defined land use type correlate well with increase in canopy and/or surface cover, as well as frequency of disturbance. Infrequently disturbed land use types such as natural vegetation, tea and banana plantations generally have low soil loss values (1 to 4 t ha\(^{-1}\) yr\(^{-1}\)), whereas frequently disturbed land use types such as intercropped cane and vegetables have moderate (13 t ha\(^{-1}\) yr\(^{-1}\)) to very high (80 t ha\(^{-1}\) yr\(^{-1}\)) soil loss rates, respectively.

Crop diversification will have a considerable influence on soil erosion. RUSLE predicts a mean soil loss of 42 t ha\(^{-1}\) yr\(^{-1}\), 20 t ha\(^{-1}\) yr\(^{-1}\), and 0.2 t ha\(^{-1}\) yr\(^{-1}\) under vegetables, pineapple, and forest, respectively. When compared to current conditions, the mean soil loss for the catchment will double under pineapple, and quadruple under vegetables. Future vegetables or pineapple plantations seem to be associated with moderate to extremely high erosion hazard. Results illustrate that it is the combination of extreme gradients and intense rainfall events which makes the RDAC sensitive to soil erosion under vegetable and pineapple scenarios. Therefore, vegetables and pineapple will not be viable on the steep slopes of the valley and upper catchment area. In contrast, no appreciable erosion damage should occur in the RDAC under forested land. Natural as well as commercial forests in the upper catchment area and along the steep valley slopes should remain undisturbed.

Current developments include extension of this work to the rest of the island with an update wherever changes in land use have occurred, validation of the estimations of the RUSLE by measuring actual erosion using field plots, and measurement of rainfall intensity. Future research includes investigations on intercropping, crop suitability, and crop productivity.
ACKNOWLEDGEMENTS

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Agricultural diversification under changing land use: Modelling the Riviere Des Anguilles catchment. JJ Le Roux et al.


WATER BUDGET ESTIMATION FOR WATER BASINS OF MAURITIUS


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ABSTRACT

Water is a resource of vital importance for the economic and social development of a country. Issues on water resources constantly recur in the media whenever the increased demand and/or the vagaries of climate top the local agenda. It is evident that a sound knowledge of water recharge, spatial distribution, and its partitioning into ground infiltration, surface runoff, and evapotranspiration, is indispensable. The spatial and temporal variations of the hydrological cycle are key inputs that determine the rainfall patterns in Mauritius. Few studies have been carried out on numerical modelling of water budget in Mauritius. Water balance calculations using such methods as Thiessen’s mean method or the Isohyetal method have revealed poor estimates of the water budget. In this paper we report a novel, high performing, image-processing technique for the purpose of water budget calculations for a selected basin. The basin selected for this work is restricted to the Bel-Air/Rivière Sèche catchment. Our results show satisfactory results so that the technique is now being extended to other basins in Mauritius.

Keywords: Water Balance calculations, Kriging, precipitation, Basins

INTRODUCTION

Water is one of the most important natural resource of a country. An enormous pressure on the water resources all around the globe exists due to the increasing population growth rate and increasing needs for irrigated lands. There are different kinds of precipitation, which differ from one another by characteristics such as the total amount, intensity and the duration. Precipitation occurs in Mauritius mainly in the form of rain following condensation of atmospheric water vapour. Due to physical restrictions in the real world, it is basically impossible to collect thorough data at every desired point. The mean precipitation over a drainage basin has been so far estimated using numerical averages and weighted averages such as the Thiessen’s Mean method (Thiessen, 1911) and the Isohyetal method (Custodio and Llamas, 1983). The Thiessen’s mean method is a formal method of describing settlement patterns based on territorial divisions centred on a single site, representing for instance a rainfall station. In fact, polygons are created by first drawing line segments between pairs of neighbouring sites in order to construct their perpendicular bisectors so that they are then linked to form the Thiessen polygons as shown in Figure 1 (Chow et al., 1988).

Figure 1 Bisecting lines to get Voronoi polygons
The mean rainfall over a basin of area $A$ is estimated by \( P = \frac{\sum_{i=1}^{n} A_i P_i}{A} \), where $A$ has been partitioned into $n$ polygons such that \( A = \sum_{i=1}^{n} A_i \). In the Isohyetal methods, isohyets, which are the contours of equal rainfall, are drawn on a map, after the rainfall at each station is plotted. The mean precipitation $P$ of an area $A$ is governed by the same formula (1) where $A_i$, $i = 1, 2, \ldots, n$, are the respective areas found between adjacent isohyets. Since these estimations depend on the distribution of the rainfall stations, a major drawback is that computed results are not accurate enough when the stations are concentrated over a small part of the region considered. In this paper the Kriging method is used for estimating the precipitation over a drainage basin. Image processing techniques are then used to compute the water budget for a selected basin. The basin selected for this work is Bel-Air/Rivière Sèche catchment; chosen for its simplicity and availability of relevant data. Our results show satisfactory results so that the technique can be extended to other basins in Mauritius.

**Estimating rainfall over Mauritius**

A point-to-point interpolation method is imperative for the proper generation of digital contour images. The Kriging method, a geostatistical gridding method, is an interpolation technique which uses variogram to express the spatial variation and applies minimization techniques to reduce error of predicted values (Kitanidis, 1997). Kriging interpolation generates contour maps from irregularly spaced data (optimal spatial prediction). In this work, Surfer8™ has been used to generate these images (Figure 2) after application of the island mask.

![Figure 2 Typical rainfall image over Mauritius](image)

The generation technique first involves the plotting the contours in gray scale image (0 to 255) – the pixel intensity value relating to the amount of rainfall followed by the application of a mask (Mauritius) to crop region of contours inside the island.

The precipitation, average precipitation and volume due to precipitation can be calculated for the whole of Mauritius or for any catchment's area using the intensity values of the pixels. We make use, for the purpose, of the gray-scale i.e. intensity values between 0 (black) and 255 (white) corresponding to the minimum and maximum precipitations. The precipitation can be thus be obtained from the image only after a linear re-scaling the intensity values of the pixels i.e. if the minimum precipitation or rainfall is $P_{\text{min}}$ and the maximum precipitation is $P_{\text{max}}$, then for any intensity value of a pixel found in the $i^{\text{th}}$ row and $j^{\text{th}}$ column, its corresponding precipitation $P(i,j)$ is given by
The total precipitation over a region of interest (ROI) can be estimated by computing the total of all intensity values for pixels located in the region and rescale them as above. The total precipitation $P_{\text{tot}}$ in the ROI is then given by the equation

$$P_{\text{tot}} = \sum_{i=1}^{N} P_i,$$

where $N$ is the total number of pixels found in the ROI and $P_i$ is the precipitation over area covered by the $i^{th}$ pixel.

The average precipitation $P_{\text{av}}$ over the ROI is given by

$$P_{\text{av}} = \frac{P}{N}.$$

The volume of water collected in mm$^3$ due to precipitation at the region represented by the $i^{th}$ pixel is the product of the precipitation (height of water level in mm) and the pixel area $a (= A/N)$. Therefore,

$$V_i = P(i, j) \times a.$$

Hence, the total volume due to precipitation over the ROI is given by

$$V_{\text{tot}} = \sum_{i=1}^{N} V_i = V_{\text{tot}} = P_{\text{av}} \times A.$$

The above calculations are thus more precise as number of pixels gets larger.

Our computed annual mean precipitation over Mauritius for the period November 1991 to October 1995 amounts to 2014 mm (in agreement with the value of 2100 mm reported by the MMS). The computed mean precipitation over Mauritius is 1350 mm in summer and 664 mm in winter, which represent 67% and 33% of the annual mean precipitation respectively. Figure 3 shows the average rainfall over Mauritius for the period November 1993 to October 1994.

**Figure 3** Average Precipitation in mm of Mauritius for period Nov 93 - Oct 94

**Evapotranspiration**

Evapotranspiration (E.T) is the process during which water is lost as vapour during evaporation from the soil and transpiration from plants and is affected by parameters such as wind speed, amount of sunshine, humidity and temperature. Thornthwaite (1948) and Wilm et al. (1944) developed an empirical equation for approximating the potential evapotranspiration from a reference grass surface. This equation is also well used, as it requires only two physical parameters such as the mean monthly temperature and the day length estimates. There is indeed a more direct physical relationship between potential evapotranspiration (P.E.T) and radiation than between P.E.T and temperature, but due to lack
of information and need for tedious calculations, there will be no reliable result from inaccurate data measurements and hence, Thornthwaite’s method will be used in this work. The fact that the equation considers only the relationship between P.E.T and temperature, factors such as: humidity, reflection coefficient or Albedo, wind and pressure are assumed to be constant. The Potential Evapotranspiration is given in mm/month and without adjustment in the length of the day can be calculated by using:

\[
P.E_i = \begin{cases} 
0 & \text{for } T < 0^\circ C \\
16 \left[ \frac{10T_i}{I} \right]^a & \text{for } 0 \leq T < 26.5^\circ C \\
-415.85 + 32.24T_i - 0.43T_i^2 & \text{for } T \geq 26.5^\circ C 
\end{cases}
\]

where \( T \) is the mean surface air temperature in month \( i \) (°C) and \( I \) is defined as the heat index and is given by

\[
I = \frac{\sum_{i=1}^{N} (T_i / 5)^{1.514}}{N} 
\]

and \( a \) is the heat index function given by (Garg, S.K., 2000):

\[
a = 6.7 \times 10^{-7} (I^3) - 7.71 \times 10^{-5} (I^2) + 1.79 \times 10^{-2} (I) + 0.49.
\]

After adjusting the day length and the number of days, the adjusted evapotranspiration or corrected evapotranspiration is \( APE_i = PE_i \left[ \left( d / 30 \right) \left( h / 12 \right) \right] \), where \( APE \) is in (mm/month), \( d \) is length of the month in days, and \( h \) is the duration of daylight in hours on the fifteenth day of the month. Table 1 shows the corrected coefficients that are used to calculate the actual evapotranspiration.

**Table 1 Corrected coefficients for calculating actual evapotranspiration**

<table>
<thead>
<tr>
<th>Latitude (Deg.)</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
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<tbody>
<tr>
<td>10 S</td>
<td>1.08</td>
<td>0.97</td>
<td>1.05</td>
<td>0.99</td>
<td>1.01</td>
<td>0.96</td>
<td>1.00</td>
<td>1.01</td>
<td>1.00</td>
<td>1.06</td>
<td>1.05</td>
<td>1.10</td>
</tr>
<tr>
<td>20 S</td>
<td>1.14</td>
<td>1.00</td>
<td>1.05</td>
<td>0.97</td>
<td>0.96</td>
<td>0.91</td>
<td>0.95</td>
<td>0.99</td>
<td>1.00</td>
<td>1.08</td>
<td>1.09</td>
<td>1.15</td>
</tr>
<tr>
<td>30 S</td>
<td>1.20</td>
<td>1.03</td>
<td>1.06</td>
<td>0.95</td>
<td>0.92</td>
<td>0.85</td>
<td>0.90</td>
<td>0.96</td>
<td>1.00</td>
<td>1.12</td>
<td>1.14</td>
<td>1.21</td>
</tr>
</tbody>
</table>

The computation of evapotranspiration is similar to that of mean precipitation. Knowing the mean monthly temperature at different stations, contour images are created using the Kriging method in Surfer™ (Figure 4). Using the Thornthwaite’s equation, the potential evapotranspiration and hence the corrected evapotranspiration are then calculated (Figure 5).
The mean PET for the period of November 1991 to October 1995 was estimated to be about 1101.93 mm. The PET during summer is 671.89 mm and the winter PET is 430.04 mm. In fact, the amount of water lost in evapotranspiration during summer is about 60.1% of the annual PET and 39.9% of the annual PET during the winter season. Our results agree with those reported by the Water Resources Unit of the Ministry of Public Infrastructures, whereby the annual potential evapotranspiration ranges from 1100 mm to 1600 mm annually with small inter annual variations (Hydrology Data Book, 1992-1995).
Water budget estimation of Bel-Air/Rivière Sèche catchment (region D)

The water budget is a computational technique that balances water input and output, taking into consideration the change in storage. The hydrological equation for a catchment’s area is given by (Garg, S.K., 2000):

\[
\text{Inflow (or recharge)} - \text{Outflow (Discharge)} = \text{Change in Storage}
\]

and thus

\[
P = AET + R_s + R_g + U + \Delta S_m + \Delta S_g + \Delta S_s,
\]

where:

- \( P \) = Precipitation
- \( AET \) = Actual evapotranspiration
- \( R_s \) = direct surface runoff
- \( R_g \) = groundwater discharge, including interflow.
- \( U \) = underflow
- \( \Delta S_m \) = change in soil moisture storage.
- \( \Delta S_g \) = change in groundwater storage.
- \( \Delta S_s \) = change in surface storage.

It is seen that Inflow (I) consists of precipitation and groundwater inflow while Outflow (O) consists of stream discharge, evapotranspiration losses and groundwater outflow. The water balance equation reduces to

\[
\text{Precipitation} = \text{Evapotranspiration} + \text{Total Runoff} + \text{Fluctuations of water stored in soil}
\]

or simply

\[
P = E + R + \Delta S,
\]

where \( E \) = Evapotranspiration, \( R \) is the total runoff \((R_s + R_g)\), \( \Delta S \) is the sum of changes in soil moisture storage, ground water storage and assuming that any underflow is directed towards surface river flow.

The catchment area of Bel-Air/Rivière Sèche (Figure 6) is the most suitable basin to carry out a water budget since almost all data for the parameters involved in the budgeting equation are available. The total runoff recorded at different gauge stations is available in the Hydrological Data Book 1992-1995.

**Figure 6** Basins in Mauritius (source: Hydrology Data Book 1992-1995)
Information concerning the change in the water level of the only borehole SW 155 found in that region and the surface runoff recorded at the gauge station D01 found at Bel-Air/Rivière Sèche and gauge station E020 (diversion at Melrose) can be obtained from the Hydrology Data Book 1992-1995. Once the images over Mauritius for both precipitation and evapotranspiration are generated, the mean precipitation (Figure 7a) and potential evapotranspiration (Figure 7b) for region D are computed after applying the mask for region D.

Figure 7a  Average precipitation (in mm) for region D (Nov 93-Oct 94)

![Figure 7a](image)

Figure 7b  Average P.E.T. (in mm) for region D (Nov 93-Oct 94)

![Figure 7b](image)

The change in ground water storage can now be estimated using the simplified water budget equation. Estimation of the change in the ground water storage in the region of Bel-Air/Rivière Sèche agrees with the change in water level of the borehole S 155 found in the same region (Figure 8); note the increases in the borehole values in the months of February.
CONCLUSION

In this work, a novel approach to estimate the water budget of a catchment area has been described involving image-processing technique. The technique was applied to the Bel-Air/Rivière Sèche catchment (region D). Results for the period Nov 91 to Oct 95 analysed reveal that the computed water borehole level correlated well with the actual water level. Work is underway to extend this work to all other catchment areas of Mauritius and Rodrigues.

ACKNOWLEDGEMENTS

We are indebted to the Mauritius Meteorological Service and the Water Resources Unit for providing the relevant data. We are also grateful to the University of Mauritius for providing the necessary facilities for this research.
REFERENCES


DELINEATION OF MAJOR DRAINAGE BASINS OF MAURITIUS

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ABSTRACT
It is important to delineate the boundaries of drainage basins and watersheds. Mauritius is currently known to be divided into 25 main catchment areas or river basins, each corresponding to a main river, 22 minor ones with coastal zones drained by streams or rivulets. A consistent database of river networks and their catchments can be useful for a wide range of applications, including mapping, monitoring and modelling activities, offering support to a variety of hydrological, agricultural, ecosystem and climate models. In this paper we report the delineation of drainage basins using digital data for drainage basins of Mauritius. The digital method requires digital data of contour lines of elevation and river networks as well as a 3D grid of elevation and a vector map of slope. The digital method yields more accurate results than the conventional method based on paper maps. Based on the digital data (1:100000 and 1:250000 scales), 70 rivers were identified and classified as the major rivers for the island. Our studies also yielded 3 additional catchments located inland, where water drains to pits/lakes. Difficulties were encountered in delineating some basins either due to poor spatial resolution when dealing with flat terrain and/or due to unavailability of data (such as discontinuous river networks).

Keywords: Basins, Catchments, GIS, Delineation, Watersheds

INTRODUCTION

The availability of digital data on rivers and lakes and their drainage basins (catchments), including information on the characteristics of these entities, is important for the analysis of environmental pressures and their impact on water resources. As such pressures on landscapes increase, land managers are continually looking for new methods of managing and monitoring landscape "health." In order to analyse the properties of a landscape, indeed, in order to monitor any object, it is necessary to break that object into manageable units. In the past, landscapes have been managed on an ownership basis. However, experience has shown that the old methods of land management do not make biological sense. Most biological processes do not stop at an ownership boundary. Animal species migrate across private and public lands (as long as they can get across the fences). Contiguous forested lands may traverse much ownership. Streams flow across different ownerships and political boundaries. A logical unit of land management is the watershed. The American Heritage Dictionary defines watersheds as: "The region draining into a river, river system, or body of water." Watersheds are always physically delineated by the area upstream from a given outlet point. This generally means that for a stream network, the contributing area goes upstream to stop at a ridgeline. Ridgelines separate watersheds from each other.

Delineation of the boundaries of drainage basins and watersheds is not only important, but it must be also a methodically easy process, where the watersheds for any dam or station can be delineated systematically as the user wishes. Traditional method of delineation of these areas involves a paper map of contours lines of elevation at a suitable topographical scale (2D map of elevation) and river networks. The digital method reported in this paper involves, in addition to the previous two \textit{but now in digital format}, a 3D grid of elevation and a vector map of slope. It is therefore obvious as to why the digital method will yield more accurate results (given that there are more inputs). In the era of the information age, manual delineation of drainage basins using digital data can work side by side with the systematic delineation of drainage basins using GIS software. So, any user planning to delineate watersheds or drainage basins can adopt both methods. In this work, the method of manually delineating drainage basins using digital data has been applied to the island of Mauritius for identifying the major drainage basins.
METHODOLOGY

It is important to define major and minor drainage basins as different definitions are currently in use in the literature. A major drainage basin will be defined here to enclose a complete stand-alone major river and all its tributaries and a minor catchment is one that encloses a minor river with its tributaries. When ridges between major drainage basins have been delineated then the watershed delineation of sub-catchments can be performed. Based on the digital data of rivers from the Ministry of Housing and Land that were produced from 1:100000 and 1:25000 map series, 70 rivers were identified from the 1:100000 map series and these were classified as the major rivers for the island (Figure 1). Three additional catchments forming inland water catchments, where water drained to pits and formed lakes, were also identified in this work.

Figure 1 Part of Port Louis on a 1:25000 scale with 10m contours

A tangential plane measures the rate of change at a point on a 3D surface at that point; the rate of change thus has a magnitude (called gradient) and a direction (called aspect); the two components measure one property of the surface called slope (Chrisman, 1996). Cayley (1859) provided early mathematical insights into the structure of surfaces. Warntz (1966) applied Cayley’s structure to geographic (topographic) surfaces and whose terminology we used. At most places on the 3D surface, slopes are parallel, but at others the slopes converge and diverge (Figure 2).

Figure 2 Topology of a surface, labelled with Warntz’s (1966) terminology
At the tops of hills (peaks), slopes diverge in every direction. As the slopes continue downhill, they eventually meet the slopes from another hill, causing convergence. Warntz (1966) termed this a course, because in a water-eroded landscape, the streams (watercourses) would end up following this line. Similarly, from peak to peak, there is a line (a ridge) where the slopes diverge. The network of ridges divides the region into a set of areas, the watersheds. The two networks-ridges around watersheds and courses around hills-constitute the topology of the topography. Therefore, the topology of a 3D surface is defined by the local behaviour of the surface-patterns of convergence and divergence. Gravity-powered flow of water (such as rainfall surface runoff) over the 3D surface is strongly controlled by this structure. Water will remain contained by the watershed in which it falls. Ridges create drainage divides that separate river systems from each other. As water flows over the surface, it will converge into the course lines and eventually forms streams. At the lower ends of the flow, the water will fill up any pits, forming lakes.

For Mauritius, the projection of the digital data is Lambert Conical Orthomorphic and the spheroid is Clarke 1880. The false coordinates of origin, Le Pouce, are 1000000m East and 1000000m North. Our 3D grid of elevation and rivers and road networks are based on this false origin and accordingly the x and y coordinates are in meters. Elevation increments in step of 10m (obtained from 1:25000 digital data) from 0 m representing the coastline to reach the highest elevation of 820 m. Digital data used were 10m interval contours, roads and rivers networks. Software used were AutoCAD R14® and Surfer®®, Global Mapper and ArcGIS 8.3. More than 1.2 million of xyz coordinates were software-extracted to form the 10m contours map. These data points were imported in Surfer®® where the geostatistic method Kriging was used to generate a 75X75 m grid for the island. A 3D surface plot was then made from the grid (Figure 3).

**Figure 3** 3D surface plot of Mauritius
RESULTS & DISCUSSIONS

The resulted hydrological basins delineated were classified as physical (laboratory) models, based purely on the hydrological processes occurring on the surface (Barrett and Curtis, 1992). From the 3D surface, the vector maps showing direction and magnitude of slope at points on the 3D surface were generated. The grid data for the island was used to generate the vector map with an overlay of the river networks on the 3D surface. It was possible to delineate the ridgelines between major drainage basins manually by using a digitiser function. The ridgelines delineating the major drainage basins were digitised and saved as CAD polylines consisting of x and y coordinates. The drainage boundaries can be edited and added to the map. The arrow symbol points in the “downhill” direction and the length of the arrow depends on the magnitude, or steepness, of the slope. This is illustrated in Figure 4 for regions where the delineation for the catchments is shown on the 3D surface with rivers, contours and road overlay.

**Figure 4a:** Region of Grand Sable-Petit Sable showing 3D surface with overlay of vectors, 10m contours, rivers, roads and drainage basins boundaries. The image on the right shows the drainage basins delineated and added to the map (in blue)

**Figure 4b** River networks overlaid on Shaded Relief Map
This simple procedure permitted the boundaries of the 73 major drainage basins (shown in Figure 5) to be delineated. For coding purposes of the catchments areas, the country was divided into five regions namely: North, East, West, South and Inland (Figure 5); the grey lines show the boundaries between the different regions, the normal black lines are the boundaries for the catchments and the bold black lines are the boundaries for the inland catchments. The procedure adopted for labelling the catchments and the full listing of the major rivers and their codes, their catchments, sizes and whether they have been delineated or not will be published elsewhere. The three inland catchments are Mare-aux-Vacoas basin, Mare-Longue basin, and La Nicolière.

Figure 5 Major drainage basins boundaries of Mauritius

<table>
<thead>
<tr>
<th>(a) Old catchments (Hydrology Data Book 92-95)</th>
<th>(b) New catchments derived from this work</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Old catchments" /></td>
<td><img src="image2" alt="New catchments" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(c) Major drainage boundaries with all river networks (Cyan lines)</th>
<th>(d) Regions for which difficulties were encountered</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3" alt="Major drainage" /></td>
<td><img src="image4" alt="Difficulties encountered" /></td>
</tr>
</tbody>
</table>
Difficulties were encountered in delineating 28 of the 73 major catchments either due to the poor spatial resolution when dealing with flat terrains and/or due to unavailability of data (such as discontinuous river networks). In flat terrain, it was difficult to find a direction for the ridgelines since the vectors did neither converge nor diverge. Therefore, in these areas no ridges were identified by the vectors alone and thus a combination of vectors and contour lines had to be used. This difficulty affected 12% of the catchments delineated (see Table 1). Two of the twenty-eight catchments that poses problem was due to lack of accurate data. The presence of imprecise data (such as man-made irrigation channels) in the river network made it difficult to find reasonable and precise path for ridgelines. It was decided not to delete polylines that appeared as irrigation channels because some other polylines appeared to be irrigation channels as well as rivers. In this case the data was left as it was. A future field survey will be set up to check for these imprecise and inaccurate data. Moreover, the latter coupled with the poor spatial resolution when dealing with flat terrains brought about the greatest problem in delineating the boundaries for these catchments. This combination affected 26% of the 73 drainage basins delineated (see Table 1). Data of greater precision and accuracy is therefore essential to delineate all the catchments of the island. The 2m contours map actually in preparation by the Ministry of Housings and Lands for certain regions of the island will be useful for this purpose.

**Table 1** Delineation status using 10m contours

<table>
<thead>
<tr>
<th>Regions</th>
<th>North</th>
<th>West</th>
<th>South</th>
<th>East</th>
<th>Inland</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of major catchments</td>
<td>13</td>
<td>21</td>
<td>23</td>
<td>13</td>
<td>3</td>
<td>73</td>
</tr>
<tr>
<td>No. of major catchments delineated</td>
<td>4</td>
<td>13</td>
<td>15</td>
<td>10</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>Problems encountered due to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor spatial resolution of flat terrains</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Inaccurate data</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Poor spatial resolution of flat terrains and inaccurate data</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Paper maps are not the only sources for the manual delineation of drainage basins. Digital data can also be used for this purpose. In this paper a simple methodology has been developed for the purpose. The method has proved to yield more accurate results than the method for paper maps, because the method incorporate the methods of paper maps in addition to using digital data in 3D and vector maps of slope. Our research however revealed two main problems in delineating drainage basins manually using digital data; the poor spatial resolution when handling flat terrains and imprecise and inaccurate data (such as discontinuous river networks) or a combination thereof. Thus, even though the 73 catchments have been successfully delineated, the degree of accuracy for 28 catchments (out of 73) is low. A mosaic of grids made from 10m contours and 2 m contours (for flat terrains) is currently being made together with field surveys to check for discontinuities present in river networks.

**ACKNOWLEDGEMENTS**

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REFERENCES


A FARMER-PARTICIPATORY APPROACH FOR THE IMPLEMENTATION OF A DEVELOPED INTEGRATED PEST MANAGEMENT SYSTEM AGAINST PLUTELLA XYLOSTELLA IN CRUCIFER CULTIVATION IN MAURITIUS

C. Dunhawoor and D. Abeeluck

Agricultural Research and Extension Unit

ABSTRACT

The diamondback moth (DBM), Plutella xylostella is a major pest of cabbage and cauliflower and had become resistant to many insecticides during 1980’s. In 1997, an IPM package was eventually developed and focussed primarily on DBM monitoring and control with selective insecticides when threshold level is attained. The prevailing extension method (training and visits (T&V)) was not successful in promoting the IPM package for implementation by growers. About 86 % of growers could not recognise pests and natural enemies and monitor DBM population in their fields. This paper describes two case studies in which the developed IPM package was validated in cabbage and cauliflower cultivation and the Farmers’ Field School (FFS) approach tested for IPM training in crucifer cultivation. The FFS approach was used to train forty one crucifer growers during the first crop cycle of cabbage and cauliflower cultivation. After training, 90 % of growers could recognise DBM and Cotesia plutellae, could use the scouting method to monitor DBM population and treat their fields when threshold level was attained. DBM control in the non-IPM plot was about 2.4 times more expensive than the IPM plot in cabbage and about 3.9 times in cauliflower cultivation. In the IPM plot, a 50 % reduction in insecticide use was achieved and the parasitoid, C. plutellae was more abundant. Marketable yield in the IPM and non-IPM plot was above 85 %. The developed IPM package was still effective against DBM and the FFS approach is an effective tool to promote the IPM package for implementation by crucifer growers

Keywords: Diamondback moth, IPM package, Farmers’ Field School, farmer-participatory approach

INTRODUCTION

The diamondback moth, Plutella xylostella is a key pest of cabbage and cauliflower grown on over 350 hectares by about 700 growers yearly. During the 1980’s, P. xylostella became resistant to insecticides and up to 80 % of crop losses were incurred. An IPM project was then set up to develop and implement an IPM package to solve the problem of pesticide abuse and pesticide resistance problem. A workable IPM package was developed which focussed primarily on monitoring of larval population by scouting method and implementation of control measures when specific threshold level was reached (Dunhawoor and Abeeluck, 1997). Control measures included releases of parasitoids, use of biopesticide and lepidoptera specific chemical insecticides.

From 1998 to 2002, the prevailing extension methodology (training and visits (T&V)) was used to promote the IPM package for implementation by growers. It was based on regular visits by Extension Officers to farmers’ fields, technological review meetings/workshops and on-farm demonstrations by research staff. Though there had been considerable research and demonstration-plot data to show that the package was workable, there had been a lingering doubt in growers as to its reliability. This method was thus not effective to convince growers to change their practice from a simple application of a recipe to the IPM package.

The inappropriateness of the traditional extension method for transferring technical knowledge of IPM programmes has been highlighted by the success of Farmers’ Field School (FFS) (Waage et al., 1995; Bruin and Meerman, 2001). The FFS is primarily a learning approach in which groups of growers are trained in fields during a season-long activity. Growers learn about the ecology of the field by regular observation, analysis of field condition and application of ecologically safe and effective methods to
A farmer-participatory approach for the implementation of a developed Integrated Pest Management System against *Plutella xylostella* in crucifer cultivation in Mauritius. *C Dunhawoor and D Abeeluck*

manage pests. Such experiential learning approach is reported to facilitate “grower empowerment” (Kenmore, 1991).

The best example of such a FFS approach to IPM is that of the rice farming system in Asia (Waage et al., 1995). It has been successfully implemented in Indonesia, Philippines, Vietnam and India making a national commitment to the training of rice growers through FFS system.

This paper describes two case studies on a pilot project in Plaine Wilhems District where the developed IPM package was validated and the FFS approach tested for IPM training in crucifer cultivation.

The first study was conducted in cabbage fields in Plaine Sophie within Plaine Wilhems from July 2003 to October 2004. This site is about 5 km away from other crucifer growing area. Every year, some 150 growers are involved in cultivation of major crops (eg., potato, carrot, coriander, greens, chinese cabbage, cabbage and cauliflower) on a rotational basis on some 140 hectares of the land.

The second study was conducted in cauliflower fields at Carreau Laliane from July 2004 to October 2004. Carreau Laliane is situated in the suburb of Vacoas and about 80 growers cultivate cabbage and cauliflower in rotation with creepers, squash and tomato on about 40 hectares yearly.

In each study, a non-IPM block (7 fields of about 3 hectares) away from the IPM block was selected to determine the cost of DBM control in both blocks.

**METHODOLOGY**

As a first step to initiate the FFS approach, data was collected from a total of 68 growers on a questionnaire-based survey in Plaine Wilhems from May to June 2003. Information collected was based on their current practices and needs. A training programme was then prepared and consisted of: (1) pest recognition - (DBM adult and larva), *Croccidolomia binotalis* (larva), *C. plutellae* (cocoons) (2) DBM larval scouting (select 10 plants in a field at equal intervals along each diagonal; examine selected plants; record the number of larvae on them and determine the average number of larvae per plant (total number of larvae/20 plants), (3) release of *C. plutellae* (4) Field treatment when the average number of larvae/plant equals or exceeds one and (5) Field sanitation.

As incentives to implement the IPM package, growers were provided IPM products such as, biological pesticide (*Bacillus thuringiensis*) and lepidoptera specific chemical insecticides (indoxacarp and lufenuron). From August to December 2003, batches of 500 adults of *Cotesia plutellae* were released twice per week in existing fields.

This training programme was run with groups of motivated growers one week before programme implementation and then individual fields during the 1st crop cycle. The primary objective was to develop grower’s aptitude in pest and parasitoid recognition and pest monitoring.

**Case Study 1: Participatory Approach to implement IPM Package at Plaine Sophie**

The IPM programme consisted of 2 phases: (1) training of motivated growers during the 1st cabbage crop cycle and determination of the cost of DBM control in IPM and non-IPM blocks at Plaine Sophie and La Marie and (2) determination of the rate of adoption of the IPM package by trained growers in the 2nd and 3rd crop cycle.

**Season-long training of growers**

During the 1st crop cycle (July 2003 to April 04), 28 growers were trained in scouting and decision making in their fields either singly or in groups not exceeding 5 growers.
A farmer-participatory approach for the implementation of a developed Integrated Pest Management System against *Plutella xylostella* in crucifer cultivation in Mauritius. *C Dunhawoor and D Abeeluck*

*Determinant of the cost of DBM control in IPM and non-IPM blocks*

From July to September 2003, 2 blocks (an IPM from Plaine Sophie and a non-IPM (growers’) from La Marie) were selected. Each block consisted of 3 hectares of cabbage fields. At Plaine Sophie, treatment was made when threshold level was reached whereas at La Marie, growers sprayed as per their own practices.

In both blocks, the scouting method was used to record the number of larvae and *Cotesia* cocoons at 7 day-intervals. The number of insecticide treatments was recorded as well.

*Determinant of the rate of adoption of the IPM package*

During April to July 04, 12 trained growers were involved in the 2nd crop cultivation on 6 hectares. They themselves monitored the larval population by scouting method and implemented control measures when threshold level was reached without any assistance. Weekly visits were effected to monitor their performance.

From July to October 2004, the practice of 6 trained growers on DBM management in their 3rd crop on 4 hectares was monitored as well.

**Case Study 2: Participatory Approach to Implement IPM Package at Carreau Laliane**

This programme also consisted of 2 phases. During May to July 04, 13 trained growers were assisted in scouting and decision making in their 1st crop cycle on 4.4 hectares. The cost effectiveness of DBM control in a selected IPM and non-IPM block (each of 4 ha in size) was determined as per method described above. The blocks were at a distance of about 200 metres from one another.

During the 2nd crop cycle (July to October 04), 8 of the trained growers having cauliflower plantations performed scouting (without assistance) on 3.3 hectares. Their practice was monitored as per method mentioned above.

**RESULTS**

**Farmers’ profile**

The 68 interviewed growers operated on a small scale and their land was made of disparate parcels (from 0.6-0.8 hectares) with mixed cropping. All growers were literate (19.1 % attended up to 6 years of schooling; 76.5 % up to 11 years & 4.4 % up to tertiary level).

As per age, they were categorised in 4 groups (I, II, III, & IV) (*Table 1*). The percentage of growers in Group I, II, III, and IV was 1.4 %; 35.2 %; 25 % and 38.2 % respectively. Irrespective of age groups and educational background, few growers could correctly recognize pests and parasitoids in their fields. 70.5 % of them sprayed their fields every week, 16.1 % at 3-day intervals and 13.2 % treated their fields based on scouting.
A farmer-participatory approach for the implementation of a developed Integrated Pest Management System against *Plutella xylostella* in crucifer cultivation in Mauritius. C Dunhawoor and D Abeeluck

**Table 1** Profile of sixty eight crucifer growers interviewed during the survey conducted in Plaine Wilhems District from May to June 2003

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of planters</th>
<th>Highest educational background</th>
<th>Pest &amp; parasitoid recognition</th>
<th>Insecticide application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary</td>
<td>Secondary</td>
<td>Tertiary</td>
</tr>
<tr>
<td>I (18 – 21)</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>II (22 – 35)</td>
<td>24</td>
<td>-</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>III (36 – 45)</td>
<td>17</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV (&gt; 46)</td>
<td>26</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Training of growers**

Forty-one growers (28 cabbage and 13 cauliflower growers) were trained in pest and parasitoid recognition, procedures to conduct larval scouting and treat their fields with IPM products during the first crop cycle on about 18.3 hectares (12.9 ha under cabbage and 5.4 under cauliflower cultivation).

During the 1st crop at Plaine Sophie, 235 visits were effected in 28 fields and 28 growers were trained in pest recognition and assisted in DBM scouting. By April 2004, 90 % of them could recognize larvae, pupae and adult DBM, *Crocidolomia* larvae and cocoons of *C. plutellae*. 90 % could perform larval scouting and select appropriate products to spray their fields when the threshold level was attained.

During the 1st crop at Carreau Laliane, 218 visits were effected in 21 fields and 13 growers were trained as at Plaine Sophie. After the 1st crop cycle, 90 % of them could recognize pests and cocoons of *C. plutellae* and 90 % could perform larval scouting and take decision when to spray their fields (Table 2).

**Table 2** Percentage of growers capable of identifying DBM and *Cotesia* cocoons and performing larval scouting in their fields after training

<table>
<thead>
<tr>
<th>Commodity</th>
<th>No. of planters trained</th>
<th>% of growers capable of identifying pest and parasitoid recognition and performing larval scouting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DBM</td>
</tr>
<tr>
<td>Cabbage</td>
<td>28</td>
<td>75</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>13</td>
<td>90</td>
</tr>
</tbody>
</table>
**DBM larval scouting and control at Plaine Sophie**

Four of the 28 fields were not treated because larval numbers were below threshold level. Eleven fields were treated once, 8 fields twice, 4 and one fields were treated three and four times respectively. The number of treatments during the 2nd and 3rd crop (monitored by growers themselves) did not exceed four (Table 3).

<table>
<thead>
<tr>
<th>No. of fields (ha)</th>
<th>Frequencies at which threshold level was exceeded</th>
<th>No. of treatments effected per field and products used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scouting performed by Researcher/Grower from 30/07/03 – 07/04/04 (1st crop):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (1.8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11 (4.5)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8 (4.1)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4 (2.5)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1 (0.2)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Scouting performed by Growers from 16.02 – 03/08/04 (2nd crop):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (1.4)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4 (1.7)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4 (1.9)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1 (0.8)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Scouting performed by Growers from 16.02 – 03/08/04 (3rd crop):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 (0.8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2 (0.4)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2 (1.9)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1 (1.6)</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Determination of the cost of DBM control in IPM and non-IPM blocks**

The IPM block consisted of 7 fields and 17 treatments were effected during the crop cycle with 3 products (lufenuron, *Bacillus thuringiensis* and indoxacarb) used alternately. An average of 2 treatments was effected per field. Lufenuron, *Bacillus thuringiensis* and indoxacarb act against lepidopterous larvae.

In the non-IPM block, 45 treatments were effected in the 7 fields with 9 products (abamectin, *Bacillus thuringiensis* cartap, flufenoxuron, deltamethrin, indoxacarb, lambda cyhalothrin, lufenuron and profenofos). An average of 6 treatments was made per field either singly or in mixtures.

The estimated cost of DBM control per hectare (insecticides & man labour) in the IPM and non-IPM blocks was Rs 913 (69 US dollars) and Rs 2216 (167 US dollars) respectively. Marketable heads in both blocks were above 85 %.
A farmer-participatory approach for the implementation of a developed Integrated Pest Management System against *Plutella xylostella* in crucifer cultivation in Mauritius. C Dunhawoour and D Abeeluck

**DBM larval scouting and control at Carreau Laliane**

Insecticidal treatments during the 1st and the 2nd crop cycle were low in IPM block. Only 1 field was treated four times (Table 4).

**Table 4** Frequencies at which larval numbers exceeded threshold level in a crop cycle during weekly monitoring 13 cauliflower fields at Carreau Laliane from May to October 2004.

<table>
<thead>
<tr>
<th>No. of fields (ha)</th>
<th>Frequencies at which threshold level was exceeded</th>
<th>No. of treatments effected per field and products used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assisting scouting performed from May to July 2004 (1st crop)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (4.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4 (0.8)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 (0.8)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Scouting performed by Growers from July to October 2004 (2nd crop)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 (0.5)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3 (1.3)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3 (1.5)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1 (0.3)</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Determination of the cost of DBM control in IPM and non-IPM blocks**

Eight treatments (lufenuron and *Bacillus thuringiensis*) were effected in the 7 fields of the IPM Block. In the non-IPM block, 50 treatments with 8 products (abamectin, *Bacillus thuringiensis*, cartap, deltamethrin, indoxacarb, lambda cyhalothrin, lufenuron and profenofos) were applied.

The estimated cost of pest DBM control per hectare (insecticides & man labour) in the IPM and growers’ blocks was Rs 1068 (80 US dollars) and Rs 4080 (309 US dollars) respectively. Marketable curds in both blocks were above 90 %.

**DBM incidence at Plaine Sophie and Carreau Laliane**

About 3.5 hectares of land was under permanent cabbage cultivation every month during summer and winter in Plaine Sophie. DBM larvae were present at varying numbers throughout the study period.

During November and December, the larval population was high in cabbage fields. Larval numbers exceeded threshold levels on 16 out of 64 scouting dates. From January to April, the threshold level was exceeded on 7 out of 80 scouting dates.

A similar trend in larval population in cauliflower fields was observed in Carreau Laliane during the early winter (May to July 2004). The threshold level was exceeded in 20 out of 117 scouting dates. In late winter (July to September), threshold level was exceeded in 13 out of 39 scouting dates. Larvae were most abundant only in September.

DBM larval population fluctuations were similar within the cabbage and cauliflower crop cycle in summer and winter (Figure 1 and 2). The average number of larvae per plant ranged from 0 to 0.4 during the 1st week after transplantation. An increase in numbers was noted from the 2nd to 6th week in cabbage and from 3rd to 9th week in the cauliflower fields.
DBM incidence during a crop cycle

During the cabbage crop cycle, the larval numbers reached up to 1.2 per plant at the 3rd week (at 4-6 leaf stage) and reached a maximum of 1.4 at the 7th week (primordial stage). This stage is critical when larvae can cause up to 100% damage to young plants and/or newly formed heads.

On the 7th day of transplantation, the average number of larvae per plant was 0.3 during winter (May to October) and 0.4 during summer (November to April) (Figure 1). This tends to indicate that treatment is not warranted in the 1st week if DBM free plants are used at transplantation.

**Figure 1** Average number of larvae per plant per week recorded during a cabbage crop cycle at Plaine Sophie

![Graph showing DBM incidence during a cabbage crop cycle](image1)

In the cauliflower crop cycle, larval numbers were high during the 4th week (curd formation stage) to 9th week. In both periods, the average number of larvae per plant ranged from 0.5 to 1.9 (Figure 2). Severity of DBM larvae was higher whenever tender leaves were abundant.

**Figure 2** Average number of larvae per plant per week recorded during a cauliflower crop cycle at Carreau Laliane 2004.

![Graph showing DBM incidence during a cauliflower crop cycle](image2)
A farmer-participatory approach for the implementation of a developed Integrated Pest Management System against *Plutella xylostella* in crucifer cultivation in Mauritius. C Dunhawoor and D Abeeluck

Incidence of *Cotesia* cocoons in IPM and non-IPM blocks at Plaine Sophie

In the IPM block, *Cotesia* cocoons were present in higher numbers. The average number of cocoons per plant ranged from 0.2 to 1 on 39 out of 56 scouting dates. In the non-IPM block, cocoons were recorded only on 7 out of 56 scouting dates and the average number ranged from 0.1 to 0.4 per plant.

During the cabbage crop cycle, the average number of cocoons per plant from the 2nd to 9th week varied from 0.5 to 1 (Figure 3). DBM larvae were highest in the 4th week (2.2/plant) and controlled by *Bacillus thuringiensis*. During the 3 weeks after treatment, an average of 0.7 cocoons per plant was observed.

**Figure 3** Average number of DBM larvae and *Cotesia* cocoons per plant per week during one crop cycle in the IPM block at Plaine Sophie during November and December 2003

DISCUSSION

The information collected on growers’ profile indicates that 86.8 % of the interviewees were not in a position to differentiate between pest (DBM) and natural enemy (*Cotesia plutellae*) and could not use the scouting method to monitor DBM population in their fields. This shows that previous training (workshops, field demonstrations in Plaine Wilhems), did not increase growers’ knowledge in DBM management.

The FFS approach was an effective method to promote the developed IPM package for implementation by cabbage and cauliflower growers through the “season-long” training activity. Such success had been previously obtained in developing a trapping method for leaf miners with onion growers (Unmole et al., 1999) and for *Stomoxys* flies with deer ranch owners (Abeeluck et al., 2001).

During a single crop cycle, more than 75 % of the trained growers were capable of recognising pest and parasitoids, monitor DBM population and treat with IPM compatible products whenever threshold level was attained. However, these growers need further support to assure that their crops do not require weekly application of insecticides.

DBM control in the non-IPM plot was about 2.4 times more expensive than the IPM plot in cabbage cultivation and about 3.9 times in cauliflower. It is also remarked that non-IPM growers used unnecessarily a wide range of chemical insecticides against *P. xylostella*. The population of *C. plutellae* fields of such growers was comparatively lower.

CONCLUSION

The FFS approach is an effective method to promote the developed IPM package for implementation among cabbage and cauliflower growers. After training, growers were capable of monitoring their fields and spray according to threshold level. This FFS approach is to be extended islandwide.

ACKNOWLEDGEMENTS

The authors wish to thank growers of Plaine Sophie and Carreau Laliane who participated actively in the IPM study. We would like to extend our gratitude to Ireland Blyth Limited who provided IPM products and other incentives to growers during the implementation of the 2nd case study at Carreau Laliane. The collaboration of Extension Officers and the assistance of the staff of the Entomology Division during implementation of the study are also acknowledged.

REFERENCES


BIOLOGICAL CONTROL OF THE SPIRALLING WHITEFLY, ALEURODICUS DISPERSUS

B. Gungah, S.I. Seewooruthun, P. Nundloll and M. Rambhunjun

Ministry of Agro-Industry and Fisheries

ABSTRACT

The spiralling whitefly, Aleurodicus dispersus Russel, was reported for the first time in Mauritius in July 2000. This pest is native to Central America, the Caribbean region and the Pacific islands. It has the potential to become widespread and it has steadily extended its geographical distribution to Southeast Asia, Australia, Africa and the Indian Ocean. It is highly polyphagous and surveys carried out in Mauritius revealed that the pest attacks fruit trees, shade trees, ornamentals and to a lesser extent vegetable crops. Its extensive host range covers more than 77 plant species in Mauritius. The main hosts include acalypha, poinsettia, guava, frangipani, papaya, Indian almond and banana. Field observations have shown that the spiralling whitefly population is most abundant in coastal areas and other localities where the temperature is relatively high. Attempts to control the pest using pesticides were not found feasible due to its wide host range and high infestations and therefore a classical biological control approach has been adopted. Surveys were carried out island-wide to search for existing natural enemy complex attacking Aleurodicus dispersus. One exotic coccinellid predator, Nephaspis bicolor which is specific to aleurodids was introduced from Trinidad in 2003. This paper provides information on the pest, the biocontrol agent and the implementation of the biological control effort.

Keywords: Biological control, whitefly, Aleurodicus dispersus, coccinellid, Nephaspis bicolor

INTRODUCTION

The spiralling whitefly, Aleurodicus dispersus Russel (Homoptera : Aleurodidae) was reported in Mauritius in July 2000. It was observed for the first time in the west coast of the island. Since then the pest has rapidly spread and is now established all over the island, being most abundant in coastal areas and regions with relatively high temperatures. It has also spread to Rodrigues in 2001. Initial measures to control the pest using insecticides were found difficult due to its wide host range and high infestations. This would also involve high costs and generally the widespread use of pesticides has a negative impact on the environment. Hence, classical biological control was suggested as a sustainable long-term solution. Based on excellent results in controlling this pest following introduction of natural enemies in Hawaii and elsewhere from Trinidad, prospects for success appeared good (Cock, 1985).

Prior to the importation of natural enemies surveys were conducted island-wide to investigate for the existence of predators and parasitoids since in some countries where the pest was introduced, it arrived together with its natural enemies. During surveys several species were found attacking the spiralling whitefly (Table 1). However, they apparently did not provide adequate control. The coccinellid predator Nephaspis bicolor which is one of the species that has co-evolved and has specialised in preying upon A. dispersus was imported from the Caribbean and Latin American Regional Centre of CAB International, Trinidad in 2003. The predator is being reared locally and releases are made in all infested regions. This paper provides information on the pest, the bio-control agent and the implementation of the biological control programme.
Biological control of the spiralling whitefly, *Aleurodicus dispersus*. R Gangah et al.

The Pest

**Origin and distribution**
The pest is native to Central America, the Caribbean region and the Pacific islands. It was first observed in Florida in 1957 (Russel, 1965) and was reported in Hawaii on the Oahu island in 1978 (Paulson and Kumashiro, 1985). Since then it started to spread rapidly to most tropical regions of the world, reaching Papua New Guinea in 1987 (Waterhouse and Norris, 1989).

It was found in Continental Africa in 1992 where a severe outbreak was reported in Nigeria. From there the pest has steadily spread to other western African countries. It was also reported from India in 1993 from Kerala (Palaniswami et al., 1995) and later from other parts of peninsular India (Reddy and Chandurkar, 1999). In 1998 it was discovered in Australia (Lambkin, 1998). In Mauritius it was reported in 2000 (Anon, 2000). It was found in the Seychelles in 2001. It has also been recently detected in Reunion island (pers.comm., Philippe Ryckewaert 2005 CIRAD-FLHOR).

**Biology, damage and host plants**
The adult female lays its eggs in a typical spiral pattern under the leaf surface. The developmental biology at 26°C ± 2°C and 60 ± 5% relative humidity takes about 32 to 38 days to complete the total life cycle, with the egg stage lasting 7-12 days, each of the first three instars 4-6 days, 3-5 days, 6-10 days, respectively and the fourth instar or pupa 10-15 days.

Like other aleyrodids, damage is caused both by the immature stages and adults, which feed by sucking plant sap. Direct feeding can cause premature leaf drop, reduces plant vigour and yields. However, injury caused by heavy infestations is usually insufficient to kill plants. Indirect damage is due to excreted honeydew that encourages growth of black sooty moulds which interfere with photosynthesis. The copious production of wax as well as honeydew and associated sooty moulds make affected plants look unsightly.

*Aleurodicus dispersus* is highly polyphagous and is capable of infesting a wide range of host plants. These include fruit trees, shade trees, ornamentals, weeds and to a lesser extent vegetable crops that are grown commercially. This may be due to the repeated application of pesticides in field condition. Its extensive host range covers more than 77 plant species in Mauritius. The most preferred hosts include acalypha, poinsettia, guava, frangipani, papaya, Indian almond and banana (*Table 2*).

*A. dispersus* is found more or less island-wide but is practically absent at altitudes above 400 metres. Population levels in the cooler regions such as Curepipe, Forest side and Trou aux Cerfs are negligible.

**Surveys for Natural enemies in Mauritius**
Before implementing the biological control programme of the spiralling whitefly, part of the initial activities was to search for natural enemies occurring locally. The surveys revealed that some natural enemies were found attacking *A. dispersus* (*Table 1*). But they were mainly generalist predators which exploited the new abundant food resource, and were not effective in bringing *A. dispersus* under control. One aphelinid parasitoid was also detected. It was identified by the Natural History Museum (NHM) as *Encarsia hispida*.

---

**Table 1: Natural enemies of *Aleurodicus dispersus* in Mauritius**

<table>
<thead>
<tr>
<th>Natural enemy</th>
<th>Family</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predators</td>
<td>Coccinellidae</td>
<td><em>Chilochorus nigritus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Thea variegata</em></td>
</tr>
<tr>
<td></td>
<td>Chrysopidae</td>
<td>2 Unidentified sp</td>
</tr>
<tr>
<td></td>
<td>Syrphidae</td>
<td>Unidentified sp</td>
</tr>
<tr>
<td>Parasitoid</td>
<td>Aphelinidae</td>
<td><em>Encarsia hispida</em></td>
</tr>
</tbody>
</table>

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308
Table 2: Main hosts of *Aleurodicus dispersus* in Mauritius

<table>
<thead>
<tr>
<th>Type of Plant</th>
<th>Common Name</th>
<th>Botanical Name</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit trees</td>
<td>Guava</td>
<td><em>Psidium guajava</em></td>
<td>Myrtaceae</td>
</tr>
<tr>
<td></td>
<td>Papaya</td>
<td><em>Carica papaya</em></td>
<td>Caricaceae</td>
</tr>
<tr>
<td></td>
<td>Banana</td>
<td><em>Musa sapientum</em></td>
<td>Musaceae</td>
</tr>
<tr>
<td>Ornamental</td>
<td>Acalypha</td>
<td><em>Acalypha sp</em></td>
<td>Euphorbiaceae</td>
</tr>
<tr>
<td></td>
<td>Poinsetia</td>
<td><em>Euphorbia pulcherrina</em></td>
<td>Euphorbiaceae</td>
</tr>
<tr>
<td></td>
<td>Frangipani</td>
<td><em>Plumeria rubra</em></td>
<td>Apocynaceae</td>
</tr>
<tr>
<td>Shade trees</td>
<td>Indian almond</td>
<td><em>Terminalia catappa</em></td>
<td>Combretaceae</td>
</tr>
</tbody>
</table>

An island-wide survey was carried out in order to study the impact of the parasitoid on the pest. The parasitoid was found to be rather localised. Surveys were carried out throughout the summer period 2001/2002 since *A. dispersus* thrives in hot summer temperatures. It was found that the parasitism was extremely low and the parasitoid had not dispersed from its site of collection, i.e Réduit. Attempts to help its dispersal by collection and release at different sites also gave poor results. Other general predators present included reduviid bugs, spiders, lizards and birds: these attacked the whitefly, but did not have any significant impact.

**The Biocontrol Agent: *Nephaspis bicolor***

**Taxonomy and Biology**
The coccinellid predator *Nephaspis bicolor* belongs to the order Coleoptera, family Coccinellidae and subfamily Scymninae. Both males and females are very small and the sizes vary from a length of 1.3 to 1.6 mm and width 0.79 to 1.15 mm. The body is convex and pubescent. Males have yellow prothorax and the colour of the female pronotum is black with a wide yellow area on each side (Gordon, 1982).

Female of *N. bicolor* preferentially lays most eggs near host insects. Thus most of the eggs are found under the flocculent material produced by the whiteflies. The larvae usually emerge between day 4 and day 7. The larvae develop through 4 instars with no significant morphological differences between the different instars. All stages are pale white to cream in colour. The first instar lasts 2-7 days, second instar 1-4 days, third instar 1-6 days, fourth instar 2-5 days. A short pre-pupal period lasts 1-2 days during which the larva stops feeding and prepares for pupation. Duration of the life cycle from egg to adult ranges from 21 to 28 days (Lopez et al., 1997a).

**Biological control procedures**
The biological control effort was initiated in 2003. The Caribbean and Latin American Regional Centre of CAB International in Trinidad was contacted and the introduction of the coccinellid predator *Nephaspis bicolor* was recommended. This species had shown a strong preference for whiteflies. Natural enemy that had co-evolved with *Aleurodicus* sp would be expected to have specialized in preying upon this pest and thus provide good levels of control. Based on excellent results following the introduction of this natural enemy in Hawaii (Kumashiro et al., 1983) and other islands in the Pacific, *N. bicolor* was definitely considered for introduction from CAB International in Trinidad. An agreement was thus signed between CAB International - CLARC and the Ministry of Agriculture, Food Technology and Natural resources for the supply of natural enemy.

Recent years have seen increased concerns over the potential impact of introduced agents on non-target species (Howarth, 1991). With the ratification of the International Code of Conduct for the Import and Release of Exotic Biological Control Agents (FAO, 1996) as an International Standard for Phytosanitary Measures, it was expected that provision of this code would apply in the implementation of the biological control programme of the spiralling whitefly. The code provides guidelines to be followed when making introductions of natural enemies by both importing and exporting agencies. The present project is carried out in full compliance with the code. To this end a dossier was prepared for *Nephaspis bicolor* (Lopez et al., 1997b). The purpose of the dossier was to enable our Quarantine Services to make an informed judgement on whether or not to introduce the natural enemy. In the dossier relevant information on the pest and the predator has been summarised. This included an assessment of potential risks posed to non-target organisms (particularly to other whitefly species and...
beneficial insects), to human and animal health. It is also reported that the predator has a narrow host range.

**Importation of *Nephaspis bicolor***

*Introduction, rearing and release of N. bicolor*

Authorisation for importation of biocontrol agent was obtained from the Quarantine Committee following the submission of the comprehensive Dossier on the pest and predator. The CABI - CLARC proceeded with field collection of the predator and laboratory multiplication such that only certified laboratory reared *N. bicolor* could be despatched to Mauritius. Two shipments of *N. bicolor* were received. The first consignment comprised about 100 adults which arrived in July 2003 and the second consignment comprising 80 adults was sent in November 2003.

The predators were carefully examined upon arrival and multiplied at the Entomology Division for one generation under quarantine conditions in a controlled- temperature room before field release. The first release was effected in the month of September 2003. An average of about 250 to 300 adults has been released every month and releases are on-going. The total number of adult predators that were released till end of 2004 was 3939 (Table 3). Release sites were identified 1 to 2 weeks before the adults were due to emerge in the laboratory. The choice of release sites was mainly on locations where infestations occurred first and also where heavy infestations were found.

Two release strategies were adopted: a few adults were released into sleeve cages while the rest was released in the open. The latter proved more successful.

**Table 3: Release of *Nephaspis bicolor* in different regions of Mauritius**

<table>
<thead>
<tr>
<th>Region</th>
<th>Total number released*</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>631</td>
</tr>
<tr>
<td>South</td>
<td>658</td>
</tr>
<tr>
<td>East</td>
<td>498</td>
</tr>
<tr>
<td>West</td>
<td>1677</td>
</tr>
<tr>
<td>Lower Plaine Wilhems</td>
<td>475</td>
</tr>
</tbody>
</table>

* Denotes total number of predators released during period September 2003 to December 2004

The Entomology Division has developed the capability to rear the coccinellid predator and it is envisaged that releases would be continued over a few years.

**Current status**

Regular surveys were carried out at selected sites. Population counts of *A. dispersus* were made by sampling leaves of guava and acalypha at locations where releases were made. These have shown a distinct and significant decrease in whitefly population. There is evidence that the predator has become established and is spreading from initial release sites. They have been recovered at a distance of 3 to 4 kilometres away from the release points.

**CONCLUSION**

*Aleurodicus dispersus* has become well established in and around release sites mainly in the coastal regions and localities where high temperatures persist. During the period 2000 to 2003 there was a flare up of the whitefly populations and several complaints were received from the public on a regular basis. However the releases made over several localities have proved quite effective in suppressing the pest populations. It is envisaged to continue releases over a few years until the predator is well established throughout the island. A more comprehensive evaluation of the effectiveness of the introduced natural enemy is required and assessment is being conducted in different localities.
ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help and encouragement rendered by the Ministry of Agriculture, Food Technology and Natural Resources for implementing the project. We thank Ms Vyu Lopez and Dr M.T.K. Kairo of CAB International in Trinidad for their valuable suggestions and advice prior to the implementation of the programme. We also wish to thank the acting Principal Research and Development Officer of the Entomology Division for providing various facilities.

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ABSTRACT

The cypress aphid, *Cinara cupressivora* was first reported in Mauritius in 1999. This pest is native to the region from eastern Greece to south of the Caspian Sea. *C. cupressivora* feeds mainly on members of the conifer family Cupressaceae including species in the genera Cupressus, Juniperus, Widdringtonia and to a lesser extent Thuja. The aphid causes heavy economic losses in the African countries whilst in Mauritius, the aesthetic value of cypress trees is affected. Field surveys revealed that the main host of the pest is *Juniperus bermudiana*. The aphid exploits a wide range of feeding sites ranging from green branches to woody stems. Damage is characterised by dieback and severe infestation causes death of mature trees. Surveys show that cypress aphid populations are strongly influenced by climatic conditions and temperature. The population builds to a peak during the dry winter season and is markedly reduced during summer and rainy season. The same trend has been observed since 1999. The most effective time to apply control measures is when the aphids are beginning to build up in numbers that is in May-July before symptoms of attack appear. Control strategies for the pest include chemical control, use of resistant varieties and silvicultural techniques. The best effective management option of the aphid is integrated pest management. However, since cypress aphid is an exotic pest, classical biological control seems to be the most suitable and permanent solution for control. A very promising natural enemy *Pauesia juniperorum* which is a highly specific braconid wasp of the aphid, was introduced from Kenya in 2001, 2003 and 2004 for mass-rearing and release. Since laboratory rearing proved to be difficult, no release was possible during the first introduction. The first field releases were made in August 2003. About 1500 parasitoids were released in the open and in sleeve cages in 2003 and 2004. Surveys to monitor establishment of the biological agent showed presence of mummies and newly emerged adults in the field a few weeks after release. However, no mummies were recorded thereafter. Further surveys will be pursued to determine the survival rate and dispersal of the parasitoid when the aphid population is low. Moreover, more parasitoids will be introduced in the subsequent years for release.

**Keywords:** cypress aphid, *Cinara cupressivora*, biological control, *Pauesia juniperorum*, parasitoids

INTRODUCTION

The cypress aphid *C. cupressivora* was first reported in Mauritius in January 1999. The pest feeds on sap of cypress plants. Initially the pest was present in the upper Plaines Wilhems but within a few months, it had spread to all parts of the island. Surveys indicated that the preferred host was *Juniperus bermudiana* which covers about 20 ha of forest land and is mainly grown as a decorative plant in gardens, private yards and public areas. The pest is of temperate origin and thrives best at 18-22º C.

In September/ October 1999, browning symptoms of huge cypress trees were observed in many places. This also led to death of many trees. Following major public concern, emergency control measures were prompted which mainly involved massive chemical spraying in areas of high density of cypress. However, this measure was implemented as a rapid and short-term treatment since repeated use of hazardous chemicals in the environment, would cause health and environmental problems. A more environmental friendly method was thereby envisaged for long-term control. Biological control turned out to be the ultimate rescue to this devastating pest.

Field observations revealed the presence of several natural enemy predators including coccinellid beetles (Coleoptera: Coccinellidae), syrphid flies (Diptera: Syrphidae), chrysopids (Neuroptera: Chrysopidae) and spiders (Arachnida). However, these biological agents are classified as generalist
natural enemies that cannot keep the pest population below economic levels. In other countries e.g. in Europe and Africa various insects were identified for the control of this pest, and one which proved out to be a particularly very effective natural enemy of C. cupressivora was the braconid wasp, Pauesia juniperorum. This parasitoid has given successful control in Kenya, Malawi, Uganda, Burundi, Tanzania and other countries.

Following approval by the Quarantine committee for importation of biological agents, P. juniperorum was imported from Kenya (Kenya Forestry Research Institute) in 2001 with the objective of rearing it in quarantine prior to release. Rearing in the laboratory proved to be difficult and very few parasitoids were recovered. In 2003, another consignment of the natural enemy was again imported and due to death of many parasitoids and difficult rearing, approval for direct release was sought and release was done. In 2004, more parasitoids were imported and adults were released directly after careful screening. Surveys at sites of release revealed successful parasitism in the field after 4-5 generations and newly emerged adults were also observed.

The Pest

The cypress aphid, C. cupressivora (Homoptera: Aphididae), an important alien aphid of the cypresses, cedars and junipers invaded Africa in the late 1980’s. It was first reported in Malawi in 1986 (Murphy et al., 1994) and rapidly spread to many countries in Africa. It is now considered as a major forestry pest in southern, eastern and central Africa. The pest was formerly identified as C. cupressi (Buckton). However, from morphometric analysis C. cupressivora was found to belong to a species complex (Watson et al., 1999). Its origin is most likely to be in the region from eastern Greece to the south of the Caspian Sea (Watson et al., 1999) where it is not considered as a pest due to the state of biological equilibrium existing with its natural enemies. In Africa, C. cupressivora is considered as a serious pest of commercial cypress. By 1991, it was estimated that the aphid had killed US $ 41 million worth of trees in Africa (Day et al., 2003) and was causing an annual loss of growth increment worth US $ 13.5 million (Murphy, 1996). In Mauritius, a first report of the pest was made in 1999 (MAFTNR, 1999). Locally, it is not considered as a pest of economic importance.

Description, Biology, Damage And Host Range

C. cupressivora is a brownish soft-bodied aphid ranging from 2-5 mm in length, often with a grey waxy coating. Adults can be either winged or wingless. Usually, they occur in colonies on twigs and branches of infested trees. Adults produce young nymphs rapidly hence causing a rapid build-up in population. Reproduction occurs mostly by parthenogenesis and adults give birth to live nymphs. Three nymphal instars occur, lasting for about eleven days. Adults survive for a period of about fifteen days and the entire lifespan extends over approximately 25 days (Alleck and Seewooruthun, 2002).

C. cupressivora has a wide host range. It attacks cypresses, cedars and junipers. Worldwide, it feeds on various trees from the following genera: Cupressus, Juniperus, Thuja, Callitris, Widdringtonia, Chamaecyparis, Austrocedrus, and the hybrid Cupressocyparis (Day, 1999; Baldini and Aguayo, 2005).

The aphid exploits a wide range of feeding sites varying from green branches to woody stems (Day et al., 2003). Damage mainly occurs by sap feeding hence causing yellowing and browning of foliage. The saliva produced is phytotoxic and leads to necrosis in the phloem resulting subsequently in twig withering. Feeding retards new growth and causes desiccation of the stems with a progressive dieback of heavily infested trees. The overall effect ranges from partial damage to eventual death of the entire tree depending on the severity of the infestation. Death of mature trees can occur within three months in case of severe infestation (Owuor, 1991). Large amount of honeydew is also produced favouring growth of sooty mould which thereby hinders photosynthesis and gas exchange.
Pest Status

Immediately following its first detection, monitoring of the pest, its natural enemies and the damage it caused was carried out. Since it is not practical to attain upper branches of tall trees for sampling, sub-sampling of lower branches of 15 centimetres long from the terminal end was done. This gives the density of the aphids per unit of tree canopy.

In 1999, a high population was observed during the months of January and February. After the cyclone Daniella passed over Mauritius in March 1999, a rapid decline in the population was observed. A few months later, population increase was noted again and the pest rapidly spread to all parts of Mauritius. Later as from October onwards, population decline was observed again (Figures 1 & 2).

**Figure 1** Cypress aphid population density at Salazie in 1999

**Figure 2** Cypress aphid population density at Vacoas in 1999
Cypress aphid populations are strongly influenced by weather conditions. During heavy rains and high temperatures, cypress aphid populations decline. The pest is present throughout the year with a low population during the warm months (from January to April) and a population rise during the cooler months (from May to September). This is explained by a population temperature dependent relationship (Figure 3). Temperature affects longevity, development time and fecundity (Kairo and Murphy, 1999) of Cinara species, all of which influence intrinsic rate of increase. Rate of development is high between 20 - 25°C (Kairo and Murphy, 1999) hence causing a rapid population growth during winter. Reduction in the population caused by the death of aphids occurs above 28°C which usually prevails from October to March. The same trend in the pest population has been observed in the subsequent years (Figures 4-6).

**Figure 3** Mean monthly maximum and minimum temperature at Vacoas in 1999

(MMS, 1999)

**Figure 4** Cypress aphid population density at Vacoas in 2000
Browning symptoms start to appear as from September at the end of winter. Timing of biological control measures depends on presence of aphids in the field. From survey results, the best time to begin release of parasitoids is when the aphid population starts to build up in May, June and July.

Management Of C. cupessivora

Different control options exist for the pest namely:

1. Physical / Silvicultural
   Thinning reduces the density of trees and shady conditions which are favourable to the aphid.

2. Chemical
   Treatment with insecticides of low toxicity to humans and other insects can be effected. Nevertheless, chemical control is not practical on huge trees and involves handling and regular spraying of toxic substances. In addition, this may lead to problems associated with environmental contamination, health safety and the possibility of resistance development in the pest.

3. Genetic
   Resistant varieties of cypresses and junipers can be planted to replace dead and infested trees. Genetic resistance to C. cupressivora in Cupressus lusitanica has been found and investigated in Kenya (Kamunya et al., 1999; Orondo and Day, 1994).

4. Biological
   Several generalist natural enemies may help to regulate the pest population to a certain extent. Other natural enemies which can be used as potential biological agents of Cinara spp. are Pauesia cupressobii and P. juniperorum (Hymenoptera: Braconidae) and Aphidus spp., a parasitoid which has been found attacking C. cupressivora in Germany (Mwangi, 2002). Biological control seems to be the most promising option for a long term management of this pest.

Biological Control

Since cypress aphid is an exotic pest, classical biological control is the most suitable option for controlling it. P. juniperorum was selected as a potential agent for the biological control programme. This parasitoid was introduced in Africa in the 1990’s from UK and France and released in several countries. In 1999 the parasitoid was observed in the field and is now widespread in countries where it was released.

A decline in the severity of damage and the aphid population has been observed since the introduction of the parasitoid in the African countries. However, this could not be attributed solely to the action of the parasitoid. Impact assessment is being carried out to evaluate the effectiveness of the parasitoids.

Biology of P. juniperorum

P. juniperorum is a braconid wasp of the sub-family aphidiinae. It is a solitary endo-parasitoid (Kairo and Murphy, 1993) which has a narrow host range restricted to Cinara spp. in the subgenus Cupressobium (Stary, 1976). It will not attack other parasitoid species. Adults are about 10 mm long, with a black head, brown-black thorax, yellow legs and a yellowish abdomen which becomes darker in older insects. Adults mate soon after emerging and immediately start parasitising aphids. The female deposits only one egg inside the host aphid. Eggs hatch 48 hours after oviposition and developing larvae pass through three instars in eight days before pupation. The larvae feed on the internal organ of the host and eventually only the cuticle remains. The parasitoid pupa remains inside the host cuticle which stretches, darkens and becomes hard and gets attached to the twigs. At this stage, the parasitized aphid is referred to as a mummy. The pupal period lasts for about six days. After full development, the adult parasitoid cuts an incomplete circular hole at the posterior end of the mummy to emerge. The total development time from egg to adult is about 14 days at 22°C (Mutitu and Ogembo, 2000). Adults can live up to 7 days.
Release of *P. juniperorum*

In Mauritius, the biological control programme was initiated in 2000. A dossier was prepared according to the FAO Code of Conduct (FAO, 1996) for the importation and release of the parasitoid. The timing for release of the biocontrol agent is very crucial to maximize the chances for survival and establishment. Through the population monitoring figures, it is concluded that the optimal time for release would be when the aphid population starts to build up or when it is most abundant in the field, that is from May onwards till August.

Two consignments of about 1000 parasitoids were imported in August 2001 from Kenya Forestry Research Institute with the objective of rearing it and carrying out host specificity tests before release. The parasitoids were carefully screened for hyperparasites and reared in the laboratory till the third generation. However very few parasitoids were recovered in the third generation and no release was possible.

In 2003, two more consignments of about 1000 parasitoids were again imported and reared in the laboratory. Rearing proved to be difficult again due to poor quality of cypress seedlings resulting in low quality aphids which are not attractive to the parasitoids. *P. juniperorum* parasitises only healthy aphids (Day, 1999). Due to the rearing difficulty of the parasitoid, direct release of the adults. (Table 1) was done in the field following approval by the Quarantine Sub-Committee. Direct releases of adult parasitoids do not represent any risks as the material imported is laboratory reared and certified. Close monitoring of the released parasitoids showed signs of mummification two weeks after release in the field though no more mummies were observed thereafter.

<table>
<thead>
<tr>
<th>Sites of release</th>
<th>Number of parasitoids released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salazie</td>
<td>50</td>
</tr>
<tr>
<td>Belle Rive</td>
<td>20</td>
</tr>
<tr>
<td>Vacoas</td>
<td>50</td>
</tr>
<tr>
<td>Mare-Longue</td>
<td>50</td>
</tr>
<tr>
<td>Réduit</td>
<td>60</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>230</strong></td>
</tr>
</tbody>
</table>

In 2004 two more consignments of parasitoids were imported in July for direct release. Both adults and mummies were shipped. Release of adults were carried out in several localities (Table 2). Release was done in sleeve cages and directly on aphid colonies on cypress trees. Mummies were kept in the laboratory for emergence before release was done.

<table>
<thead>
<tr>
<th>Sites of release</th>
<th>Number of parasitoids released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salazie</td>
<td>466</td>
</tr>
<tr>
<td>Belle Rive</td>
<td>90</td>
</tr>
<tr>
<td>Vacoas</td>
<td>203</td>
</tr>
<tr>
<td>Mare-Longue</td>
<td>44</td>
</tr>
<tr>
<td>Réduit</td>
<td>254</td>
</tr>
<tr>
<td>Quinze Cantons</td>
<td>99</td>
</tr>
<tr>
<td>Quatre-Bornes</td>
<td>29</td>
</tr>
<tr>
<td>Curepipe</td>
<td>93</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1278</strong></td>
</tr>
</tbody>
</table>
Establishment of Parasitoids

Mummies were observed at Vacoas and Quinze Cantons two weeks after release. Mummies were again observed in August 2004 at Vacoas and Quinze Cantons and a few adults from the first generation were observed at Quinze Cantons in August. Thereafter no mummies were recorded during the month of September. However, in November and December mummies were again found at Vacoas at the release site, though a low number was recorded for December during which the aphid population was low in most surveyed areas. No mummies were observed in January 2005 most probably due to the heavy mortality of cypress aphid which is observed every year during this period as a result of a rapid rise in temperature.

CONCLUSION

At this stage, it is difficult to determine the establishment of the parasitoids in the field. It is expected that successful establishment will be attained after several releases. It is very encouraging to note that mummified aphids were observed two weeks after the first releases in 2003 and in 2004, soon after release and again after 3-4 months. This observation shows that the parasitoids are adapting to our local conditions. In other countries such as Kenya, Malawi and Uganda, no signs of establishment were observed after the first releases of the parasitoids (Day et al., 2003; Mutitu, 2002; Kiwuso, 2002). It is only after several years that parasitoids and mummies were recovered in the field and now, the parasitoid is well established in African countries where P. juniperorum has been released (Day et al., 2003).

More parasitoids will be imported and released during 2005 and 2006. Since mummies were recovered several months after release, there is hope that successful establishment will be attained following repeated releases.

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**MMS (Mauritius Meteorological Service).** 1999. Annual report of climatological summaries.


ASSESSMENT ON THE POPULATION OF CRYPTOPLHEBIA PELTASTICA (MEYRICK) (LEPIDOPTERA: TORTRICIDAE) AND FRUIT DAMAGE IN LITCHI ORCHARDS AND BACKYARDS IN MAURITIUS

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ABSTRACT

Cryptothelbia peltastica (Meyrick) is an important pest of litchi, Litchi chinensis Sonn. in Mauritius. Very little is known about C. peltastica and control has so far been effected without prior knowledge of its biology and ecology. This study reports on the first attempt at assessing adult population of C. peltastica and damage on litchi fruits in commercial orchards and backyards.

4 types of pheromones (A, B, C and D) were tested. Type C, a combination of (Z)-8-dodecenyl acetate and (E)-8-dodecenyl acetate in a ratio 3:1, was attractive to male moths and used in population studies in 4 orchards and 6 backyards. The average number of moths per trap per week varied from 0 to 2 in all sites. In one orchard, a 2-fold increase in trap catches was observed at the start of fruiting and catches remained high until after harvest.

74 clusters totaling 1480 fruits from 16 litchi trees were examined every week as from fruit set until harvest. Eggs of C. peltastica were recorded on fruits whose sizes varied from 2 mm to 27 mm in diameter. In most cases, a single egg was observed per fruit but up to 3 eggs on one fruit had been recorded. Damage occurred at any time during the fruiting period, starting from an early fruit stage (3 mm) and peaked at ripening stage. The estimated cumulative damage for the litchi season of 2004 ranged from 7% to 22%.

At harvest time, 3579 fruits were randomly selected and examined in orchards and backyards in the north and central plateau. Damage varied from 0% to 21%. There was no significant difference in damage levels between north and central plateau. However, orchards had significantly higher damage levels compared to backyards.

458 damaged fruits were observed individually in laboratory. 146 adults with a sex ratio 1:1 (male:female) emerged from 139 fruits. On average, one adult was recorded per damaged fruit but a maximum of 2 adults per fruit has also been recorded. A hymenopteran parasitoid was recovered from 1.5% of the damaged fruit samples.

Keywords: Cryptothelbia peltastica, litchi, pheromone, fruit damage

INTRODUCTION

The genus Cryptothelbia contains five species that attack fruits of Litchi chinensis Sonn. throughout the world (Waite and Hwang, 2002). Cryptothelbia peltastica is considered an important pest in Mauritius, South Africa and other Indian Ocean islands (Abeeluck et al., 2002; Mamet and Williams, 1993; Waite and Hwang, 2002). In Mauritius, C. peltastica is recorded on 13 plant species from 4 different families (Mamet and Williams, 1993).

Abeeluck et al., (2002) and Quilici et al., (1988) described briefly the biology of C. peltastica. The adult moth is small (8 mm long and 20 mm wide), grey in colour and is recognized by a prominent tornal spot on its forewings (Quilici et al., 1988). The female moth lays eggs on the fruit. On hatching, the larva feeds on the fruit skin and then tunnels towards the seed. Such feeding favours fungal growth and other infestation leading to fruit rot. The mature larva is about 15 mm long, pinkish in colour and has a black head capsule. Pupation usually occurs within the fruit. But if an infested fruit is stored for some time, the puparium can be seen partially protruding out.

In Mauritius, about 80% of fruits are produced from backyards (Abeeluck et al., 2002). Since early 1990’s, there has been increasing interest among people in setting up commercial orchards due to attractive local and export markets (Ramburn, 1997). Fruit production however can be seriously
Assessment on the population of *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. A Mantrakhan et al.

hampered by insect pests. Damage due to *C. peltastica* renders fruits unmarketable. Moreover, attacked fruits when packaged with undamaged ones can lead to further rotting during transport.

Little is known on *C. peltastica* in Mauritius. It is therefore important to understand the biology and ecology of the pest for its management in orchards and backyards.

The use of pheromones is widely used to monitor many pests such as fruit flies (dipterans) and pink bollworm *Pectinophora gossypiella* (Saund.) (Lepidoptera) (Cunningham, 1989; Dent, 1991). Newton et al., (1993) has identified an attractant for *C. peltastica* in South Africa. He found that blends of (E)-8-dodecenyl acetate and (Z)-8-dodecenyl acetate can result in catches of *C. peltastica* when containing 70% or more (Z)-dodecenyl acetate. In Reunion Island, Quilici et al., (1988) did not record catches of male *C. peltastica* in traps baited with commercially available pheromone of *C. leucotreta*.

The objectives of this study were to: (1) test 4 types of synthetic pheromones (2) determine the abundance of *C. peltastica* in orchards and backyards with the identified pheromone, (3) establish the time when fruits are susceptible to attack and (4) assess fruit damage in orchards and backyards.

**MATERIALS AND METHODS**

**Testing of synthetic pheromones**

*Description of pheromone and traps*

Four types of synthetic pheromones (types A, B, C and D) from Pherobank, Plant Research International (PRI), Netherlands were used. Type A consisted of 1 mg (Z)-8-dodecenyl acetate. Types B, C and D were 1 mg each of two component blends of (Z)-8-dodecenyl acetate and (E)-8-dodecenyl acetate containing 98%, 75% and 50% of (Z)-8-dodecenyl acetate respectively. Each pheromone type was impregnated in a red natural rubber dispenser.

The trap used was the transparent plastic delta trap (21 x 11 x 9 cm) from PRI, Netherlands. A single pheromone dispenser was suspended from a hook in the centre of each trap. The lure was positioned 2–3 cm above a disposable sticky insert (17 x 9 cm) placed at the bottom of the trap. One type of pheromone was placed in one trap.

*Experimental lay out*

The trapping experiment was set in a litchi orchard (0.5 ha) in Réduit Crop Research Station (CRS) from December 2003 to April 2004. The delta trap with the pheromone dispenser was placed on a litchi tree at 1.5 m above ground level. Traps were placed 9 m from each other.

There were 2 replicates for each pheromone type in the orchard. Trap catches were recorded every week and caught insects identified. Traps and dispensers were replaced on a monthly basis.

**Determination of the abundance of *Cryptophlebia peltastica***

In May 2004, 4 orchards and 6 backyards were selected to run the experiment. 2 commercial orchards (Darichy Vale and Poudre D’Or) and 3 backyards were selected in the north of Mauritius. Two orchards (1 commercial at Beau Songes and 1 experimental at Réduit CRS) and 3 backyards were selected in the central plateau. Orchards were untreated except at Darichy Vale where 2 insecticide treatments were effected at the beginning of the season. Backyards were also untreated except for one at Cottage where 2 insecticide treatments were effected, one at the beginning of the fruiting season and one close to harvest.

Delta traps with pheromone type C were set on litchi trees in orchards and backyards as per method described above. 4 traps were placed in each orchard (0.5 ha) at Darichy Vale, Poudre D’Or and Reduit and 8 at Beau Songes over an area of 1 ha. Traps were placed at intervals of 30-50 m. One trap was placed in each backyard.
Assessment on the population of *cryptophlebia peltastica* (Meyrick) (lepidoptera: tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. *A Mantrakhan et al.*

Every week, trap catches were recorded and caught insects identified. Traps and pheromone dispensers were replaced every month.

**Susceptibility of fruits to damage**

2 trees (variety Tai So) were selected randomly and earmarked from each of the 3 commercial orchards at Poudre D’Or, Vale and Beau Songes and 4 from Réduit CRS. One tree was selected from each of the 6 earmarked backyards.

At each of the four cardinal points on a tree, a cluster with 20 fruits (1.5 m above ground level) was selected and tagged. If the clusters contained less than 20, additional fruits from the nearest clusters were selected and tagged.

At Réduit CRS, fruit clusters were examined at two levels (1.5 and 2.5 m above ground level) on each earmarked tree. Therefore additional clusters of fruits at 2.5 m from ground level were selected and tagged on each tree.

A total of 74 clusters were selected and tagged. 1480 fruits were examined every week from September to December 2004. Eggs and larvae present on fruits were recorded and fruit size measured. Damaged fruits were kept in individual plastic containers and emerged adults were identified and sexed.

**Fruit damage assessment at harvest time**

Five orchards (3 in the north and 2 in the central plateau) and 5 backyards (2 in the north and 3 in the central plateau) were earmarked to assess damage on harvested fruits during November and December 2004.

At harvest time, 600 fruits from each orchard and 100 fruits from each backyard were randomly selected and examined. Fruits were categorized according to size and colour (green, 1/3 red, 2/3 red or full red). Samples of attacked fruits were collected and kept in individual plastic containers in laboratory. Emerging adults were identified and sexed.

**Statistical analysis**

Data were analysed by Analysis of Variance (ANOVA) with means separations conducted using the Tukey’s HSD test (SAS Institute 2003). Data on trap captures were log (x+1) transformed and data on percent of fruits containing eggs or larvae were arcsine transformed in order to stabilise variances.

**RESULTS**

**Efficacy of types of formulated pheromones**

The pheromone type C (a mixture of (Z)-8-dodecenyl acetate and (E)-8-dodecenyl acetate containing 75% of (Z)-8-dodecenyl acetate) was significantly more attractive to male *C. peltastica* ($F = 4.54; \text{df} = 3, 60; P = 0.01$) (Figure 1). Type A consisting of 100% (Z)-8-dodecenyl acetate and type B, two component mixture containing 98% of (Z)-8-dodecenyl acetate also caught *C. peltastica* but in lower numbers compared to type C. Type D which is a mixture of (Z)-8-dodecenyl acetate and (E)-8-dodecenyl acetate in a 1:1 ratio did not catch any *C. peltastica*. 
Assessment on the population of *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. A Manrakhan et al.

**Figure 1** Catches of male of *Cryptophlebia peltastica* in delta traps baited with 4 types of pheromones in a litchi orchard at Reduit Crop Research Station from December 2003 to April 2004

Type C attracted other species of moths in higher numbers compared to *C. peltastica*, though differences in catches were not significant ($F = 2.67$; $df = 3, 60$; $P = 0.06$). Other insects recorded in traps baited with type C were Diptera in comparatively lower numbers than Lepidoptera. Few coleopterans were captured only in traps with pheromone type B. The pheromone type C was therefore used in population studies of adult *C. peltastica*.

**Abundance of Cryptophlebia peltastica in orchards and backyards**

Male *C. peltastica* were caught in low numbers (varying on average from 0 to 2 moths/trap/week) in traps placed at all sites (Figure 2). Except at one backyard in Palma, a maximum of 13 males were recorded in one week during harvest time.

**Figure 2** Average number of *Cryptophlebia peltastica* caught per trap per week in backyards and orchards from May to December 2004
For comparison of male catches in traps at different fruit stages, 3 orchards were selected: Réduit CRS, Darichy Vale and Poudre D’Or. These sites were selected since population monitoring was carried out at different times during the litchi season and in each of these orchards there were 4 traps representing 4 replicates at any trapping date. In Darichy Vale, a 2-fold increase in catches in male moths was observed soon after fruit set and catches remained high even after harvest (F=11.79; df=4, 198; P<0.0001) (Figure 3). In contrast, at Réduit and Poudre D’Or, catches stayed more or less constant at different phenological stages (Réduit: F = 1.55; df = 4, 204; P = 0.19; Poudre D’Or: F = 0.05; df = 2, 45; P = 0.95).

**Figure 3** Average number of *Cryptophlebia peltastica* caught per trap per week at different phenological stages of *Litchi chinensis* from May to December 2004.

* Data collected till 16/12/04

**Susceptibility of fruit to damage**

1480 selected fruits were examined weekly as from very early fruit stage (1 mm in diameter) until mature and ripe stage (35 mm in diameter). In total, 306 fruits were found to contain eggs. The smallest and largest sizes of fruits on which eggs were recorded were 2 mm and 27 mm in diameter respectively (Figure 4). There were no preferred size of fruits on which eggs were present (F = 0.50; df = 22, 76, P = 0.97). A maximum of 3 eggs were recorded per fruit. The percentage of fruits containing 1, 2 and 3 eggs per fruit were 90%, 9% and 1% respectively.

There was no significant difference in percentage of fruits containing eggs at two different height levels, 1.5 m and 2.5 m above ground level (t = 0.43; df = 22; P = 0.67).

There were significant differences among sites in the percentage of fruits containing eggs. Orchards in Beau Songes and Poudre D’Or had significantly higher percentages of fruits containing eggs than other sites (F = 15.81; df = 5, 93, P<0.0001).

Only 7% of fruits containing eggs had larvae inside and were therefore damaged due to larval feeding. Damage symptoms on fruits due to larvae were seen as frass (brown faecal pellets). In the case of mature fruits, the first sign of damage are usually seen as punctures on the fruit skin with juice oozing out, usually entailing fungal growth on the surface.

Damage occurred at any time during the fruiting period, starting from 3 mm and peaked at ripening stage (F = 4.44, df = 30, 115, P<0.0001) (Figure 5). Damage levels below harvest size (20 mm) varied between 0% and 5%. Damage levels above harvest size varied between 0% and 22%. Damage by *C. peltastica* therefore not only resulted in unmarketable or bad quality mature fruits but also included damage at the immature stage. The cumulative damage by *C. peltastica* for the litchi season of 2004 in Mauritius therefore varied from 7% to 22% (Figure 6).
The first litchi harvests were carried out at Poudre D’Or and Vale at the beginning and mid November respectively. Cumulative damage was found to be lower in these two sites compared to other backyards in the north and central plateau and other orchards in Beau Songes and Réduit.

**Figure 4** Average percentage of fruits containing eggs with respect to fruit size.

**Figure 5** Average percentage damage due to Cryptophlebia peltastica with respect to fruit size.
Assessment on the population of *cryptophlebia peltastica* (Meyrick) (lepidoptera: tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. *A Manrakhan et al.*

**Figure 6** Cumulative fruit damage during 2004 litchi season from August to December in 4 orchards and backyards from 2 areas.

There was no significant difference in fruit damage at different height levels (*t* = 0.60; df = 22; *P* = 0.55). This showed that fruits were liable to same level of damage irrespective of height level on the tree.

**Level of damage at harvest**

3579 fruits were examined at harvest time in all sites. Sizes of fruits at harvest varied from 20 mm to 32 mm. 61% of the fruits were 2/3 red in colour, 31% were full red, 7% were 1/3 red and 1% was green.

Damage at harvest varied from 0% to 21% depending on locality. There was no significant difference in percentage fruit damage between the two areas (north and central plateau) in either orchards or backyards (*t* = 0.27; df = 8; *P* = 0.79). According to data combined for the two areas, damage levels in orchards were found to be higher compared to backyards, though the difference was not significant (*t* = 1.18; df = 8; *P* = 0.27).
Assessment on the population of cryptophlebia peltastica (Meyrick) (lepidoptera: tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. A Manrakhan et al.

At harvest, there were no significant differences in % damage between different fruit sizes (F = 0.40, df = 3, 24, P = 0.75) and the two main colours (2/3 red and full red) (t= 1.09; df = 16; P = 0.29).

**Sex ratio of C. peltastica reared from damaged fruits**

458 damaged fruits were observed individually in laboratory. A total of 146 C. peltastica adults emerged from 139 fruits. The average number of adults per damaged fruit was 1.07 (± 0.03). A maximum of 2 adults were recorded from one fruit. A sex ratio of 1:1 (male:female) was observed among emerged adults.

**Parasitism**

A hymenopteran parasitoid was recovered from 7 out of the 458 damaged fruits kept in the laboratory. The identity of the parasitoid is yet to be determined. The parasitism rate was higher on smaller fruits than bigger ones. Parasitoids were recovered from 6.7% of damaged fruits that were below the harvest size (20 mm in diameter). The rate of parasitism in fruits that have reached the harvest size was 0.5%.

**Other insect pest**

Other insect pest recorded from damaged fruits was Ceratitis rosa Karsch. Adults of C. rosa, were recorded from 2 out of 458 damaged fruits observed in laboratory. The first fruit fly adult was recorded along with a C. peltastica male while the second one was recorded with the undetermined hymenopteran parasitoid.

**DISCUSSION**

The trial on evaluation of pheromones showed that the pheromone type C was attractive to males of C. peltastica and substantiates findings of Newton et al. (1993) that C. peltastica are attracted to blends of (Z)-8-dodecenyl acetate and (E)-8-dodecenyl acetate, when containing 70% or more. This pheromone (type C) showed population trends of male moths at study sites. Male catches increased during the fruiting period and remained high at and after harvest.

Though pheromone type C was the best candidate attractant for C. peltastica, male catches ranged from 0 to only 2 per trap per week during most of the monitoring period. Moreover, other lepidopterans were caught in comparatively higher numbers. The low catches indicate that either male population was low during that period or the pheromone had a low efficiency and/or specificity.

Another important finding was the presence of eggs on fruits whose sizes range from 2 mm to 27 mm in diameter. This shows that the female tends to oviposit indiscriminately on fruits, irrespective of size and stage. This finding is however in contrast to Newton & Crause (1990) who found that oviposition rate of females was low during the early litchi season and increased rapidly to a maximum after the ripening phase commenced. Litchi fruits in our study were harvested at early ripening stage and this can possibly explain why our results were not concurrent with that of Newton & Crause (1990).

Fruit damage below harvest size varied from 0% to 5%. Young damaged fruits were often found to dry up and drop on the ground. Quilici et al., (1988) found that C. peltastica contributed to about 20% of fruit drop from untreated litchi trees. Such fallen fruits are breeding sites of C. peltastica. A peak in damage levels was observed at the ripening stage. Damage levels for mature ripe fruits at harvest were found to vary from 0% to 21%, depending on locality. However, damage may continue to occur further if fruits with eggs/larvae are packaged with undamaged ones.

Laboratory investigations indicated that life cycle of C. peltastica was approximately 2 months. Egg hatch occurred within 4-8 days. The larval stage was about 2 - 4 weeks depending on fruit size, the smaller the fruit the longer the larval stage. Pupal stage lasted for about 1-2 weeks and adults (fed on 10% honey solution) lived for about 1 week. Given that the litchi season from fruit set to harvest takes about 2-3 months, it is likely that damage of young fruits was caused by migrating females from nearby host plants such as Bauhinia spp and Tamarindus indica L. (Mamet and Williams, 1993).
Assessment on the population of cryptophlebia peltastica (Meyrick) (lepidoptera: tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. A Mantrakhan et al.

latter were fruiting just before start of litchi fruiting season. The fruit damage at ripening stage could be due to the first generation of adults produced within the litchi orchard and backyard.

Interestingly in this study, parasitism in damaged fruit samples (fruit samples containing larvae) was 6.7% early in the season (when fruits were below harvest size). Parasitism decreased as fruiting progressed.

CONCLUSION

There is a potential in using pheromones (such as type C) to detect and monitor C. peltastica in commercial orchards and backyards. It is also essential to carry out fruit sampling soon after fruit set for early detection of eggs or larvae. The mere presence of eggs on fruits indicates damage potential and therefore control actions should be initiated soon after detection. Protection of mature fruits should be carried out to reduce fruit damage at harvest as well as during transport. The possible use of the unidentified hymenopteran parasitoid can be another option to manage C. peltastica in litchi orchards, backyards and on other nearby hosts.

FUTURE RESEARCH

It is proposed to carry out testing of various other blends of (Z)-8-dodecenyl acetate and (E)-8-dodecenyl acetate in the search for a more specific and efficient pheromone for C. peltastica. Possibilities to test other substitutes of rubber dispensers could be considered. Pheromones impregnated into rubber dispenser were found by La Croix et al., (1985) to be less effective in attracting C. leucotreta than those impregnated into polyethylene vials.

It is suspected here that very young fruits when damaged dry up and drop on the ground as recorded by Quilici et al., (1988). The contribution of C. peltastica to fruit drop should also be quantified for a more precise determination of total damage level.

Finally, rearing of damaged fruits in laboratory provided some background data on the life cycle of C. peltastica. There is a need to collect more data on the life table of C. peltastica in order to better understand population fluctuations and to provide background information for better management of C. peltastica.

ACKNOWLEDGEMENTS

We are grateful to owners of orchards and backyards for their collaboration in conducting this study. We would like to extend our gratitude to Mr Willem Stol and Dr Frans Griepink, Pherobank, Plant Research International, Netherlands who provided pheromones for testing. We are grateful to Mr R Ramnauth for statistical advice. Finally we would like to thank the staff of the Entomology Division, AREU for their assistance during the study.

Assessment on the population of cryptophlebia peltastica (Meyrick) (lepidoptera: tortricidae) and fruit damage in litchi orchards and backyards in Mauritius. A Mantrakhan et al.

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ASSESSMENT OF ATTRACTANTS FOR FRUIT FLY (DIPTERA: TEPHRITIDAE) MANAGEMENT

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Ministry of Agro-Industry and Fisheries

ABSTRACT

Fruit flies are severe constraint to fruit production in Mauritius. The Peach fruit fly, Bactrocera zonata (Saunders), the Natal fly, Ceratitis rosa (Karsch), the Medfly, C. capitata (Wiedmann) are the main pests of fleshy fruits. Studies were conducted to find the most effective combination of attractant and lures for females. A first set of trials was conducted during the period November 2002 to March 2003 and then repeated during the period October 2003 to March 2004 in mixed orchards. Sex and food based attractants were tested in plastic McPhail traps following a standard coordinated experimental protocol. The attractants tested included Nulure, ½ Ammonium Acetate (AA) patch, 2 AA, Di-Ammonium phosphate, Ammonium sulphate, AA + Putrescine (PT) + Trimethylamine (TMA) and Torula. In the first set of trials (November 2002 to March 2003) and in one trial during the period October 2003 to March 2004, the treatment AA + PT + TMA captured the highest number of B. zonata females, males or females and males together as compared to the other treatments. The treatment Torula captured significantly more female B. zonata in trials carried out during the period November 2002 to March 2003.

Keywords: Fruit fly, Attractant, traps

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are recognized as major pests of horticultural crops worldwide. Eight pest species of fruit flies are known in Mauritius: the peach fruit fly Bactrocera zonata (Saunders), the natal fly Ceratitis rosa (Karsch), the medfly C. capitata (Wiedmann), the melon fly, B. cucurbitae (Coquillett), Carpomya vesuviana Costa, Neoceratitis cyanescens (Bezzi), Dacus demmerezi (Bezzi) and D. ciliatus (Loew).

The importance of fruit flies has been evoked in 1960 (Orian and Moutia, 1960). Control efforts in the past have been focused on cover sprays of insecticides, biological control and the Sterile Insect Technique SIT (Hammes, 1980).

More recently, control has been directed towards area-wide management which is more effective in that an entire area is treated, instead of individual efforts where reinvasions from neighbours’ fields continuously occur. An area-wide control was thus proposed, following a study by Landell Mills (Landell Mills, 1991). A programme based on this concept is operational since 1994, initially with financial and technical assistance from the European Union, and subsequently through sole Mauritian Government funding. Control actions are currently being carried out in major fruit growing areas.

The possibility of eradicating the major fruit fly species, B. zonata has been contemplated and a feasibility study is currently ongoing with technical and financial assistance from the International Atomic Energy Agency, on integrated management of fruit flies using the Sterile Insect Technique (SIT).

Management of fruit flies by SIT or other techniques require population estimation methods which accurately reflect changes in population levels. Monitoring for adult flies is conducted through use of traps, usually combined with sexual lures or food odours. While sexual lures have been quite effective, depending on the fruit fly species, female attractants have been less effective. The need was therefore felt for developing trapping systems geared towards female flies (Heath et al., 1995) particularly so as to improve efficacy of SIT and monitoring its effectiveness. An international network research project for the development of a female attractant system for medfly trapping was subsequently operated under IAEA/FAO, as a five year Coordinated Research Programme (CRP), as
from 1995 (IAEA, 1999). The CRP enabled the testing of ammonium acetate, putrescine and trimethylamine in different combinations and in different types of traps. Significant achievements were made in the development of a female detection system for the Mediterranean fly. The three-component synthetic female food attractant was accepted as a means of assessing the effectiveness of SIT programme efforts (IAEA, 1999).

Another 5-year CRP complementary to the above focusing on the development of improved attractants and their integration into SIT fruit fly management programmes, was initiated in 2000 and is due for completion this year. Trials are geared towards the genera Anastrepha, Ceratitis and Bactrocera which attack over 300 species of fruits and vegetables in tropical, subtropical and temperate climates on five continents. The objectives of the CRP are mainly to develop and compare female-biased food attractants in different environments, to provide a standardized detection system among fruit fly pest species and regions, and to develop female targeted bait/kill stations. Experimentation is being conducted in three phases.

This paper presents results of using different food-based attractants for trapping of three fruit fly species, B. zonata, C. rosa and C. capitata which infest tree fruits. Trials were conducted according to a common protocol devised by the IAEA, and agreed by the different participating countries which are from Latin America, Europe, Africa and Indian Ocean.

MATERIALS AND METHODS

The experiment was conducted at two sites: Pointe aux Sables (5-20 m ASL) and Beau Bassin (290-355 m ASL) during the period November 2002 to March 2004. Each trial was repeated twice at each site. The targeted fruit flies were B. zonata, C. rosa and C. capitata and the main hosts during the period of study were Indian Almond, Loquat, and Peach. Trials were set in backyard gardens as adequate orchards are not available for experimentation.

The traps used were of the McPhail type used as a wet trap. The attractants tested were: Nulure (hydrolysed protein); Ammonium acetate, putrescine, and trimethyl amine, each contained in a patch; di-amminium phosphate; ammonium sulphate; and torula yeast.

The trial consisted of seven treatments replicated five times in a randomized block design. The treatments were as follows:

(i) Plastic Multilure McPhail type trap (PMT) baited with 300 ml of a solution containing 9% Nulure, 3% borax, 88% water (by weight)
(ii) PMT as a wet trap baited with lure ammonium acetate (AA) ½ patch, 300 ml of water and Triton (1-2 drops)
(iii) PMT as a wet trap baited with lure ammonium acetate (AA) 2 patch, 300 ml of water and Triton (1-2 drops)
(iv) PMT as a wet trap baited with Di-ammonium phosphate (50gms/L)
(v) PMT as a wet trap baited with ammonium sulphate (30gms/L)
(vi) PMT as a wet trap baited with lure ammonium acetate (AA) patch, putrescine (PT) patch, trimethylamine (TMA) patch, 300 ml of water and Triton (1-2 drops)
(vii) PMT as a wet trap baited with 3 tablets torula yeast with 300 ml of water

Traps were hung on fruit trees, 1 – 2 metres above the ground, in the lower half of the south-eastern part of the tre canopy. At each site, traps were set in five lines of seven traps. The distance between two traps varied between 25 to 50 m in any one line. Traps were serviced twice a week and all tephritids and beneficial insects captured were collected in 70% alcohol. Fruit flies were identified, sexed and recorded. After data collection, traps within a line were rotated sequentially. During weekly renewal, the old liquid baits of PMT traps were collected in a plastic bucket to avoid interference with traps. Similarly, synthetic lures that were changed after four weeks were collected in a plastic bag. The traps were rinsed with water before the addition of fresh bait. A sample of the females collected by each bait was dissected to determine their fertility status. The trials were run for eight weeks as required by the agreed protocol.

Trap catches were log transformed prior to analysis of variance and means were separated with least significant test.
RESULTS

In three trials (Tables 1a, 1b and 1d), the treatment AA+PT+TMA captured significantly more B. zonata male, female or male + female flies as compared to the other treatments. In the trial at Pointe aux Sables (Table 2c) the treatment 2AA trapped the highest number of male, female or male + female B. zonata flies. In all four trials, the treatments captured more female than male B. zonata flies and the treatment AA+PT+TMA captured the highest number of female B. zonata. The percentage of female captured by the treatment AA+PT+TMA was above 60%.

Table 1a Trap catches of Bactrocera zonata at Pointe aux Sables Nov/Dec 2002

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day *</th>
<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>136</td>
<td>54</td>
<td>190</td>
<td>0.49 c</td>
<td>0.19 c</td>
<td>0.68 c</td>
<td>13</td>
<td>10</td>
<td>72</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>50</td>
<td>43</td>
<td>93</td>
<td>0.18 f</td>
<td>0.15 d</td>
<td>0.33 e</td>
<td>5</td>
<td>8</td>
<td>54</td>
</tr>
<tr>
<td>2 AA</td>
<td>80</td>
<td>42</td>
<td>122</td>
<td>0.29 e</td>
<td>0.15 de</td>
<td>0.44 d</td>
<td>8</td>
<td>8</td>
<td>66</td>
</tr>
<tr>
<td>Di-Ammonium</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>0.01 g</td>
<td>0.01 f</td>
<td>0.02 f</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Ammonium</td>
<td>94</td>
<td>38</td>
<td>132</td>
<td>0.34 d</td>
<td>0.14 e</td>
<td>0.47 d</td>
<td>9</td>
<td>7</td>
<td>71</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>461</td>
<td>237</td>
<td>698</td>
<td>1.65 a</td>
<td>0.85 a</td>
<td>2.49 a</td>
<td>43</td>
<td>45</td>
<td>66</td>
</tr>
<tr>
<td>Torula</td>
<td>239</td>
<td>110</td>
<td>349</td>
<td>0.85 b</td>
<td>0.39 b</td>
<td>1.25 b</td>
<td>22</td>
<td>21</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 1b Trap catches of Bactrocera zonata at Beau Bassin Jan/Mar 2003

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day *</th>
<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>402</td>
<td>114</td>
<td>516</td>
<td>1.44 e</td>
<td>0.41 f</td>
<td>1.84 d</td>
<td>9</td>
<td>9</td>
<td>78</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>686</td>
<td>187</td>
<td>873</td>
<td>2.45 c</td>
<td>0.67 d</td>
<td>3.12 b</td>
<td>16</td>
<td>15</td>
<td>79</td>
</tr>
<tr>
<td>2 AA</td>
<td>782</td>
<td>171</td>
<td>953</td>
<td>2.79 b</td>
<td>0.61 e</td>
<td>3.40 b</td>
<td>18</td>
<td>14</td>
<td>82</td>
</tr>
<tr>
<td>Di- Ammonium</td>
<td>748</td>
<td>221</td>
<td>969</td>
<td>2.67 b</td>
<td>0.79 b</td>
<td>3.46 a</td>
<td>18</td>
<td>18</td>
<td>77</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>184</td>
<td>56</td>
<td>240</td>
<td>0.66 f</td>
<td>0.20 g</td>
<td>0.86 e</td>
<td>4</td>
<td>4</td>
<td>77</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>787</td>
<td>304</td>
<td>1091</td>
<td>2.81 a</td>
<td>1.09 a</td>
<td>3.90 a</td>
<td>18</td>
<td>24</td>
<td>72</td>
</tr>
<tr>
<td>Torula</td>
<td>669</td>
<td>201</td>
<td>870</td>
<td>2.39 d</td>
<td>0.72 c</td>
<td>3.11 c</td>
<td>16</td>
<td>16</td>
<td>77</td>
</tr>
</tbody>
</table>

Values in each column not followed by the same letters are not significantly different according to Duncan’s Multiple range test (P=0.05)
Table 1c Trap catches of *Bactrocera zonata* at Beau Bassin Jan/Mar 2004

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day</th>
<th>Av. ♂/Trap/Day</th>
<th>Total/Trap/Day</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>152</td>
<td>65</td>
<td>217</td>
<td>0.54 e</td>
<td>0.23 c</td>
<td>0.78 e</td>
<td>9</td>
<td>10</td>
<td>70</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>190</td>
<td>59</td>
<td>249</td>
<td>0.68 d</td>
<td>0.21 d</td>
<td>0.89 d</td>
<td>11</td>
<td>9</td>
<td>76</td>
</tr>
<tr>
<td>2 AA</td>
<td>320</td>
<td>127</td>
<td>447</td>
<td>1.14 c</td>
<td>0.45 b</td>
<td>1.60 c</td>
<td>19</td>
<td>20</td>
<td>72</td>
</tr>
<tr>
<td>Di-Ammonium phosphate</td>
<td>129</td>
<td>57</td>
<td>186</td>
<td>0.46 f</td>
<td>0.20 e</td>
<td>0.66 f</td>
<td>8</td>
<td>9</td>
<td>69</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>132</td>
<td>42</td>
<td>174</td>
<td>0.47 f</td>
<td>0.15 f</td>
<td>0.62 g</td>
<td>8</td>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>410</td>
<td>152</td>
<td>562</td>
<td>1.46 a</td>
<td>0.54 a</td>
<td>2.01 a</td>
<td>25</td>
<td>24</td>
<td>73</td>
</tr>
<tr>
<td>Torula</td>
<td>337</td>
<td>126</td>
<td>463</td>
<td>1.20 b</td>
<td>0.45 b</td>
<td>1.65 b</td>
<td>20</td>
<td>20</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>1670</td>
<td>628</td>
<td>2298</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)

Table 1d Trap catches of *Bactrocera zonata* at Pointe aux Sables Oct/Dec 2003

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day</th>
<th>Av. ♂/Trap/Day</th>
<th>Total/Trap/Day</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>89</td>
<td>28</td>
<td>117</td>
<td>0.32 d</td>
<td>0.10 d</td>
<td>0.42 d</td>
<td>9</td>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>186</td>
<td>72</td>
<td>258</td>
<td>0.66 b</td>
<td>0.26 c</td>
<td>0.92 b</td>
<td>18</td>
<td>19</td>
<td>72</td>
</tr>
<tr>
<td>2 AA</td>
<td>246</td>
<td>88</td>
<td>334</td>
<td>0.88 a</td>
<td>0.31 a</td>
<td>1.19 a</td>
<td>24</td>
<td>23</td>
<td>74</td>
</tr>
<tr>
<td>Di-Ammonium phosphate</td>
<td>66</td>
<td>27</td>
<td>93</td>
<td>0.24 e</td>
<td>0.10 d</td>
<td>0.33 e</td>
<td>6</td>
<td>7</td>
<td>71</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>88</td>
<td>25</td>
<td>113</td>
<td>0.31 d</td>
<td>0.09 e</td>
<td>0.40 d</td>
<td>9</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>161</td>
<td>62</td>
<td>223</td>
<td>0.58 c</td>
<td>0.22 c</td>
<td>0.80 c</td>
<td>16</td>
<td>16</td>
<td>72</td>
</tr>
<tr>
<td>Torula</td>
<td>181</td>
<td>74</td>
<td>255</td>
<td>0.65 b</td>
<td>0.26 b</td>
<td>0.91 b</td>
<td>18</td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>1017</td>
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<td></td>
<td></td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)
As regards trap catches for *C. rosa*, the treatment AA+PT+TMA captured the highest number of females in the trial at Pointe aux Sables period Nov/Dec 2002 (Table 2a), Beau Bassin period Jan/Mar 2003 (Table 2b), Pointe aux Sables period Oct/Dec 2003 (Table 2c) and Beau Bassin Period Jan/Mar 2004 (Table 2d).

### Table 2a Trap catches of *Ceratitis rosa* at Pointe aux Sables Nov/Dec 2002

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day *</th>
<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>24</td>
<td>20</td>
<td>44</td>
<td>0.09 c</td>
<td>0.07 c</td>
<td>0.16 c</td>
<td>15</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>14</td>
<td>8</td>
<td>22</td>
<td>0.05 f</td>
<td>0.03 d</td>
<td>0.08 e</td>
<td>9</td>
<td>6</td>
<td>64</td>
</tr>
<tr>
<td>2 AA</td>
<td>8</td>
<td>12</td>
<td>20</td>
<td>0.03 e</td>
<td>0.04 de</td>
<td>0.07 d</td>
<td>5</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>Di-Ammonium</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0.01 g</td>
<td>0.00 f</td>
<td>0.01f</td>
<td>1</td>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>Ammonium</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>0.02 d</td>
<td>0.03 e</td>
<td>0.05 d</td>
<td>5</td>
<td>6</td>
<td>47</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>63</td>
<td>55</td>
<td>118</td>
<td>0.22 a</td>
<td>0.20 a</td>
<td>0.42 a</td>
<td>41</td>
<td>42</td>
<td>53</td>
</tr>
<tr>
<td>Torula</td>
<td>37</td>
<td>27</td>
<td>64</td>
<td>0.13 b</td>
<td>0.10 b</td>
<td>0.23 b</td>
<td>24</td>
<td>21</td>
<td>58</td>
</tr>
</tbody>
</table>

*Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)*

### Table 2b Trap catches of *Ceratitis rosa* at Beau Bassin Jan/Mar 2003

<table>
<thead>
<tr>
<th>Treatments</th>
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<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day *</th>
<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>370</td>
<td>181</td>
<td>551</td>
<td>1.32 e</td>
<td>0.65 e</td>
<td>1.97 e</td>
<td>9</td>
<td>10</td>
<td>67</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>433</td>
<td>205</td>
<td>638</td>
<td>1.55 d</td>
<td>0.73 d</td>
<td>2.28 d</td>
<td>11</td>
<td>11</td>
<td>68</td>
</tr>
<tr>
<td>2 AA</td>
<td>433</td>
<td>235</td>
<td>668</td>
<td>1.55 d</td>
<td>0.84 c</td>
<td>2.39 d</td>
<td>11</td>
<td>13</td>
<td>65</td>
</tr>
<tr>
<td>Di-Ammonium</td>
<td>1187</td>
<td>438</td>
<td>1625</td>
<td>4.24 a</td>
<td>1.56 a</td>
<td>5.80 a</td>
<td>29</td>
<td>24</td>
<td>73</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>141</td>
<td>49</td>
<td>190</td>
<td>0.50 f</td>
<td>0.18 f</td>
<td>0.68 f</td>
<td>3</td>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>863</td>
<td>428</td>
<td>1291</td>
<td>3.08 b</td>
<td>1.53 a</td>
<td>4.61 b</td>
<td>21</td>
<td>24</td>
<td>67</td>
</tr>
<tr>
<td>Torula</td>
<td>656</td>
<td>259</td>
<td>915</td>
<td>2.34 c</td>
<td>0.92 b</td>
<td>3.27 c</td>
<td>16</td>
<td>14</td>
<td>72</td>
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<tr>
<td></td>
<td>4083</td>
<td>1795</td>
<td>5878</td>
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<td>100</td>
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</table>

*Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)*
### Table 2c Trap catches of *Ceratitis rosa* at Pointe aux Sables Oct/Dec 2003

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day*</th>
<th>Av. ♂/Trap/Day*</th>
<th>Total/Trap/Day*</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>47</td>
<td>25</td>
<td>72</td>
<td>0.17 c</td>
<td>0.09 d</td>
<td>0.26 c</td>
<td>17</td>
<td>14</td>
<td>65</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>23</td>
<td>13</td>
<td>36</td>
<td>0.08 e</td>
<td>0.05 f</td>
<td>0.13 e</td>
<td>8</td>
<td>7</td>
<td>64</td>
</tr>
<tr>
<td>2 AA</td>
<td>26</td>
<td>30</td>
<td>56</td>
<td>0.09 d</td>
<td>0.11 c</td>
<td>0.20 d</td>
<td>9</td>
<td>17</td>
<td>46</td>
</tr>
<tr>
<td>Di-Ammonium phosphate</td>
<td>20</td>
<td>14</td>
<td>34</td>
<td>0.07 f</td>
<td>0.05 e</td>
<td>0.12 f</td>
<td>7</td>
<td>8</td>
<td>59</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>18</td>
<td>4</td>
<td>22</td>
<td>0.06 g</td>
<td>0.01 g</td>
<td>0.08 g</td>
<td>7</td>
<td>2</td>
<td>82</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>65</td>
<td>54</td>
<td>119</td>
<td>0.23 b</td>
<td>0.19 a</td>
<td>0.42 a</td>
<td>24</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>Torula</td>
<td>75</td>
<td>40</td>
<td>115</td>
<td>0.27 a</td>
<td>0.14 b</td>
<td>0.41 b</td>
<td>27</td>
<td>22</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>274</td>
<td>180</td>
<td>454</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
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</tr>
</tbody>
</table>

* Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)

### Table 2d Trap catches of *Ceratis rosa* at Beau Bassin Jan/Mar 2004

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day*</th>
<th>Av. ♂/Trap/Day*</th>
<th>Total/Trap/Day*</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>259</td>
<td>80</td>
<td>339</td>
<td>0.93</td>
<td>0.29</td>
<td>1.21</td>
<td>20</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>134</td>
<td>53</td>
<td>187</td>
<td>0.48</td>
<td>0.19</td>
<td>0.67</td>
<td>11</td>
<td>10</td>
<td>72</td>
</tr>
<tr>
<td>2 AA</td>
<td>191</td>
<td>94</td>
<td>285</td>
<td>0.68</td>
<td>0.34</td>
<td>1.02</td>
<td>15</td>
<td>17</td>
<td>67</td>
</tr>
<tr>
<td>Di-Ammonium phosphate</td>
<td>78</td>
<td>31</td>
<td>109</td>
<td>0.28</td>
<td>0.11</td>
<td>0.39</td>
<td>6</td>
<td>6</td>
<td>72</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>93</td>
<td>30</td>
<td>123</td>
<td>0.33</td>
<td>0.11</td>
<td>0.44</td>
<td>7</td>
<td>5</td>
<td>76</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>316</td>
<td>168</td>
<td>484</td>
<td>1.13</td>
<td>0.60</td>
<td>1.73</td>
<td>25</td>
<td>31</td>
<td>65</td>
</tr>
<tr>
<td>Torula</td>
<td>205</td>
<td>92</td>
<td>297</td>
<td>0.73</td>
<td>0.33</td>
<td>1.06</td>
<td>16</td>
<td>17</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>1276</td>
<td>548</td>
<td>1824</td>
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<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)
The population of *C. capitata* was the smallest in all trials as compared to the populations of *B. zonata* or *C. rosa*. The treatment AA+PT+TMA captured significantly more females at Pointe aux Sables period Nov/Dec 2002 (Table 3a) and at Beau Bassin period Jan/Mar 2004 (Table 3d). There was no significant difference between female trap catches at Beau Bassin period Jan/Mar 2003 (Table 3b) and at Pointe aux Sables period Oct/Dec 2003 (Table 3c).

**Table 3a** Trap catches of *Ceratitis capitata* at Pointe aux Sables Nov/Dec 2002

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day *</th>
<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>0.014 d</td>
<td>0.011 e</td>
<td>0.025 f</td>
<td>5</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>4</td>
<td>12</td>
<td>16</td>
<td>0.014 d</td>
<td>0.043 b</td>
<td>0.057 c</td>
<td>5</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>2 AA</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>0.018 c</td>
<td>0.014 d</td>
<td>0.032 e</td>
<td>6</td>
<td>7</td>
<td>56</td>
</tr>
<tr>
<td>Di-</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0.011 e</td>
<td>0.000 f</td>
<td>0.011 g</td>
<td>3</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium</td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>0.018 c</td>
<td>0.021 c</td>
<td>0.039 d</td>
<td>6</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>39</td>
<td>28</td>
<td>67</td>
<td>0.139 a</td>
<td>0.100 a</td>
<td>0.239 a</td>
<td>45</td>
<td>47</td>
<td>58</td>
</tr>
<tr>
<td>Torula</td>
<td>26</td>
<td>6</td>
<td>32</td>
<td>0.093 b</td>
<td>0.021 c</td>
<td>0.114 b</td>
<td>30</td>
<td>10</td>
<td>81</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>86</td>
<td>59</td>
<td>100</td>
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<td></td>
<td></td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3b** Trap catches of *Ceratitis capitata* at Beau Bassin Jan/Mar 2004

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
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<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>13</td>
<td>5</td>
<td>18</td>
<td>0.05 d</td>
<td>0.02</td>
<td>0.06 d</td>
<td>14</td>
<td>14</td>
<td>72</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>16</td>
<td>4</td>
<td>20</td>
<td>0.06 b</td>
<td>0.01</td>
<td>0.07 c</td>
<td>17</td>
<td>11</td>
<td>80</td>
</tr>
<tr>
<td>2 AA</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>0.02 e</td>
<td>0.00</td>
<td>0.02 e</td>
<td>8</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Di-</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.01 g</td>
<td>0.00</td>
<td>0.01 f</td>
<td>2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>0.02 f</td>
<td>0.01</td>
<td>0.02 e</td>
<td>5</td>
<td>6</td>
<td>71</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>34</td>
<td>17</td>
<td>51</td>
<td>0.12 a</td>
<td>0.06</td>
<td>0.18 a</td>
<td>37</td>
<td>49</td>
<td>67</td>
</tr>
<tr>
<td>Torula</td>
<td>15</td>
<td>7</td>
<td>22</td>
<td>0.05 c</td>
<td>0.03</td>
<td>0.08 b</td>
<td>16</td>
<td>20</td>
<td>68</td>
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<td><strong>Total</strong></td>
<td>92</td>
<td>35</td>
<td>127</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

* Values in each column not followed by the same letters are not significantly different according to Duncan’s Multiple range test (P=0.05)
Table 3c Trap catches of *Ceratitis capitata* at Beau Bassin Jan/Mar 2003

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day *</th>
<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
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<td>Nulure +</td>
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<td>16</td>
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<td>0.011</td>
<td>0.057</td>
<td>6</td>
<td>3</td>
<td>81</td>
</tr>
<tr>
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<td>10</td>
<td>6</td>
<td>16</td>
<td>0.036</td>
<td>0.021</td>
<td>0.057</td>
<td>4</td>
<td>6</td>
<td>63</td>
</tr>
<tr>
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<td>10</td>
<td>1</td>
<td>11</td>
<td>0.036</td>
<td>0.004</td>
<td>0.039</td>
<td>4</td>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td>Di-Ammonium</td>
<td>27</td>
<td>11</td>
<td>38</td>
<td>0.096</td>
<td>0.039</td>
<td>0.136</td>
<td>12</td>
<td>11</td>
<td>71</td>
</tr>
<tr>
<td>Ammonium</td>
<td>12</td>
<td>4</td>
<td>16</td>
<td>0.043</td>
<td>0.014</td>
<td>0.057</td>
<td>5</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
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<td>37</td>
<td>108</td>
<td>0.254</td>
<td>0.132</td>
<td>0.386</td>
<td>30</td>
<td>36</td>
<td>66</td>
</tr>
<tr>
<td>Torula</td>
<td>90</td>
<td>40</td>
<td>130</td>
<td>0.321</td>
<td>0.143</td>
<td>0.464</td>
<td>39</td>
<td>39</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>233</td>
<td>102</td>
<td>335</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
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</tr>
</tbody>
</table>

* Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)

Table 3d Trap catches of *Ceratitis capitata* at Pointe aux Sables Oct/Dec 2003

<table>
<thead>
<tr>
<th>Treatments</th>
<th>♀</th>
<th>♂</th>
<th>Total (♀ + ♂)</th>
<th>Av. ♀/Trap/Day *</th>
<th>Av. ♂/Trap/Day *</th>
<th>Total/Trap/Day *</th>
<th>% ♀</th>
<th>% ♂</th>
<th>% ♀/Trap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulure</td>
<td>18</td>
<td>14</td>
<td>32</td>
<td>0.06</td>
<td>0.05</td>
<td>0.11</td>
<td>16</td>
<td>22</td>
<td>56</td>
</tr>
<tr>
<td>1/2 AA</td>
<td>13</td>
<td>6</td>
<td>19</td>
<td>0.05</td>
<td>0.02</td>
<td>0.07</td>
<td>12</td>
<td>9</td>
<td>68</td>
</tr>
<tr>
<td>2 AA</td>
<td>21</td>
<td>8</td>
<td>29</td>
<td>0.08</td>
<td>0.03</td>
<td>0.10</td>
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<td>Di-Ammonium phosphate</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>4</td>
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<td>67</td>
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<tr>
<td>Ammonium sulphate</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>4</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>AA + PT + TMA</td>
<td>25</td>
<td>12</td>
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<td>0.04</td>
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<td>68</td>
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<td>100</td>
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</tr>
</tbody>
</table>

* Values in each column not followed by the same letters are not significantly different according to Duncan's Multiple range test (P=0.05)
DISCUSSION AND CONCLUSION

Insect trapping is essential for population studies or for use in insect pest control programmes. Estimation of population size, detection of newly introduced species and evaluation of population reproductive ability are necessary components for any control system (Economopoulos and Haniotakis, 1994). This study focused on seeking a trap and lure combination that will be appropriate as both a sensitive trapping system for monitoring low numbers of fruit flies and as a control option (bait station). Traps baited with the 3 component food-based synthetic attractant (ammonium acetate, putrescine and trimethylamine lures) showed remarkable performance in tests conducted in Guatemala (Heath et al., 1997). In our tests, AA+PT+TMA was the most effective female attractant for B. zonata, C. rosa or C. capitata. The 3 component food-based synthetic attractant performed better than the single AA attractant (Tables 2a, 2b, 2d, 3a, 3b, 3c, 4a and 4d).

The food-based attractants/lures tested in this study were more female specific. In tests conducted in Israel, IPMT traps baited with the 3 component synthetic lure captured about 2 times more female C. capitata than male in tests in citrus orchards (Gazit et al., 1998).

Area-wide integrated application of the SIT for fruit fly pests is being implemented in several countries and such a programme is also envisaged in Mauritius. The current programme under IAEA will determine the feasibility of using SIT against B. zonata as part of an integrated approach. The usual method of monitoring wild populations has been dependant on male trapping. The female selectivity of the synthetic attractant observed will be of considerable value in SIT programmes by removing feral females without eliminating sterile males. Such a trapping system, if used on a large scale during the envisaged SIT programme, will no doubt enhance its success.

ACKNOWLEDGEMENT

We are grateful to the FAO/IAEA and the Ministry of Agriculture, Food Technology & Natural Resources, without whose support this research/participation would not have been possible.

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FIELD EVALUATION OF SYNTHETIC SEX ATTRACTANTS OF
THE TOMATO FRUIT WORM, HELICOVERPA ARMIGERA
HUBNER (LEPIDOPTERA: NOCTUIDAE) IN MAURITIUS

L. Unmole and D. Abeeluck

Agricultural Research and Extension Unit

ABSTRACT

The tomato fruitworm, Helicoverpa armigera, is a major pest in tomato and up to 30% of fruit damage is reported. Growers rely heavily on insecticides to control H. armigera. Since 2001, research has been conducted to develop environmentally safe and sound methods for the management of H. armigera. This paper reports the first attempt at field evaluation of the efficacy of synthetic pheromones in luring male moths and its potential in monitoring H. armigera population in tomato cultivation. The efficacy of the pheromone septa from Plant Research International (PRI), Netherlands and Pest Control India (PCI) to lure male moths was evaluated in tomato fields. Both septa were effective in luring males of H.armigera. Five delta traps with PRI septa were set in 0.4 ha of tomato field at Khoyratty and 32 in a block of 3.4 ha at Plaine des Papayes and trap catches monitored every 7 days. Eggs and larvae were also recorded from a determined plant sample (1st 4 compound leaves from 3 branch tips & 3 flower clusters) on randomly selected tomato plants. There was a positive correlation between trap catches and egg numbers in plant samples (r= 0.7) at both sites. Relative Humidity showed a positive correlation with trap catches as well. Catches were low at 58% relative humidity but high above 70%. Three types of traps (delta (PRI), funnel (PCI) and a local plastic bottle trap) were evaluated for use in Helicoverpa management. The delta & plastic bottle trap caught comparatively higher numbers of male moths. The plastic bottle is a cheap alternative to delta trap.

Keywords: Helicoverpa armigera, tomato, pheromone, septa, trap

INTRODUCTION

The tomato fruit worm, Helicoverpa armigera, is recorded from at least 60 cultivated and 67 wild plants worldwide (Reed and Pawar, 1982). In Mauritius, H. armigera occurs on tomato, maize, tobacco, pea, sunflower, cotton, potato, carnation and roses (Mamet & Williams 1993; Anon, 1995; Ganeshan et al., 1997). Growers undertake up to twenty insecticide treatments per crop cycle to prevent fruit damage in tomato fields (Anon, 1997). Such treatments are not always effective because they are very often not well timed and young larvae are able to penetrate into fruits prior to treatment. Moreover, such practice could lead to the development of resistance to insecticides (Riley, 1989; Sawicki, 1989).

Up to 30% fruit damage has been reported in tomato cultivation (MSIRI, 1994). In an attempt to develop an Integrated Pest Management (IPM) system, research was conducted on the use of pheromone lures as an option to manage H. armigera.

Pheromone has been previously used to study, monitor and control H. armigera in crops such as chickpea and cotton. (King et al., 1990; Prasad et al., 1993; Srivastava et al., 1991; Dhanorkar and Puri, 1993; Kehat and Dunkelblum, 1993; Kehat et al., 1998).

This paper reports on (1) evaluation of the efficacy of synthetic pheromones to lure male moths (2) determination of the potential of the pheromone in monitoring H. armigera in tomato field (3) evaluation of the efficacy of the local plastic, delta (Plant Research International, PRI) and funnel (Pest Control India, PCI) traps for capturing males of H. armigera.
Field evaluation of synthetic sex attractants of the tomato fruit worm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in Mauritius. *L Unmole and D Abeeluck*

**MATERIALS AND METHODS**

**Evaluation of the efficacy of synthetic pheromones to lure male moths**

(i) *Evaluation of the efficacy of PRI pheromone septa for luring male moths*

*Description of trap, lure and experimental lay out:*
The trap used was the delta trap (from PRI), which is a transparent plastic trap (21 x 11 x 9 cm) with a disposable sticky insert placed (17 x 9 cm) at the bottom. The pheromone lure was impregnated in a 2 cm red natural rubber septa.

The experiment was carried out in 2 fields (each of 0.2 ha in size and at flowering stage) from January to March 2003. One field was located at Richelieu Crop Research Station (CRS) and the other at Réduit CRS.

A delta trap with pheromone septa was suspended on a wooden stake and placed in the field at 1 m from ground level and 30 m from another one. Three sets of such traps were set in each field at different dates (21/01/03, 11/02/03 and 27/02/03).

Trap catches were recorded every 7 days for 7 weeks and caught insects identified.

(ii) *Investigation on the relative attractiveness of the PRI and PCI pheromone septa*

*Description of lures:*
Two types of lures were tested: the PRI lure (described above) and the lure from PCI impregnated in a 2 cm black rubber septa (Helilure as tradename). Each of the pheromone septa was set in a PRI delta trap (described above).

Six delta traps (3 with PRI and 3 with PCI septa) were set in a 0.4 ha field (at flowering and fruiting stage) 30 m from one another at Triolet. A similar set of 6 traps was placed in a tomato plot of 0.1 ha (at flowering and fruiting stage) at Réduit CRS. Trap catches were recorded every 7 days and septa renewed every 14 days. On each monitoring date, traps were rotated clockwise.

**Determination of the potential of PRI pheromone in monitoring male moths**

Two trials were conducted in the District of Pamplemousses: the 1st one in a 0.4 ha tomato field (variety MST 32/1) at Khoiratty from June to September 2003 and the 2nd in a block of 3.4 ha (variety MST 32/1) at Plaine des Papayes from February to April 2004.

At Khoiratty, 5 delta traps with pheromone septa (PRI) were placed in the field 18 Days After Transplantation (DAT) as per method described above. Trap catches were recorded every 7 days. 15 plants were selected randomly. Eggs and larvae were recorded every week from a determined plant sample (1st 4 compound leaves from 3 branch tips & 3 flower clusters) on each selected plant. Septa were renewed every 14 days. At 109 DAT, the crop was in senescence and the field was abandoned by the planter. However, trapping of moths was pursued for another 21 days up to 130 DAT.

At Plaine des Papayes, 32 delta traps with pheromone septa were placed at 30 m intervals in the block at 28 DAT. Trap catches were recorded every 7 days. Eggs and larvae were recorded on 30 randomly selected plants (as per method described above) from the 3.4 ha field. Septa were renewed every 14 days.
Evaluation of three trap types for use in Helicoverpa management

Description of traps:
Three types of traps were used: (i) delta from PRI (described above) (ii) funnel from PCI and (iii) plastic bottle trap constructed locally.

(i) Funnel trap:
It consisted of a cover, a funnel with handle, an ‘O’ ring to fasten a plastic bag on the perimeter of the funnel, and a long plastic bag. The pheromone septa was placed on the underside of the cover and the open end of a long plastic bag wrapped around outer edges of the funnel. The two parts were attached together by 3 pegs moulded on the funnel.

(ii) Plastic bottle trap:
It consisted of a transparent plastic bottle (1.5 L) cut on both sides to make an open cylinder. A string was attached on top of the cylinder held horizontally and a PRI sticky insert was fixed at the bottom. The pheromone septa was placed in the middle of the sticky insert.

From November 2004 to January 2005, three sets of the trap types baited with PRI septa were placed at a distance of 20 m in a 0.4 ha field at Boulingrin. Catches were recorded every 7 days and septa renewed every 14 days. On each monitoring date, traps were rotated clockwise. The trapping experiment was repeated at Richelieu CRS during the same period.

RESULTS AND DISCUSSION

Evaluation of the efficacy of synthetic pheromones to lure male moths

(i) Evaluation of the efficacy of PRI pheromone septa in luring male moths

The PRI pheromone septa attracted males of *H. armigera*. 128 males were captured in the 9 traps exposed for 49 days at Réduit CRS and Richelieu CRS.

Catches at both sites were high in the 1st week but gradually decreased in the 2nd and 3rd. No catches were recorded from the 4th to 7th week. Except in the 1st set, a very low catch (0.3/trap/week) was recorded at Richelieu CRS in the 5th week. Pheromone septa is reported to be effective for 21 days by Sinha and Mehrotra (1993) and 13 by Loganathan et al., (1999). Based on their findings and the very low captures in the 3rd & 5th week (Table 1) in our study, the time interval set for renewal of septa was 14 days.

Table 1 Average number of males of *Helicoverpa armigera* caught per trap in a tomato field at Réduit and Richelieu Crop Research Stations from January to March 2003

<table>
<thead>
<tr>
<th>Week</th>
<th>Average number of male moths per trap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Réduit</td>
</tr>
<tr>
<td></td>
<td>Set</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
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<tr>
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<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>
Field evaluation of synthetic sex attractants of the tomato fruit worm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in Mauritius. L Unmole and D Abeeluck

95% of catches were males of *H. armigera*. Other small moths and dipterans constituted the remaining proportion of the catches. The delta trap and sticky insert lasted 7 and 3 weeks respectively.

**(ii) Investigation on the relative attractiveness of the PRI and PCI pheromone septa**

The PRI and PCI lures were both attractive to males of *H. armigera*. The average number of male moths per trap per week with the PRI and PCI lure was 3.3 and 2.6 respectively at Réduit CRS and 5.6 and 3.3 respectively at Triolet. No significant difference between catches with PRI and PCI lures was observed at both sites (P-values at Réduit & Triolet were 0.9 & 0.6 respectively).

**Determination of the potential of PRI pheromone in monitoring male moths**

The crop cycle of the variety MST 32/1 lasted 109 days after transplantation and was characterised by 3 stages (1) vegetative (1 to 35 DAT) (2) flowering (35 to 60 DAT) and (3) fruiting (60 to 109 DAT). Flowering overlapped with the fruiting period. Results would henceforth be expressed in DAT.

At Khoyratty, male moths were captured throughout the crop cycle and 220 males were caught in the 5 traps during 16 weeks. The average number of males per trap per week in the vegetative, flowering and fruiting stage was 1.2, 1.3 & 3.1 respectively. During the crop cycle, catches were highest at 46 DAT (2.4 moths/trap), 67 DAT (6.6 moths/trap) and 109 DAT (6 moths/trap), which coincided with peak bloom, fruiting and senescence of plants respectively (Figure 2a). The average number of eggs at flowering (0.3) and fruiting stage (0.7) was 1.5 to 3.5 times higher than that at vegetative stage (0.2) respectively (Figure 2a). Flowers and fruits have been found to provide strong oviposition cues to Heliothinae females (Johnson et al., 1975, Latheff et al., 1991 and Zalom et al., 1983) and this explains the high number of eggs observed during the flowering and fruiting stages in our study.

**Figure 2a** Average number of male moths captured per trap and average number of eggs and larvae recorded per plant sample from 25 to 130 DAT at Khoyratty.

![Figure 2a](image)

The average number of eggs per plant sample was highest at 67 DAT (1.9/sample). There was a positive correlation between trap catches and egg numbers (r= 0.74) (Figure 2a).

At Plaine des Papayes, 462 male moths were caught in 32 traps during 11 weeks. Similar to Khoyratty, a positive correlation was observed between trap captures and egg numbers on plant sample (r= 0.75). In the tomato plant samples, the egg numbers were maximal at 67 DAT (2.2/sample) and coincided with high trap catches (2.7/trap) (Fig. 2b). Similarly, a positive correlation between trap catches and egg numbers on pigeon pea was reported by Dayakar and Rao (2000) and on tomato and carnation by Izquierdo (1996). Bues et al. (1985) noticed a time coincidence between catches in pheromone traps and *H. armigera* oviposition on tomato in France.

Field evaluation of synthetic sex attractants of the tomato fruit worm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in Mauritius. *L Unmole and D Abeeluck*

**Figure 2b** Average number of moths captured per trap and average number of eggs per plant sample from 39 to 109 DAT at Plaine des Papayes.

![Graph showing average number of moths and eggs](image)

Among weather parameters, only the relative humidity showed a significant positive correlation ($r=0.6$) with trap catches. Similar results were obtained by Patil & Kulkarni (1997). A drop in relative humidity coincided with a decrease in trap catches. Catches were highest at relative humidity above 70% (**Figure 2c**). Fluctuations in relative humidity, appears to influence the flight activity of moths.

**Figure 2c** Relative humidity and average trap catches in the tomato field at Khoyratty from June to Sept 2003.

![Graph showing relative humidity and trap catches](image)
In this study and in that of Nathawut and Fememore (1991), temperature and rainfall did not influence moth catches.

The average number of larvae/sample varied from 0 to 0.7 and was highest at 88 DAT at Khoyratty. At Plaine des Papayes, it varied from 0.1 to 1.2 with highest numbers at 46, 74 and 102 DAT. No significant correlation was observed between trap captures and numbers of larvae on plant samples at both sites. Peak moth captures did not coincide with peak larval numbers on plant samples (Fig. 2a and 2b). Similarly, Kehat et al., (1982) had also found that the levels of larval infestation could not be accurately predicted using trap catches.

According Hnimina (1986), the life cycle of H. armigera (from egg to egg) is completed in about 71.3 days at 20°C and 39.8 days at 25°C. At Khoynraty and Plaine des Papayes, the average temperature during the whole crop cycle was 20.6°C (± 0.3) and 26.2°C (± 0.2) respectively. Based on the temperature data, it can be deduced that eggs on plants at Khoynraty were deposited by females from adjoining fields. Such eggs deposited at the vegetative phase could give rise to adults only after 71 days. Trap catches as from 96 DAT may represent a proportion of these adults.

At Plaine des Papayes, H. armigera had a shorter life cycle. Eggs on plants as from 39 DAT gave rise to adults after 79 DAT. Trap catches from 79 to 109 DAT also included males emerging within the field.

During winter (at temperature below 20°C) a new tomato field is prone to attack by ovipositing females from neighbouring fields only. But in summer (>25°C), a new field is first attacked by females from neighbouring fields and after 79 DAT by both migrating and those emerging from the field.

**Evaluation of three trap types for use in Helicoverpa management**

Males of H. armigera were caught in the delta, funnel and plastic bottle traps with PRI pheromone septa. At Boulingrin, the average number of males per trap per week caught in delta, funnel and plastic bottle traps was 3.4, 1.0 and 3.3 respectively. The funnel trap caught significantly lowest numbers (P value = 0.03). There was no significant difference between catches in plastic bottle and delta traps.

At Richelieu, trap catches per week in delta, funnel and plastic bottle traps was 2.1, 3.2 and 4.0 respectively. No significant difference (P value = 0.29) was observed among catches in the 3 trap types.

The funnel trap was broken within 3 weeks of exposure in the field. On the other hand, the plastic and delta traps were used up to 7 weeks and were easier to service in the field. The plastic bottle trap is a good alternative to delta trap taking into consideration cost and availability of the trap locally.

**CONCLUSION**

There is a potential in using pheromones (PRI & PCI) to detect and monitor H. armigera population in tomato crop. Plastic bottle trap is an effective and cheap alternative to the funnel & delta traps.

**FURTHER RESEARCH**

It is now desirable to pursue research into the investigation on the potential of pheromone traps for mass trapping at higher trap density and the possible use of pheromone for mating disruption of H. armigera moths in tomato growing areas.

Field evaluation of synthetic sex attractants of the tomato fruit worm, Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) in Mauritius. L Unmole and D Abeeluck

ACKNOWLEDGEMENTS

We are grateful to the tomato growers of Khoiratty and Plaines des Papayes, staff of the Réduit Research Station & Richelieu Research Station for their collaboration in conducting this study. We would like to extend our gratitude to Mr Willem Stol and Dr Frans Griepink, Pherobank, Plant Research Institute, Netherlands, to Mr Nikhil Chatterjee, Pest Control India, India who provided pheromones for testing. Finally we would like to thank the staff of the Entomology Division, AREU for their assistance during research.

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Field evaluation of synthetic sex attractants of the tomato fruit worm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in Mauritius. *L Unmole and D Abeeluck*


SYSTEMIC AND CONTACT EFFECTS OF SOME PLANT EXTRACTS ON THE SERPENTINE LEAFMINER, L. TRIFOLII, IN POTATOES

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ABSTRACT

With a view to enlarging the available spectrum of insecticides effective against the serpentine leafminer, L. trifolii, ethanolic extracts of six locally available plants were studied for their systemic and contact pest controlling potential. Effect of systemic application on adult feeding and oviposition: Petioles of untreated, uninfested potato leaves were dipped in one of five concentrations of ethanolic extracts of either neem (Azadirachta indica), vetiver (Vetiveria zizanioides), ayapana (Eupatorium ayapana), lemon grass (Cymbopogon citratus), vieille fille (Lantana camara) or custard apple (Annona squamosa), air-dried and placed in test tubes, in thrice-replicated laboratory trials. Adult L. trifolii released in these cages fed on, and laid a greater number of eggs in, the untreated control leaves than in the treated leaves, the order of non-preference for both feeding and oviposition being: A. indica > V. zizanioides > A. squamosa > C. citratus > L. camara > E. ayapana

Effect of systemic application on egg and larval development: Petioles of untreated potato leaves infested with either eggs or larvae of L. trifolii were dipped in one of five concentrations of ethanolic extracts of one of the six plant spp. listed above, in test tubes, in thrice-replicated laboratory trials. Degree of reduction in larval and pupal survival was in the order: A. indica > V. zizanioides > E. ayapana = C. citratus = A. squamosa > L. camara.

Effect of contact application on egg and larval development: Untreated potato leaves infested with either eggs or larvae of L. trifolii were dipped in one of the five concentrations of ethanolic extracts of one of the six plant spp. listed above, and air-dried. Their petioles were dipped individually in water in test tubes plugged with cotton wool. Survival percentages of larvae, pupae and adults emerging from such treated leaves were found to be significantly reduced to varying degrees. The order of efficacy of the plant extracts in reducing these parameters were as follows: A. indica > V. zizanioides = C. citratus > E. ayapana > A. squamosa > L. camara.

Dipping untreated potato leaves infested with L. trifolii larvae in one of the five ethanolic extracts as described above significantly reduced larval, pupal and adult survival as compared to the untreated control. Results showed that the contact treatments were more effective than the systemic ones and that the treatments at the egg stage were more effective than were those at the larval stage. The order of efficacy of the treatments were as follows: Contact treatment at egg stage > contact treatment at larval stage > systemic treatment at egg stage > systemic treatment at larval stage. Furthermore, results showed that E. ayapana exhibited a greater systemic mode of action, C. citratus was more effective as a contact pesticide, while A. indica and V. zizanioides showed both systemic and contact effects.

INTRODUCTION

The serpentine leaf miner, Liriomyza trifolii, and the pea leaf miner, L. huidobrensis, are the major insect pests of potato in Mauritius today. Considerable research effort has been deployed over the years for the control of these pests. The initial chemical control was followed by biological control, and several parasitoids were introduced into the country (Rajabalee et al., 1992). Biology-based methods involve the use of insect growth regulators, while a physical method makes use of yellow sticky traps (Govendasamy and Ganeshan, 2002). The net result is that today there is an integrated approach to the management of these pests. However, it is recognized that the problem is far from being solved. The insects develop resistance to insecticidal chemicals fairly rapidly, and the number of alternative toxic chemicals available is very limited.
Systemic and contact effects of some plant extracts on the serpentine leafminer, *L. trifolii*, in potatoes. S Facknath

The Government’s Non-Sugar Sector Strategic Plan (2003-2007) makes mention of making agricultural production in Mauritius more environment-friendly and sustainable, with the emphasis on reducing the present level of use of synthetic pesticides and fertilisers. In fact, one of the approaches advocated is Organic Farming. As has been detailed in this Plan, conversion to organic farming has to be preceded with, among other requirements, research to develop locally adapted, appropriate, site-specific organic farming practices. One such practice suggested is the use of botanical pesticides for pest, disease and weed control, not only in organic farming but also in conventional production to make the latter more eco-friendly.

In line with this strategy, the present work was undertaken to study the potential of six locally available plant species for their pest control potential against *L. trifolii* on potato. The plant species tested were *Azadirachta indica* [neem (margosa)], *Lantana camara* [periwinkle (vieille fille)], *Cymbopogon citratus* [lemon grass (citronelle)], *Annona squamosa* [custard apple (coeur de boeuf)], *Vetiveria zizanioides* [vetiver grass (vetiver)] and *Eupatorium ayapana* (ayapana).

The pest control potential of some of these plants has already been reported by several workers in Mauritius (Facknath and Lalljee, 1998; Facknath and Lalljee, 2000) on leafminers and other insect, mite and nematode pests. Neem extracts and formulations have been shown to reduce feeding and oviposition (Dimetry et al., 1995, as quoted by Weintraub and Horowitz, 1997), reduce pupation (Meisner et al., 1985; Parkman and Pienkowski, 1990; Webb et al., 1983) in *Liriomyza trifolii*.

**MATERIALS AND METHODS**

(i) Insect Culture
A laboratory colony of *L. trifolii* was maintained on potted potato plants placed in wooden cages (70 cm length x 60 cm width x 60 cm height) fitted with fine mesh netting, at 25-28°C, 65-70% R.H. and 13:11 L:D conditions. Pupae were collected and placed in Petri dishes on moist filter paper, and kept in a separate cage for emergence of adults.

(ii) Extraction
200 g of leaves of the respective plant species were extracted in 2 litres of 95% ethanol. The mixture was left overnight on a shaker for maximum extraction. The mixture was filtered through muslin cloth and the filtrate mixed with a further 2 litres of 95% ethanol. This suspension was evaporated under vacuum in a rotary evaporator at 60°C. The residue in each case was dissolved in 10 ml of 95% ethanol. From this stock solution, the various dilutions were made using distilled water and 2% Tween-20 as a surfactant.

(iii) Potato cultivation
Potato (*Solanum tuberosum*, var. Mondial) plants were grown on the University of Mauritius farm on a 30m x 30m plot from seeds obtained from the Agricultural Marketing Board. Normal agronomic practices were applied, except for pesticidal treatment. No pesticides were sprayed. Infested and uninfested leaves were plucked at random from the middle canopy of plants and brought to the laboratory for the different experiments.

(iv) Systemic Treatments

(a) Effect of plant extracts on feeding and oviposition by adult *L. trifolii*
Untreated, uninfested potato leaves were obtained from the UoM farm as described above. The petiole of each leaf (each having 5-7 leaflets) was dipped in 50 ml of one of four different concentrations (0.1%, 0.5%, 1.0%, 1.5%) of one of six plant extracts, neem (*Azadirachta indica*), vetiver (*Vetiveria zizanioides*), ayapana (*Eupatorium ayapana*), lemon grass (*Cymbopogon citratus*), vieille fille (*Lantana camara*), and custard apple (*Annona squamosa*), in test tubes and plugged with cotton wool. The treated leaves in test tubes were placed in wooden cages and twenty 2-day old *L. trifolii* adults taken from the laboratory culture were released for 4 h. The adults had previously been starved for 2 h to standardize their level of hunger. Three such cages were set up, i.e., there were 15-21 leaflets for each treatment. Control consisted of 3 potato leaves (i.e., 15-21 leaflets), with their petioles dipped in water. The number of feeding and oviposition punctures on each leaflet was counted under a microscope.

Systemic and contact effects of some plant extracts on the serpentine leafminer, *L. trifolii*, in potatoes. **S Facknath**

(b) Effect of plant extracts on survival, growth and development of *Liriomyza* spp. treated at egg stage

Untreated potato leaves showing leafminer punctures were obtained from the UoM farm as described above. The number of punctures per leaflet was counted. Preliminary observations on 50 leaves showed that the majority of punctures were made by *L. trifolii*. However, a few *L. huidobrensis* larvae were obtained, and thus the results in this section relate to both *L. trifolii* and *L. huidobrensis* species. The leaves were treated as described in section (iv) (a) above. The solution in the test tubes was replaced daily with fresh solution of the same plant extract and concentration, until L₃ mines began to appear. The number of L₃ mines and pupae emerging from the mines were counted in each leaflet. Pupae were transferred to a Petri dish lined with moist filter paper until adults emerged. Adults showing abnormalities or malformations were separated. The number of normal and deformed adults were counted separately.

(c) Effect of plant extracts on survival, growth and development of *L. trifolii* treated at larval stage

Untreated potato leaves with leafminer punctures were obtained from the UoM farm as described above. The leaves were kept in the laboratory with their petioles dipped in water in test tubes until mines appeared. Leaves having 6.0 to 6.5 L₂ mines/leaflet were chosen to avoid effects due to intraspecific competition (Parrella, 1983). *L. trifolii* larvae could be ascertained by the position and appearance of the mines being thinner, paler, convoluted and spread randomly on the leaf lamina (*L. huidobrensis* mines are darker, coalesced, and tend to follow leaf veins) (Banymandhub and Rajabalee, 1992). The mined leaves were treated with the plant extracts as described above in section (iv) (a). The number of pupae and adults developed from each leaflet were counted.

(v) Contact Treatments

(a) Effect of plant extracts on feeding and oviposition by adult *L. trifolii*

Untreated potato leaves were obtained from the UoM farm as described above. The leaflets (5-7) of each leaf were dipped in one of five different concentrations (0.1%, 0.5%, 1.0%, 1.5%) of one of the above listed six plant extracts, air-dried and placed in test tubes containing water and plugged with cotton wool. The parameters were studied as described in section (iv) (a) above.

(b) Effect of plant extracts on survival, growth and development of *Liriomyza* spp. treated at egg stage

Untreated potato leaves showing leafminer punctures were obtained from the UoM farm as described above. The leaves were treated as described in section (v) (a) above. The water in the test tubes was replaced daily with fresh water until L₃ mines began to appear. The same parameters as described in section (iv) (b) were studied.

(c) Effect of plant extracts on survival, growth and development of *L. trifolii* treated at larval stage

Untreated potato leaves with leafminer punctures were obtained from the UoM farm as described above, and subjected to the same treatments as in sections (iv) (c) and (v) (a) and (b). The same parameters as described in section (iv) (c) were studied.

RESULTS

As can be seen from Figs. 1-8, all the plant extracts studied caused significant reductions in survival and development of the different stages of the leafminer. The effects were dose-dependent in all cases, being highest at the 1.5% concentration and lowest at the 0.1% concentration. All the extracts deterred adult feeding and oviposition, the strongest effect seen in *A. indica* (almost 50% reduction compared to untreated control), followed by *V. zizanioides* (38% reduction), *A. squamosa* (27% reduction), *C. citratus* (22% reduction), *L. camara* (18% reduction) and *E. ayapana* (12% reduction) (Figure 1 and 2).
Figure 1 Percentage reduction in total and oviposition punctures by *Liriomyza trifolii* on potato plants treated systemically with plant extracts

![Figure 1]

Figure 2 Percentage reduction in total and oviposition punctures by *Liriomyza trifolii* on potato leaves dipped in plant extracts

![Figure 2]

The plant species varied in their pest reducing potential, with *A. indica* being the most effective overall (a maximum of 30% reduction in egg hatching, an 85% reduction in pupation and a 90% reduction in adult emergence, at the 1.5% concentration) (Figures 3-6). *V. zizanioides* was the next best after *A. indica* in terms of efficacy in reducing feeding, egg-laying, egg hatch, larval and pupal survival, followed by *C. citratus*, *E. ayapana*, *A. squamosa* and *L. camara*. *L. camara* was overall the least effective with a maximum of 16% reduction in adult survival at the highest dose tested.
Figure 3  Systemic effects of plant extracts on *Liriomyza trifolii* egg development in potato leaves (Ai= *Azadirachta indica*; Vz= *Vetiveria zizanioides*; Cc= *Cymbopogon citratus*; As= *Annona squamosa*; Lc= *Lantana camara*; Ea= *Eupatorium ayapana*)

**Egg hatch**

% egg hatch

Pupation

% pupation

Adult emergence

% adult emergence

Concentration of plant extracts

Figure 4 Systemic effects of plant extracts on *Liriomyza trifolii* larval development in potato leaves (Ai=Azadirachta indica; Vz=Vetiveria zizanioides; Cc=Cymbopogon citratus; As=Annona squamosa; Lc=Lantana camara; Ea=Eupatorium ayapana)
Systemic and contact effects of some plant extracts on the serpentine leafminer, *L. trifolii*, in potatoes. S Facknath

**Figure 5** Contact effects of plant extracts on *Liriomyza trifolii* egg development in potato leaves (*Ai*= *Azadirachta indica*; *Vz*= *Vetiveria zizanioides*; *Cc*= *Cymbopogon citratus*; *As*= *Annona squamosa*; *Lc*= *Lantana camara*; *Ea*= *Eupatorium ayapana*)

- **Egg hatch**
- **Pupation**
- **Adult emergence**


357
The different plant extracts affected the different life stages of the pest to varying degrees, e.g. *A. indica* reduced larval survival to a greater extent than it did egg hatching at all the concentrations tested. *L. camara* had a stronger negative effect on adult emergence than on pupation, while *E. ayapana* affected pupation more than it did adult emergence, in both contact and systemic treatments (Figure 7 and 8).
Figure 7: % reduction in life history parameters of *Liriomyza trifolii* treated with plant extracts in systemic treatment (Ai=Azadirachta indica; Vz=Vetiveria zizanioides; Cc=Cymbopogon citratus; As=Annona squamosa; Lc=Lantana camara; Ea=Eupatorium ayapana)
The plant extracts demonstrated growth disturbing activity (Table 1), with neem producing the highest proportion of abnormal adults.
Table 1 Number of normal and abnormal *L. trifolii* adults (mean ± S.D.) developed in potato leaves treated with plant extracts

<table>
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<th>Plant</th>
<th>Conc.</th>
<th>Normal treatment to leaves</th>
<th>Abnormal treatment to leaves</th>
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<th>Abnormal treatment to leaves</th>
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In practically all the plant species in this study, and at all the concentrations used, the degree of pest control efficacy was greater when the treatments were applied at the egg stage than when they were applied later, i.e. at the larval stage. This was observed in both the systemic and the contact modes of application.

With a few exceptions, in almost all cases, *A. indica*, *V. zizanioides*, *C. citratus*, *A. squamosa* and *E. ayapana* exhibited greater contact action than systemic activity at all concentrations and on all stages of the pest. *L. camara*, however, was more effective as a systemic treatment than as a contact treatment.

The effect of the recommended dose of cyromazine was highest on pupation in the contact treatment (80.95% reduction compared to untreated control), and less in the systemic treatment (72.28% reduction). The equivalent reductions in adult emergence were 66.68% and 63.76%, respectively, while the reductions in egg hatching were 1.98% and 1.58%, respectively.

The percentages of pupation and adult emergence in this study were observed to be fairly high in the untreated control. This could be because leaves infested with eggs were brought to the laboratory, and the developing larvae and pupae were not exposed to parasites, predators or unfavourable abiotic conditions. Under field conditions, successful pupation and adult eclosion are normally much lower.

DISCUSSION

All the plant species tested showed potential for the control of *L. trifoli*. The effectiveness of *A. indica* in controlling *Liriomyza* spp. has been demonstrated by various workers. Dimetry et al., (1995) reported the oviposition reducing property of neem seed kernel extracts in *L. trifoli* while Meisner et al., (1985) demonstrated the pupation reducing property of various extracts of neem on *L. trifoli*. The study of Parkman and Pienkowski, (1990) showed that neem seed extracts had adverse effects on egg laying and longevity of *L. trifoli* adults in chrysanthemum plants, while Webb et al., (1983) obtained high mortality in *L. trifoli* and *L. sativae* following applications of aqueous solutions of neem seed extract to bush lima bean leaves.

In the present study, the O/F ratios (oviposition punctures : feeding punctures) were not significantly changed by the treatments, indicating that the treatments reduced both feeding and egg laying in the adult insects. This points to the presence of antifeedant and oviposition-deterring allelochemicals in the plant extracts. These effects were more pronounced when the plant extracts were applied as surface treatments (Figure 1) than when applied systemically (Figure 2). In fact, except for the effect of *V. zizanioides* on egg hatch, pupation and adult emergence, the other plant species studied exhibited greater contact effect than systemic activity on all the developmental stages of the pest. Meisner et al., (1985) obtained different results, with about 75% reduction in *L. trifoli* populations in bean plants from the systemic action of crude aqueous extract of neem seed kernels. Larew et al., (1985) also demonstrated the systemic effect of neem seed kernel on pupation and adult emergence of *L. trifoli* in chrysanthemum plants. Weintraub and Horowitz (1997) reported that the systemic effect of a commercial neem formulation on pupation and adult emergence of *L. huidobrensis* on bean plants was greater than that of its contact effect. This difference between studies could be because in the present study, the plant extracts were not applied to roots of growing plants and hence true systemic action was not obtained. Although the use of detached plant leaves for *L. trifoli* studies has been shown to be equivalent to the use of whole plants (Larew, 1989), the uptake of the bioactive molecules would be the result of capillary/osmotic action rather than active translocation, and hence can be expected to be variable. This corroborates the study of Larew (1986) who reported that uprooted bush lima bean plants placed in 1.125 ppm azadirachtin solution for 24 h resulted in significantly fewer *L. trifoli* pupae and adults compared to uprooted plants placed in water, but this effect was less pronounced than spraying the leaves with the same level of azadirachtin.
The contact action of plant extracts applied to the leaf surface may affect the larvae inside the leaves by their translaminar activity and/or by contact toxicity when the larvae come out of the leaf mines to pupate and thereby come in contact with the chemicals. Meisner et al (1986) demonstrated that ethanolic and methanolic extracts of neem exhibit translaminar activity in bean leaves, but aqueous extracts do not.

All the plant species tested had an adverse effect on normal development. Adults formed were observed to have abnormally developed bodies with deformed or unexpanded wings, irregular pigmentation of the body, twisted legs, inability to emerge from pupal case, and pupal-adult irregularities. Similar developmental irregularities resulting from neem treatment in other insect species have been shown by Facknath and Lalljee, 1998; Gujar and Mehrotra, 1990; Mukherjee and Sharma, 1996; Meisner et al., 1986).

The contact effect of cyromazine was found to be much higher than in the systemic treatment. Cyromazine is a quick acting, discrete triazine molecule (Lim et al., 1990). Its breakdown product, melamine, is not larvicidal (Miller et al., 1996). Cyromazine has good translaminar action (Weintraub, 1999), and affects endophagous larvae internally in their leaf mines as well as when they emerge from the leaf to pupate. Hence cyromazine reduced larval survival immediately, but exhibited delayed action when applied systemically.

The bioactive allelochemicals from neem have been well-studied and documented. The azadirachtins are the most potent principles, exhibiting strong growth and development inhibiting effect on a wide variety of insects. Among the other active compounds in neem are salannin, meliantriol and limbidin, which are strong antifeedants. Nimbidin also disturbs growth and development in insects (Rembold, 1989; Saxena, 1989; Isman, 1997; Facknath and Lalljee, 2000).

The other plant species have not been the subject of investigation to the same extent as neem has been. Facknath and Lalljee (1998) have reviewed the plant spp. studied in Mauritius for their pest controlling property. A few studies have shown that V. zizanioides has insecticidal (Murty and Jamil, 1987), antifungal (Gangrade and Shrivastava, 1991) and antibacterial (Gangrade and Shrivastava, 1990) properties. The bioactive compounds identified in Vetiveria zizanioides include khusimol, alokhusiol and khusitoneol, and the sesquiterpene diol, vetidiol. Sukdeo (1993) demonstrated that Liriomyza spp. adults were deterred from feeding and ovipositing on potato leaves by ethanolic extracts of V. zizanioides.

C. citratus has been reported to have insect growth regulatory, insecticidal, bactericidal, antifungal and nematicidal properties (Mackeen et al., 1997; Amadioha and Obi, 1999; Dudai et al., 1999; Rajapakse and Van, 1997; Ketoh et al., 2000).

A. squamosa has been reported to have growth regulatory effects on Rhyzopertha dominica (Khanam and Talukder, 1998), and antifeedant and insecticidal effects on the Lepidopteran field pest, Achaea janata (Babu and Nair, 1997). Various acetogenins, among them annonin I, asiminic and isobullataticin have been identified as the biologically active compounds in A. squamosa (Alkofahi et al., 1989; Rupprecht et al., 1990; Sharma et al., 1999). Prakash et al., (1989) extracted procyanidin – B7 from the unripe fruits of A. squamosa, and showed this compound to be the precursor to the condensed tannins exhibiting antifeedant activity against some lepidopteran larvae.

In the present study, the effect of A. indica was comparable and even higher at the 1.0% and 1.5% concentration, to that of the recommended insecticide, cyromazine, in both contact and systemic treatments. While cyromazine showed greater effect on larvae, A. indica affected both larvae and pupae significantly, giving an overall higher control at the 1.0% and 1.5% concentration as compared to cyromazine, under the conditions of the study. However, the known susceptibility of azadirachtin to photodegradation and the wide variation in azadirachtin content resulting from extraction procedures, formulation and neem tree biotypes (Ermel et al., 1986; Isman et al., 1990; Mordue and Blackwell, 1993) can lead to variations in results in the field.

In all the 6 plant species, the effect on L. trifolii growth, development and survival was greater when the treatments were applied earlier, i.e. in the egg stage, than when the insects were in the larval stage. Similar results were obtained in L. huidobrensis by Weintraub and Horowitz (1997) with different concentrations of azadirachtin applied either systemically as or a leaf-dip. One reason for this could be
that the bioactive compounds can enter the egg shell through the chorion and affect adversely the developing zygote, leading to death or congenital abnormalities. The effect of neem allelochemicals on the eggs of *Dysdercus flavivis* has been shown by Facknath (1991). Newly hatched first-instar larvae of *Liriomyza*, and other insects, have been shown to be more susceptible to insecticidal allelochemicals than are older larvae (Sombatsiri and Temboonkeat, 1986; Gujar and Mehrotra, 1990; Mukherjee and Sharma, 1996). Hence, as was expected, the plant extracts studied were more effective when applied at the egg stage than when applied later.

Pesticidal extracts from plants are a complex mixture of a large number of biologically active compounds, and hence are less likely to result in resistance in the target pest. Furthermore, the majority of them (Boeke et al., 2004), and neem in particular, have been shown to be less harmful to the indigenous and introduced natural enemies of insect pests (Lowery and Isman, 1993; Schmutterer, 1999; Facknath and Lalljee, 2000b; El Shafiea and Basedow, 2003). Mansour et al. (1986) showed that neem seed kernel extracts were much less toxic to the predatory mite, *Phytoseiulus persimilis*, and the predatory spider, *Chiracanthium mildei*, than to the pest mite, *Tetranychus cinnabarinus*. In the present study, the effect of the plant extracts on the known parasitoids of *L. trifolii* were investigated. However, since no parasitoids were obtained from any of the mined leaves collected, the influence of the botanical pesticides could not be ascertained. A separate study needs to be conducted. However, studies carried out elsewhere have shown that crude preparations of neem and commercial formulations applied at the recommended dose are not harmful to *Diglyphus* spp., one of the parasitoids of *L. huidobrensis* (Weintraub and Horowitz, 1997).

**CONCLUSION**

Laboratories around the world are scouring various ecosystems, especially in the tropics and subtropics, for bioactive chemicals from plants, animals and microbes for commercial exploitation, either as active ingredients in agrochemical formulations, or as lead molecules for synthesis of pesticidal analogues. The 6 plant species reported here offer promise for the control of *L. trifolii* in potato. This study does not advocate entirely replacing cyromazine or the other recommended synthetic insecticides, with plant extracts. It aims to provide the larger array of efficacious, eco-friendly insecticides needed for the environmentally-sustainable control of *Liriomyza*. Tests of these plant species on the other leafminer, *L. huidobrensis*, on other host plants of *Liriomyza* and under field conditions are recommended.

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Systemic and contact effects of some plant extracts on the serpentine leafminer, *L. trifoli.* in potatoes. *S Facknath*


365


Systemic and contact effects of some plant extracts on the serpentine leafminer, *L. trifolii*, in potatoes. *S Facknath*


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PHYTOPHTHORA BLIGHT OF CUCURBITS AND CAPSICUM IN MAURITIUS

V. Vally and S.P. Beni Madhu
Agricultural Research and Extension Unit

ABSTRACT

Since the fall of 2003 Phytophthora blight was observed on members of the cucurbit and capsicum families in commercial plantations mainly in the central part of the island. According to estimates from the Crop Extension Services, crop losses due to fruit rot of up to 100% in chilli and of up to 75% in cucurbits were recorded. Fungal isolations were made from infected tissues which were surface sterilized in 1% sodium hypochlorite for 2 minutes, blotted dry on sterile filter paper and cultured on Potato Dextrose Agar (PDA). Plates were incubated at 25°C for 7 days. Only one fungal species was constantly isolated from diseased materials. Morphology of the fungus was consistent with that of Phytophthora capsici. A culture of the fungal pathogen was forwarded to CABI BioScience, UK for confirmation through DNA sequencing. The ITS region of the DNA from the isolate was sequenced and the result compared with a database of Phytophthora sequences. Authoritative identification of the fungus as Phytophthora capsici in Clade II was obtained, and a culture deposited as IMI 391995. This new disease, caused by Phytophthora capsici, is reported for the first time in Mauritius. The status of the disease, its predominant symptoms and interim management strategies are described in this paper.

Keywords: Phytophthora blight, chilli, cucurbits, DNA sequencing

INTRODUCTION

The Extension Unit of the Agricultural Research and Extension Unit reported a new disorder of unknown aetiology on members of the capsicum and cucurbit families since the fall of year 2003. The disorder, at that time, was localised in certain regions in the central part of the island namely, La Laura, Malinga, Nouvelle Decouverte, La Marie and Dubreuil. During the heavy rainfall season in February 2004 the symptom reappeared causing field losses of up to 100% in chilli (stem/foliar blight and fruit rot) and up to 75% in pumpkin (fruit rot). The disease gained more importance where other members of the capsicum and cucurbit species were affected. Some planters in these regions have had to abandon their commercial plantations due to serious losses encountered. There was a lot of pressure and outcry from the farming community and the Extension Unit to find ways and means to identify the causal agent of this new disease.

Disease Symptoms

The disease was found to be able to attack cucurbit and chilli plants at any stage of growth, infecting vines, leaves and fruits. Overall symptomatology encountered on capsicum and cucurbits are described below:

(i) Capsicum (chilli cipaye local, piment carri and sweet pepper)
   (a) Foliar blight

Dark brown water-soaked lesions were observed on leaves. The lesions turned chlorotic and became necrotic with a pale green circular border. The lesions expanded and coalesced covering the entire leaf, causing its death.
(b) Stem blight

On stems, initial symptoms consisted of dark brown and water-soaked lesions, which enlarged and girdled the stem and twigs, resulting in the rapid collapse and death of plants. Stem of highly infected plants had a dark brown to black coloration.

(c) Fruit rot

Fruit rot typically began as dark green and water-soaked areas that enlarged and became covered with white to grey characteristic cottony mould growth. Infected fruits dried out, became shrunken, wrinkled and finally collapsed, but remained attached to stems.

(ii) Cucurbits (pumpkin, bottlegourd, zucchini, squash and snakegourd)

(a) Vine blight (Crown rot)

Dark olive water-soaked lesions initially developed on vines. The lesions enlarged with time becoming dark brown and girdled the stem. A soft rot and watery rot is formed around the crown region causing a rapid wilt of infected plants.

(b) Fruit rot

Infection initially appeared as water-soaked spots which enlarged and became eventually covered with a thick whitish mycelial growth. Infection progressed rapidly resulting in complete collapse of infected fruits. Fruit rot occurred on immature as well as mature fruits. Symptoms appeared either on the side of fruits in contact with soil or on their upper surface.

Pathogen Identification

Identification of the pathogen as Phytophthora capsici was mainly based on isolation studies, the morphology of its sporangia and on authoritative confirmation from CABI Bioscience of UK.

(i) Isolation of the pathogen

Pieces of infected tissues from the edge of lesions were surfaced sterilized in 1% sodium hypochlorite for 2 minutes, blotted dry on sterile filter paper and cultured on Potato Dextrose Agar (PDA). Plates were incubated at 25°C for 7 days. Only one fungal species was constantly isolated from diseased materials. Growth patterns of colonies obtained were cottony, finely radiate, petaloid, rosaceous and stellate (Figure 1), conforming with growth patterns described for Phytophthora capsici (C.M.I., 1985).

Figure 1 Growth pattern of P. capsici on PDA, 7-day-old cultures
(ii) Morphology of sporangia

Identification of the fungal pathogen as *Phytophthora capsici* is mainly based on the morphology of the sporangia. Long pedicels (> 35 μm) were observed by light microscopy analysis on caducous, papillate sporangia which tapered at the base (Figure 2). The sporangia were subspherical, ovoid with one or more apices. The hyphae were fairly coarse (5-7 μm) with sporangiophores narrow, branching irregularly and widening slightly at the base of the sporangium. Zoospores production was also encountered (Figure 3).

![Figure 2](image1.png) Sporangia of *P. capsici* with long pedicel

![Figure 3](image2.png) Sporangium of *P. capsici* releasing zoospores

(iii) Pathogenicity tests

Koch’s postulates were confirmed by inoculating mature fruits of members of cucurbits and chilli in sterile trays kept at a temperature range of 23 to 26º C. Plugs from PDA Agar cultures of *P. capsici* were placed on wounds made on fruits. Wounded fruits inoculated with sterile water served as control. Dark, water-soaked lesions were visible on all the fruits inoculated with the pathogen after 3 days. After 6 days the fruits became soft, and covered with white mycelium of the fungus. Control fruits remained healthy. Re-isolation yielded *Phytophthora capsici*.

(iv) Authoritative confirmation from CABI Bioscience

A type culture of the fungal pathogen was forwarded to CABI BioScience, UK for confirmation through DNA sequencing. The ITS region of the DNA from the isolate was sequenced and the result compared with a database of Phytophthora sequences. Authoritative confirmation of the identity of the fungal pathogen as *Phytophthora capsici* in Clade II was obtained in July 2004 from the CABI Bioscience where a culture was deposited as IMI 391995.

Host Range

In Mauritius, the disease has so far been recorded on pumpkin (*Cucurbita maxima*), squash (*C. pepo*), zucchini (*C. pepo*), bottlegourd (*Lagenaria leucantha*), snakegourd (*Trichosantes anguina*) and on capsicum spp. namely sweet pepper (*Capsicum annum*), piment carri and piment cipaye locale (*Capsicum spp.*).

The pathogen is reported to have wide host range world wide. *Phytophthora capsici* is reported to infect 49 species of cultivated plants and weeds (Babadoost et al., 2004). Major hosts described by the author include pumpkin (*Cucurbita maxima*), cucumber (*Cucumis sativus*), squash (*Cucurbita pepo*), zucchini (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), gourd (*Cucurbita pepo*), honeydew melon (*Cucurbita melo*), cantaloupe (*Cucumis melo*), pepper (*Capsicum annum*), eggplant (*Solanum melongena*), tomato (*Lycopersicon esculentum*) and chillies (*Capsicum sp*) and lima bean (*Phaseolus lunatus*).
Disease Occurrence in Mauritius

In view of the occurrence of this new disease surveys were conducted to determine the incidence of the disease island-wide. Table 1 summarises the survey results in fields suspected by Crop Extension Division to be infected by the disease during the year 2004 (based on symptoms and laboratory confirmation).

**Table 1 Occurrence of the disease**

<table>
<thead>
<tr>
<th>Crop</th>
<th>North</th>
<th>South</th>
<th>East</th>
<th>Centre/ West</th>
<th>Total</th>
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<tr>
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<td>No. of suspected field surveyed (%)</td>
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<td>24</td>
<td>15</td>
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<td>80</td>
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<td>No. of fields affected (%)</td>
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<td>9 (37%)</td>
<td>12 (80%)</td>
<td>7 (26%)</td>
<td>31 (39%)</td>
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<td>5 – 25</td>
<td>1 – 50</td>
<td>5 – 75</td>
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<td>No. of suspected field surveyed (%)</td>
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<td>14</td>
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<td>No. of fields affected (%)</td>
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<td>0</td>
<td>8 (57%)</td>
<td>2 (66%)</td>
<td>10 (28%)</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>1 – 100</td>
<td>25 - 60</td>
<td>-</td>
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</table>

Highest disease incidence (75%) was recorded on pumpkin in the centre/west and in the east (50%). Both patole and squash (25%) are the only cucurbits affected in the north by the disease. The disease was recorded on cucurbits island-wide with however, year round prevalence in the east and the south. Among members of capsicum, highest disease incidence was recorded in piment cipaye locale (100%) in the east and piment carri (60%) in the centre/west zones. The disease has not been so far reported on capsicum in the North and the South.

Management of the Disease

Based on experience elsewhere, interim disease management strategies comprising a combination of measures recommended include the following:

1. Select fields
   (i) With no history of the disease
   (ii) That are well-drained and if possible free from low-lying areas, which favour water-logging conditions.
2. Scout the field for disease symptoms particularly after rainy periods.
3. Destroy initial foci of the disease (by deep ploughing or discing).
4. Avoid excessive irrigation.
5. Do not work in wet fields. Clean equipment of soil when moving from one field to another.
6. Treat preventively under very wet conditions with fungicides at 7 days intervals as follows:

   (i) **Alternate:**

   Ridomil Gold MZ 68 @ 1.5 kg / ha + Dithane M45 @ 1.2 kg / ha with Bravo 720 SC @ 1.2 L / ha
   or Proplant @ 1.5 L / ha with Bravo 720 SC @ 1.2 L / ha.
   (Good coverage with Bravo 720 SC should be ensured).

   (ii) **Pre-Harvest Intervals:**

   - Ridomil Gold MZ 68 – 7 days
   - Proplant and Bravo 720 SC - 3 days

7. Harvest healthy fruits as soon as possible from affected fields. Do not collect seeds from infected fruits
8. Keep harvested fruits dry and cool.
9. Prevent the spread of the pathogen from being moved in a new field.
10. For infected fields, rotate with non-hosts crops for at least 3 years.
CONCLUSION

The occurrence of Phytophthora blight, caused by *Phytophthora capsici* Leonian is confirmed on members of cucurbit and capsicum families in Mauritius causing foliar and stem blight, crown and fruit rots. Authoritative confirmation as *Phytophthora capsici* Leonian was obtained by CABI Bioscience, UK (IMI 391995). The disease has been detected only on members of cucurbit and capsicum species with highest incidence in piment cipaye locale (100%) and in pumpkin (75%). The disease represents a real threat to cucurbit and chilli production in Mauritius. This is a first report of Phytophthora blight, an economically important disease caused by *Phytophthora capsici* in Mauritius.

ACTION PLAN (IMMEDIATE / MEDIUM / LONG TERM)

To cope with this new disease which represents a threat to cucurbit and chilli production in Mauritius, the following actions have been taken:

1. Capacity building of Extension in the identification of the disease and its management
2. Preparation of a fact sheet on the disease for Extension Division
3. Sensitization of planters about the disease and its management by Extension
4. Devising interim management strategies

Future activities include the following:

1. Epidemiological studies
2. Monitoring of status of the disease during the summer rainy periods
3. Determination of field tolerance in chilli and cucurbit varieties
4. Host range monitoring
5. Evaluation of the performance of new eradicant fungicides for the control of the disease
6. Collection of isolates of the fungal pathogen for further studies particular on its pathogenic and genetic diversity as distinct pathogenic strains have been reported (Babadoost et al., 2004)

ACKNOWLEDGEMENTS

The authors acknowledge contributions from The Management of AREU; The Principal Biometrician; Mrs G Bacorisen for isolation studies; Mr R Gowd for photography; Dr Y Sarma (ex – Director of IIRS-(India); Officers of Crop Extension Division and Colleagues of Plant Pathology Division of AREU.

REFERENCES


PHENOTYPIC DIVERSITY OF XANTHOMONAS STRAINS ISOLATED FROM ONION BLIGHT USING METABOLIC FINGERPRINTING

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ABSTRACT

Bacterial leaf blight of onion (Allium cepa L.), termed as ‘blast’, is a serious disease affecting onion production in Mauritius. This disease is considered as being one of the major constraints towards the expansion of the local onion industry and is reported to be caused by a Xanthomonas species. Rapid detection coupled with an understanding of the exact identity and phylogenetic relationships of local Xanthomonas strains involved are important determinants towards the development of a successful blight control strategy. In this context, 15 different bacterial isolates, all bearing the morphological characteristics of Xanthomonas were isolated from blight affected onion leaf samples. Biolog GN2 microplates were used to phenotypically characterise and estimate the metabolic diversity within these isolates. Each isolate produced unique metabolic fingerprint patterns that were used to generate the Nei and Li genetic distance matrix showing pairwise genetic distances between the isolates. Cluster analyses were performed using the unweighted pair group method with arithmetic averages (UPGMA) in PAUP* software to produce a dendrogram showing the relationship among the isolates. Patterns of some other Xanthomonas spp. and pathovars were included in order to facilitate identification of the local strains. Preliminary qualitative examination of the dendrogram suggested that xanthomonads associated with bacterial onion blight were distinct from the type Xanthomonas strains and pathovars used.

Keywords: Allium cepa L., Xanthomonas sp., Biolog GN2 microplates, metabolic fingerprint, UPGMA, dendrogram.

INTRODUCTION

Bacterial leaf blight of onion, caused by Xanthomonas (Pammel) Dowson strains is a potentially devastating disease affecting many onion-producing areas in the world (Sanders et al., 2003). The disease is characterised by the formation of lenticular water-soaked lesions on onion leaves that elongate into chlorotic streaks causing tip dieback and stunting of plants eventually leading to abnormally undersized bulbs at harvest. Bacterial onion blight was initially reported in Barbados in 1971 (Paulraj and O’Garro, 1993) and Alvarez et al., (1978) were the first to characterise the causal pathogen.

Recently, Gent et al., (2004) and Roumagnac et al., (2004) identified the causal agent of bacterial onion blight as being Xanthomonas axonopodis pv. alli (X.c. pv. allii) based on polyphasic approaches involving pathogenicity testing to onion, 16S rDNA gene sequence diversity, DNA-DNA hybridisation, genomic fingerprinting, carbon source utilisation and fatty acid methyl ester profiles.

Bacterial onion blight is known to be prevalent in Mauritius and has been recognised as being among one of the major potential threats to the expansion of the local onion industry. However, little information regarding the diversity of local Xanthomonas strains associated with onion blight in Mauritius is currently available. Moreover, relationships of local strains to type Xanthomonas species and pathovars are unknown. Therefore, following the isolation of some local Xanthomonas strains from onion blight leaf samples, an attempt to characterise the extent of phenotypic diversity using the commercial Biolog identification kit has been made.
Phenotypic diversity of *Xanthomonas* strains isolated from onion blight using metabolic fingerprinting. P Nowbath et al.

Biolog GN microplates, developed by Biolog Inc (Hayward, Calif.), are widely used to identify a wide range of aerobic gram-negative bacteria (Miller and Rhoden, 1991). The test is based on the differential metabolisation of 95 carbon sources and gives a characteristic reaction pattern called the “metabolic fingerprint” which is used for identification purposes. The kit has been useful in identification of numerous phytopathogenic *Xanthomonas* species (Verniere et al., 1993).

Thus, the major objectives of this study have been the assessment the phenotypic diversity of some *Xanthomonas* strains from onion blight and determination of their relationship to some type *Xanthomonas* species and pathovars.

**MATERIALS AND METHODS**

**Isolation of *Xanthomonas***

Blight affected onion leaves were collected from fields, brought to the lab and were immersed in 0.5% sodium hypochlorite for 5 mins. Small chlorotic water-soaked sections were removed, rinsed in sterile distilled water, and were ground in 0.2ml sterile phosphate buffered saline (0.01M) using a mortar and pestle. 100µL of the resulting solution were then spread on yeast dextrose calcium carbonate (YDC) agar. Yellow mucoid colonies, characteristic of xanthomonads, were re-streaked to obtain single colonies that were subsequently grown on yeast glycerol agar.

**Biochemical testing**

Gram staining, catalase test and starch hydrolysis test were assayed as described by Verniere et al., (1991).

**Biolog testing**

Bacterial cultures were streaked onto trypticase soy broth agar and incubated at 26ºC for 24 to 48hrs. Biolog GN microplates (Biolog, Inc., Hayward, Calif) were inoculated as specified by the manufacturer, incubated at 28ºC for 18 to 24hrs and then read visually. All strains were tested in duplicates.

**Pattern analysis**

Reactions in wells were scored visually as “+” for purple colour (indicating utilisation of carbon substrate), “-” for colourless wells and “+/-” for wells with a pale purple (indicating partial use of carbon substrate) in reference to the negative control well (A1) containing no carbon source. Scores were assembled into a binary matrix in which fingerprint patterns of a few representative *Xanthomonas* species and pathovars were included. The matrix was used to estimate Nei and Li’s (1979) genetic distance coefficients (GD<sub>NL</sub>) among the various isolates. Relationships among isolates were determined by subjecting GD<sub>NL</sub> values to cluster analyses using unweighted pair group method with arithmetic averages (UPGMA) of PAUP* software version 4.0 (Swofford, 2000) to produce a dendrogram.

**RESULTS**

Yellow, mucoid, round and convex colonies, characteristic of xanthomonads were isolated form different leaf samples. Isolates were Gram negative, produced catalase and hydrolysed starch. Results of these biochemical and physiological tests strongly indicated that isolates belonged to the *Xanthomonas* genus. Significant variation in metabolic profiles was observed between local isolates and the included type *Xanthomonas* species and pathovars. In most cases, metabolic fingerprint profiles of the isolates were polymorphic enough to allow distinction between them. The metabolic profiles of some of the isolates are shown in **Plate 1** below. The isolates exhibited heterogeneity in their carbon utilisation profiles.
Phenotypic diversity of *Xanthomonas* strains isolated from onion blight using metabolic fingerprinting. P Nowbuth et al.

**Plate 1** Biolog microplates showing the metabolic fingerprint profiles of some local *Xanthomonas* strains isolated from onion blight.

All 15 *Xanthomonas* isolates used the following carbon sources in the Biolog plate: N-acetyl-D-glucosamine, cellobiose, D-fructose, gentiobiose, α-D-glucose, D-mannose, D-trehalose, methylpyruvate, cis-aconitic acid, citric acid, D-galacturonic acid, D-glucuronate acid, D-saccharic acid, succinic acid, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, L-serine, glycerol. However, none used the following carbon sources: α-cyclodextrin, N-acetyl-D-galactosamine, adonitol, i-erythritol, turanose, xylitol, γ-hydroxybutyric acid, p-hydroxyphenylactic acid, α-ketovaleric acid, sebacic acid, L-phenylalanine, 2,3-butanediol.

The GD$_{NL}$ coefficients are represented in Table 1. Genetic distance coefficients varied from 0.00 to 0.12 with a mean distance of 0.04. This significantly low mean GD$_{NL}$ indicated that local isolates were closely related to the type *Xanthomonas* species and pathovars. GD$_{NL}$ value of 0.00 was observed between isolates 3 and 15 suggesting a probable clonal relationship. Similar values were obtained for isolate pair 4 and 5. With a GD$_{NL}$ coefficient of 0.117, *X.c pv. citri* and isolate 7 constituted the most distant pair.

All the isolates were found to cluster separately from the type *Xanthomonas* species and pathovars as shown in the dendrogram in Figure 1. Isolate 11 was found to be the most distinct strain from the remaining isolates. Low homogeneity was seen among local *Xanthomonas* strains since they were grouped into several individual sub-clusters. None of the local *Xanthomonas* strain grouped with *X.c. pv. allii*.

Phenotypic diversity of *Xanthomonas* strains isolated from onion blight using metabolic fingerprinting.

Table 1 GD$_{NL}$ coefficients for 20 *Xanthomonas* strains using PAUP 4.0b

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<td>0.05</td>
<td>0.03</td>
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</tr>
<tr>
<td>19</td>
<td>X.arboricola</td>
<td>0.00</td>
<td>0.01</td>
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<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.06</td>
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<tr>
<td>20</td>
<td>X.axonopodis.pv.</td>
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<td>0.01</td>
<td>0.06</td>
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<td>0.08</td>
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<td>0.05</td>
<td>0.05</td>
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</tbody>
</table>

Highest GD$_{NL}$ value of 0.12 was between isolate 7 and X.c. pv. citri.

Lowest GD$_{NL}$ value of 0.00 was between the following pairs of *Xanthomonas* strains: X. arboricola and X.c. pv.armoracia, isolates 3 and 15, isolates 4 and 5, X. axonopodis pv. allii and X. arboricola, isolates 1 & 5, isolates 1 & 7, isolates 1 & 9, isolates 3 & 4, isolates 4 & 6, isolates 4 & 7, isolates 5 & 7, isolates 5 & 9 and isolates 7 & 9.
Phenotypic diversity of *Xanthomonas* strains isolated from onion blight using metabolic fingerprinting. P Niewbuth et al.

**Figure 1** Dendrogram showing the phenotypic relationship among the 15 isolates with various *Xanthomonas* species and pathovars based on cluster analysis of Biolog GN Microplate substrate utilisation patterns. It was obtained by subjecting the GD\_NL indices to UPGMA cluster analysis using PAUP* software version 4.0 (Swofford 2000).

**DISCUSSION**

Biolog substrate utilisation profiles revealed relatively close phenotypic relationship among the local isolates. Since strains with similar metabolic profiles are most often epidemiologically related, our results suggested that local isolates may form part of a fairly close group of *Xanthomonas* having similar host range and causing similar disease phenotype. However, it has been reported that a single disease phenotype, which in this case is blight, can be caused by *Xanthomonas* belonging to more than one genomospecies (Jones et al., 2000). Roumagnac et al., (2004) associated bacterial strains of onion blight disease to the *Xanthomonas campestris* core based on carbon utilisation profiles. However, using DNA based techniques such as DNA-DNA hybridisation, fluorescent AFLPs, 16S rDNA gene sequence diversity and genome fingerprinting, Roumagnac et al., (2004) and Gent et al., (2004) independently proposed to classify onion blight strain as *Xanthomonas axonopodis* pv. *allii*.

In this study, local isolates were found to be clearly distinct from *X.axonopodis. pv. allii* based on carbon substrate metabolism patterns. As the host range and pathogenicity of the various local isolates were not determined, the isolate(s) responsible for causing bacterial blight in onion remained unclear. Additionally, since many foliar bacterial pathogens were shown to colonise plant surfaces prior to infection (Hirano and Upper, 1983), the possibility that these local isolates could represent a subset of the natural epiphytic *Xanthomonas* population colonising onion leaf surfaces. Verniere et al.,
Phenotypic diversity of Xanthomonas strains isolated from onion blight using metabolic fingerprinting. P Nowbuth et al.

(1993) indicated that Xanthomonas campestris strains from the Mascarene Islands were atypical with substantially variable metabolic profiles. The reasons for the apparent high variation were unknown but most probably involved factors such as climate, onion cultivars and production practices leading to the selection of unique phenotypes. Our study also supported the view that a wide diversity of xanthomonads was associated with onion blight based on carbon source utilisation profiles.

Knowledge of the nutritional requirements of pathogenic xanthomonads can be used to devise biological control strategies. Pre-emptive competitive exclusion of phytopathogen due to prior utilisation of limiting nutritional resources by non-pathogenic strains has been shown to considerably reduce pathogen population sizes and subsequently disease severity and incidence (Ji and Wilson, 2002).

Correlation between relationships obtained with DNA based methods may prove to be helpful in determining the exact identities of these local isolates. Knowledge on the phenotypic diversity of Xanthomonas strains would be very useful in future epidemiological studies involving onion blight disease.

CONCLUSION

Biolog technique appeared useful in the routine characterisation of diversity among bacterial strains provided that data are carefully interpreted (Smalla et al., 1998). Metabolic variations between Xanthomonas isolates and type strains indicated the latter to represent diverse pathotypes. Further investigations on these isolates are necessary to identify their exact species and pathovar status.

ACKNOWLEDGEMENTS

We are thankful to the Tertiary Education Commission for the scholarships awarded to Mr. Prakash Nowbuth and Mrs. Shadila Venkatasamy.

REFERENCES


MAS 2005. Food and Agricultural Research Council, Réduit, Mauritius. 380
Phenotypic diversity of Xanthomonas strains isolated from onion blight using metabolic fingerprinting. P Nowbuth et al.


APPENDIX I

Composition of YGA:
5.0g yeast extract, 1.0 g K_2HPO_4, 0.5g MgSO_4, 20g glycerol, 20.0 g agar and distilled water to 1.0 L.

Composition of YDC medium:
10.0 g yeast extract, 20.0g dextrose, 20.0 g calcium carbonate, 17.0 g agar, water to 1.0 L.
RECENT AGRONOMIC ACHIEVEMENTS ON TOBACCO RESEARCH IN MAURITIUS

Y. Cadersa
Agricultural Research and Extension Unit

ABSTRACT

In its drive to meet the needs of the tobacco industry, research has developed a coordinated approach to optimise tobacco production in an environmentally sustainable manner. This paper highlights the main research achievements on tobacco during the recent years. Nine field experiments were conducted from 1995 to 2002 to evaluate and screen eight imported flue-cured tobacco varieties for higher yield, quality and resistance to major diseases. In 1999 and 2003 respectively, varieties RG13 and K326 were recommended to tobacco growers on basis of their higher cured leaf yields and grade indices. A planting date trial was conducted in 2000 to investigate the effect of three planting dates; 10th February, 20th March and 15th May on the performance of the air-cured variety Amarello. Results indicated that cured leaf yield (2,398 kg ha⁻¹) was significantly higher when Amarello was transplanted on 20th March. The lowest cured leaf yield (968 kg ha⁻¹) was obtained at the last planting date. The effectiveness of coconut oil, at three concentrations (7%, 14% and 21%) was compared with the commonly-used desuckercide butralin (Tabamex) to control axillary bud growth in flue-cured tobacco. Coconut oil at 14% was as effective as butralin in reducing the average number of suckers / plant (3.0 v/s 3.1) and the length of suckers / plant (0.96cm v/s 1.27cm) respectively. Coconut oil can effectively replace butralin by virtue of its environment friendly nature and low cost.

Keywords: Tobacco, flue-cured, cured leaf yield, season.

INTRODUCTION

In Mauritius, two types of tobacco (Nicotiana tabacum L.) are produced; namely Virginia flue-cured and Amarello air-cured. The sub-tropical climate allows the production of two crops of Virginia flue-cured annually with about 200 tonnes in the first season starting in February and about 400 tonnes in the second season starting in August. The latter crop is usually cultivated on rotational sugar cane lands. An air-cured crop of about 100 tonnes is also produced from March to September. Currently, about 400 hectares of tobacco are cultivated annually representing about 0.5% of the total cultivated land. Some 800 tonnes of leaf goes into manufacture annually (Anon, 2002).

Research on tobacco started in 1939 when the government created a Tobacco Research Station under the aegis of the Ministry of Agriculture (Anon, 1974). From thereon, research was geared towards the screening of better performing introduced varieties and the improvement of cultural practices. In 1995, the Agricultural Research and Extension Unit (AREU) was mandated to carry out research on tobacco and till now, it is providing its unflinching support to the tobacco farmers through the development and promotion of “best practices” to achieve efficiency of operations, cost effectiveness and cost reduction. This paper presents the main achievements on tobacco research during the recent years.

MATERIALS AND METHODS

Performance Testing and Varietal Improvement

After release from a seed-to-seed quarantine in 1995 and 1996, eight flue-cured tobacco varieties imported from the United States namely RG11, RG13, K326, K346, K394, Coker 176, NC 567 and Speight G126 were tested mainly on basis of their agronomic performance and quality aspects. In the first (February – July) and second seasons (August – December) of 1995, the varieties RG11 and RG13 were compared with the three commercially grown varieties NC95, RES85 and Speight G28 on a Low Humic Latosol (LHL) at the Richelieu Experiment Station (RES). From 1996 to 1998, all eight varieties were evaluated and screened in 3 trials at RES. Subsequently, in 1999, 2000 and 2002, the
Recent Agronomic Achievements On Tobacco Research In Mauritius. Y Cadets.

variety K326 was further evaluated in 4 trials. From 1995 to 2002, all trials were laid down in a Randomized Complete Block design (RCB). Details of the experimental trials conducted from 1995 to 2002 are presented in Table 1.

**Table 1** Details of experimental trials conducted from 1995 to 2002

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td>RES</td>
<td>RES</td>
<td>RES</td>
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<td>RES</td>
<td>Forbach</td>
<td>Ile D'Ambre</td>
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<td>Soil type*</td>
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<td>LHL</td>
<td>LHL</td>
<td>LHL</td>
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<tr>
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<td>66</td>
<td>66</td>
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<td>18</td>
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<td>Sub-humid</td>
<td>Sub-humid</td>
<td>Sub-humid</td>
<td>Sub-humid</td>
<td>Humid</td>
<td>Humid</td>
<td></td>
</tr>
<tr>
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<td>First</td>
<td>Second</td>
<td>First</td>
<td>First</td>
<td>Second</td>
<td>First</td>
<td>First</td>
</tr>
<tr>
<td>Design</td>
<td>RCB</td>
<td>RCB</td>
<td>RCB</td>
<td>RCB</td>
<td>RCB</td>
<td>RCB</td>
<td>RCB</td>
<td>RCB</td>
</tr>
<tr>
<td>No. of replicates</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Varieties tested</td>
<td>K394, K326, SPG126, RG11, RG13, NC95, RES85, Coker 176, NC 567, K346</td>
<td>K394, K326, SPG126, RG11, RG13, NC95, RES85, Coker 176, NC 567, K346</td>
<td>K394, K326, SPG126, RG11, RG13, NC95, RES85, Coker 176, NC 567, K346</td>
<td>NC95, K326, Coker 176</td>
<td>K326, RG13, K326, RG13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Source: (Padya, 1989) LRP: Latosolic Reddish Prairie RCB: Randomised Complete Block RES: Richelieu Experiment Station LHL: Low Humic Latosol

**Effect of date of planting on the performance of the air-cured variety Amarello**

The effect of three planting dates viz 10th February (first planting date), 20th March (second planting date) and 15th May (third planting date) on the growth and yield performance of the air-cured variety Amarello was studied at Richelieu Experiment Station in the crop season of 2000. The trial was laid down in a RCB design with 3 replicates.

**Effect of coconut oil on sucker control in Virginia flue-cured tobacco**

The effectiveness of 3 concentrations of coconut oil (7%, 14% and 21%) was compared with the commonly used desuckercide Butralin (Tabamex) at 1.5% for the control of axillary buds (suckers) in the flue-cured variety RG13. The trial was conducted in the first season of 1999 at île d’Ambre and Sans Souci in the humid and super-humid zones respectively. The design for each trial was similar to Experiment II.

In all three experiments (I, II and III), cultural practices such as weeding, fertilization, earthing up, pest and disease control, irrigation, topping and desuckering were carried out according to recommended practices for commercial plantations (Anon, 1990). A plot size of 27m² consisting of 5 rows of 6.0m long was used. Data on the growth and yield components were recorded on the inner 3 rows of 16.2m². After analysis of variance, treatments were compared with Least Significance Differences (LSD) or the Duncan Multiple Range Test (DMRT) whenever the number of treatments exceeded five.
RESULTS AND DISCUSSION

Performance Testing and Varietal Improvement

In 1995, although differences in growth and yield parameters among the five flue-cured varieties were not significant except for plant height, RG13 gave relatively higher green and cured leaf yields and scored a higher grade index (Table 2). This was attributed to its heavy leaf body, which is a desirable characteristic in cigarette manufacture.

From 1996 to 1998, both K326 and RG13 significantly outyielded NC95, but when averaged over three trials, differences in growth and yield parameters were not significant between the varieties which might be due to yield variations between the trials. However, K326 and RG13 gave relatively higher cured leaf yields and grade indices than NC95 (Table 3). In addition, both varieties were only slightly affected by weather fleck as compared to RG11 and K394, which were severely affected. NC95 was only moderately affected (data not presented). Based on the relatively high yield of RG13 in the five trials from 1995 to 1998, it was released for commercial growing as from the first season of 1999 while K326 was further evaluated. The growth and yield parameters of K326 were comparable to those of RG13 (Table 4) thus confirming its high yield potential as reported by Bowman and Glenn Tart (1997). Quality wise, the leaf chloride content of K326 was significantly lower and this may be attributed to its smaller midrib as compared to the fat and prominent midrib in RG13. Such low leaf chloride content enhances the fire-holding capacity of cigarettes. K326 was subsequently released as from 2003 for commercial growing as an additional variety to RG13.

Table 2  Growth and yield characteristics of five flue-cured varieties in 1995 (mean of 2 trials)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Grade index (Rskg⁻¹)</th>
<th>Cured leaf yield (kg ha⁻¹)</th>
<th>Green leaf yield (kg ha⁻¹)</th>
<th>Plant height at topping (cm)</th>
<th>Leaf number/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG11</td>
<td>54.3</td>
<td>2224</td>
<td>20376</td>
<td>103.6 a</td>
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<tr>
<td>RG13</td>
<td>56.9</td>
<td>2608</td>
<td>22227</td>
<td>97.5 ab</td>
<td>20.6</td>
</tr>
<tr>
<td>RES85</td>
<td>54.6</td>
<td>2213</td>
<td>21070</td>
<td>107.5 a</td>
<td>21.3</td>
</tr>
<tr>
<td>SPG28</td>
<td>52.1</td>
<td>2084</td>
<td>20803</td>
<td>90.7 bc</td>
<td>20.6</td>
</tr>
<tr>
<td>NC95</td>
<td>55.3</td>
<td>2009</td>
<td>18067</td>
<td>105.3 a</td>
<td>21.0</td>
</tr>
<tr>
<td>SEm +</td>
<td>3.7</td>
<td>296.1</td>
<td>1865.7</td>
<td>4.0</td>
<td>0.5</td>
</tr>
<tr>
<td>P(=0.05)</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>s</td>
<td>n.s</td>
</tr>
<tr>
<td>cv (%)</td>
<td>6.8</td>
<td>13.2</td>
<td>9.0</td>
<td>4.0</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Means followed by a different letter are significantly different by LSD test

Table 3  Growth and yield characteristics of 8 flue-cured varieties tested from 1996 to 1998 (mean of 3 trials)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Grade index (Rskg⁻¹)</th>
<th>Cured leaf yield (kg ha⁻¹)</th>
<th>Green leaf yield (kg ha⁻¹)</th>
<th>Plant height at topping (cm)</th>
<th>Leaf number/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>K326</td>
<td>63.7</td>
<td>2219</td>
<td>18200</td>
<td>108.2</td>
<td>20.9</td>
</tr>
<tr>
<td>RG13</td>
<td>63.6</td>
<td>2137</td>
<td>17217</td>
<td>103.1</td>
<td>19.6</td>
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<tr>
<td>SPG126</td>
<td>62.3</td>
<td>2064</td>
<td>15932</td>
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<td>20.3</td>
</tr>
<tr>
<td>K 394</td>
<td>61.3</td>
<td>1892</td>
<td>14212</td>
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<tr>
<td>RG11</td>
<td>62.5</td>
<td>2000</td>
<td>16348</td>
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<td>C176</td>
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<td>15874</td>
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<tr>
<td>N95</td>
<td>59.6</td>
<td>1715</td>
<td>13090</td>
<td>111.1</td>
<td>20.1</td>
</tr>
<tr>
<td>NC567</td>
<td>60.1</td>
<td>1846</td>
<td>13889</td>
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<td>20.4</td>
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<tr>
<td>K346</td>
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<td>1757</td>
<td>15461</td>
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</tr>
<tr>
<td>SEm +</td>
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<td>171.2</td>
<td>1891.4</td>
<td>4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>P(=0.05)</td>
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<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>cv (%)</td>
<td>3.0</td>
<td>8.8</td>
<td>12.2</td>
<td>4.2</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Recent Agronomic Achievements On Tobacco Research In Mauritius. Y Cadersa.

### Table 4
Comparative growth and yield characteristics of flue-cured varieties K326 and RG13 tested in 1999, 2000 and 2002 (mean of 4 trials)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Cured leaf yield (kg ha⁻¹)</th>
<th>Green leaf yield (kg ha⁻¹)</th>
<th>Leaf chloride (%)</th>
<th>Plant height at topping (cm)</th>
<th>Leaf number/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>K326</td>
<td>2229</td>
<td>20499</td>
<td>1.93²</td>
<td>110.2</td>
<td>21.0</td>
</tr>
<tr>
<td>RG13</td>
<td>2261</td>
<td>19662</td>
<td>2.78²</td>
<td>107.8</td>
<td>20.3</td>
</tr>
<tr>
<td>SEm +</td>
<td>75.9</td>
<td>512.4</td>
<td>0.078</td>
<td>3.7</td>
<td>0.9</td>
</tr>
<tr>
<td>P(=0.05)</td>
<td>n.s</td>
<td>n.s</td>
<td>s</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>cv (%)</td>
<td>4.8</td>
<td>14.4</td>
<td>1.3</td>
<td>4.8</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Means followed by a different letter are significantly different by LSD test.

### Effect of date of planting on the performance of the air-cured variety Amarello

Leaf number / plant, plant height, green and cured leaf yields were significantly higher for the second planting date. For the third planting date, growth was stunted as indicated by the reduced leaf number and plant height followed by a drastic decrease in yield characteristics (Table 5).

This may be attributed to the low temperature (21 – 23°C) prevailing during the months of June to August, which coincided, with the active growth period (4 – 6 weeks after transplantation) of the Amarello crop (Figure 1). Furthermore, the crop cycle was also extended. This result agrees with that of Akehurst (1981) where yield and quality of light air-cured tobacco were reduced by delaying planting in the winter season.

### Table 5
Effect of date of planting on the growth and yield characteristics of the air-cured variety Amarello

<table>
<thead>
<tr>
<th>Date of Planting (Year 2000)</th>
<th>Cured leaf yield (kg ha⁻¹)</th>
<th>Green leaf yield (kg ha⁻¹)</th>
<th>Leaf number/plant</th>
<th>Plant height at topping (cm)</th>
<th>Crop cycle (from transplanting to 1st harvest) (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 February</td>
<td>1679²</td>
<td>17180²</td>
<td>20.9²</td>
<td>98.8⁰</td>
<td>94.0</td>
</tr>
<tr>
<td>20 March</td>
<td>2398²</td>
<td>21738²</td>
<td>34.6²</td>
<td>135.6⁰</td>
<td>90.0</td>
</tr>
<tr>
<td>15 May</td>
<td>968⁰</td>
<td>10931⁰</td>
<td>21.5⁰</td>
<td>88.0⁰</td>
<td>105.0</td>
</tr>
<tr>
<td>SEm +</td>
<td>15.4</td>
<td>42.5</td>
<td>1.61</td>
<td>2.8</td>
<td>10.8</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td>42.8</td>
<td>118.0</td>
<td>4.5</td>
<td>7.8</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Means followed by a different letter are significantly different by LSD test.

### Figure 1
Mean temperature variation (°C) in relation to date of planting (months) for the air-cured variety Amarello

---

Effect of coconut oil on sucker control in Virginia flue-cured tobacco

The number of suckers/plant was significantly reduced when coconut oil was applied at 21% while leaf number/plant was significantly higher in the control (Butralin). A reduction in both green and cured leaf yields at 21% coconut oil was also observed. This may be due to its high concentration thus causing leaf breakage at the point of attachment of the petiole to the stem. However, at 14% coconut oil, the number of suckers/plant, green and cured leaf yields were at par with Butralin (Table 6).

Table 6  Effect of 3 rates of coconut oil on growth and yield characteristics of flue- cured variety RG13 at Ile d'Ambre

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of suckers/plant</th>
<th>Leaf number/plant</th>
<th>Cured leaf yield (kg ha(^{-1}))</th>
<th>Green leaf yield (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butralin @ 1.5%</td>
<td>3.1(^a)</td>
<td>20.4(^a)</td>
<td>2320(^a)</td>
<td>15710</td>
</tr>
<tr>
<td>Coconut oil @ 7%</td>
<td>3.4(^a)</td>
<td>17.9(^b)</td>
<td>1874(^a)</td>
<td>14748</td>
</tr>
<tr>
<td>Coconut oil @ 14%</td>
<td>3.0(^a)</td>
<td>18.5(^b)</td>
<td>2192(^a)</td>
<td>16513</td>
</tr>
<tr>
<td>Coconut oil @ 21%</td>
<td>2.5(^b)</td>
<td>18.0(^b)</td>
<td>1610(^b)</td>
<td>12438</td>
</tr>
<tr>
<td>SEm ±</td>
<td>0.18</td>
<td>0.45</td>
<td>217.8</td>
<td>1923.6</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td>0.44</td>
<td>1.10</td>
<td>533.0</td>
<td>4707.1</td>
</tr>
</tbody>
</table>

Means followed by a different letter are significantly different by LSD test.

On a cost comparative basis, 14% coconut oil was cheaper than either Butralin or Maleic Hydrazide (Table 7).

Table 7  Cost comparison between coconut oil and commonly used chemical desuckercides

<table>
<thead>
<tr>
<th>Desuckercide</th>
<th>Concentration (%)</th>
<th>Amount (litres ha(^{-1}))</th>
<th>Cost (Rs ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butralin</td>
<td>1.5</td>
<td>5</td>
<td>1800</td>
</tr>
<tr>
<td>Maleic Hydrazide</td>
<td>1.5</td>
<td>5</td>
<td>2500</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>14</td>
<td>35</td>
<td>1400</td>
</tr>
</tbody>
</table>

CONCLUSION

Research conducted from 1995 to 2002 has led to the release of two high yielding flue-cured tobacco varieties, namely RG13 and K326 for commercial growing. A fast adoption rate has been recorded and currently, around 90-95% of the domestic flue-cured tobacco is cultivated with both varieties in both seasons. It has also been shown that delaying planting of the air-cured variety Amarello until the winter season reduced yield drastically and that coconut oil is a good alternative to chemical desuckercides in terms of its effectiveness on sucker growth, lower cost and environment friendly nature.

ACKNOWLEDGEMENT

Thanks are expressed to the Richelieu Experiment Station, the Tobacco Inspectors of the Tobacco Board and the tobacco planters for their help in conducting the experiments. Mr. P. Hanoomanjee is gratefully acknowledged for lending results of 1995 and 1996 trials on varietal screening.
REFERENCES


EVALUATION OF A RATION FOR RUMINANTS USING
LOCALLY AVAILABLE BY-PRODUCTS.

G. Saraye
Agricultural Research and Extension Unit

ABSTRACT

Sugar cane bagasse was used with other locally available ingredients to constitute a complete ration for ruminants. The intake of the ration and the liveweight gain of weaners were determined. 19 weaners were used in the experiment which was conducted in 2 phases. The first phase lasted for 94 days after which the treatment and the control group were inversed. The second phase lasted for 104 days. The results indicate that the bagasse based ration has a good nutritive value. In the first phase animals in the treatment group had an average weight gain of 521g/day compared to 332g/day in the control group. The difference was significant (p<0.5). Whereas in the second phase, animals in the treatment group had a weight gain of 753g/day, compared to 579g/day in the control group. The difference was again significant (p<0.5). It is concluded that the bagasse based ration has a good nutritive value and can be used as roughage for ruminants in period of scarcity.

Keywords: bagasse, intake, liveweight gain, and complete ration.

INTRODUCTION

In Mauritius livestock regularly face a shortage of roughage and more acutely during periods of drought. This experiment was initiated in the quest to find a suitable alternative to fodder shortage using locally available resources. A new technique of using sugar cane bagasse in a complete ration for ruminants is proposed.

A preliminary laboratory study showed that the bagasse based ration (see Table 1) had a dry matter content of about 95%, crude protein 20% and crude fibre 18%. The in-vivo dry matter degradability was 70 % (48 hours). No significant changes in colour, odour, pH and chemical composition were observed after different periods of storage time of 30,60 and 90 days in closed plastic bags.

OBJECTIVE

The objective of the study was to assess palatability and intake of the bagasse based ration and determine the liveweight gain of weaners.

MATERIALS AND METHODS

19 weaners of around 104 kg liveweight were used to evaluate the bagasse based feed. The animals were allocated to the treatment (10) and control (9) groups randomly. Animals in the treatment group were fed only on the bagasse-based ration ad libitum. Those in the control group were fed on mixed fodder and or sugar cane tops ad libitum together with 1 kg of cowfeed (locally compounded concentrate with crude protein of 14.5%). Fresh water was available at all times. Details of the ingredients composing the bagasse based feed are shown in Table 1.
Evaluation of a ration for ruminants using locally available by-products. *G Saraye*

**Table 1** Composition of ingredients in the bagasse-based ration

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagasse</td>
<td>35.0</td>
</tr>
<tr>
<td>Molasses</td>
<td>35.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>16.0</td>
</tr>
<tr>
<td>Cotton seed cake</td>
<td>10.0</td>
</tr>
<tr>
<td>Common salt</td>
<td>0.5</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>0.5</td>
</tr>
<tr>
<td>Urea</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The liveweight was recorded monthly and feed intake was monitored daily. Samples of all feeds were periodically analysed for crude protein, crude fibre, dry matter, ether extract and ash. The feeding trial was conducted in two stages. The first stage lasted for 94 days after which animals in the control and treatment groups were inversed. The second stage lasted for 104 days. It started after a transition period of 24 days.

**RESULTS**

The animals were in good health throughout the trial. The composition of the feed was fairly uniform during the trial. The chemical analysis of the feeds used during the experiment is given in **Table 2**, based on dry matter basis.

**Table 2** Analysis of feed.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Dry matter</th>
<th>Crude protein</th>
<th>Crude fibre</th>
<th>Ether extract</th>
<th>Ash</th>
<th>Energy value MJ /Kg DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagasse feed</td>
<td>76.7 ± 5.6*</td>
<td>18.8 ± 3.5</td>
<td>19.6 ± 4.0</td>
<td>1.3 ± 0.6</td>
<td>9.11 ± 0.7</td>
<td>17.6</td>
</tr>
<tr>
<td>Cane tops</td>
<td>25.2 ± 2.3</td>
<td>6.3 ± 3.5</td>
<td>33.5 ± 4.2</td>
<td>1.35 ± 1.1</td>
<td>8.81 ± 1.7</td>
<td>17.3</td>
</tr>
<tr>
<td>Elephant grass</td>
<td>25.2 ± 1.4</td>
<td>10.6 ± 2.0</td>
<td>38.6 ± 1.3</td>
<td>1.8 ± 0.3</td>
<td>8.8 ± 1.0</td>
<td>17.6</td>
</tr>
<tr>
<td>Stargrass</td>
<td>24.8 ± 2.5</td>
<td>11.3 ± 1.5</td>
<td>35.4 ± 6.5</td>
<td>1.5 ± 0.3</td>
<td>6.8 ± 1.0</td>
<td>17.6</td>
</tr>
<tr>
<td>Cowfeed</td>
<td>85 ± 1.7</td>
<td>14.5 ± 1.1</td>
<td>6.1 ± 0.8</td>
<td>1.5 ± 0.6</td>
<td>9.4 ± 1.2</td>
<td>18.0</td>
</tr>
</tbody>
</table>

*Mean ±SD

The average daily voluntary feed intake is shown in **Table 3**.

**Table 3** Voluntary feed intake (Kg) of fresh matter

<table>
<thead>
<tr>
<th></th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>4 month</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>5.3 ± 1.0*</td>
<td>7.8 ± 1.0</td>
<td>8.3 ± 0.6</td>
<td>-</td>
<td>7.1 ± 1.0</td>
</tr>
<tr>
<td>Control</td>
<td>7.8 ± 1.1</td>
<td>10 ± 1.1</td>
<td>10.5 ± 0.5</td>
<td>-</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td><strong>Second phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>11 ± 0.7</td>
<td>11.3 ± 0.2</td>
<td>11.7 ± 0.2</td>
<td>12.7 ± 0.9</td>
<td>11.6 ± 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>14.8 ± 2.5</td>
<td>16.6 ± 1.0</td>
<td>18.5 ± 1.0</td>
<td>18.9 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± standard deviation
In the first phase the animals in treatment group had an average daily intake of about 7 Kg (DM-5.3Kg) of bagasse-based feed and those in control group consumed about 9kg (DM-2.3Kg) of fodder. During the second phase the treatment group had an average daily intake of about 12 Kg (DM-9Kg) of bagasse-based feed and the control group consumed about 17 kg (DM-4.3Kg) of fodder.

The average daily weight gain is shown in Table 4.

Table 4 Average daily weight gain

<table>
<thead>
<tr>
<th></th>
<th>Initial Live weight (Kg)</th>
<th>Final Live weight (Kg)</th>
<th>Average Daily gain (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First phase</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>100 ± 12*</td>
<td>142 ± 23</td>
<td>521 ± 187</td>
</tr>
<tr>
<td>Control</td>
<td>109 ± 13</td>
<td>133 ± 25</td>
<td>332 ± 120</td>
</tr>
<tr>
<td><strong>Second phase</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>147 ± 24</td>
<td>225 ± 31</td>
<td>753 ± 86</td>
</tr>
<tr>
<td>Control</td>
<td>156 ± 22</td>
<td>216 ± 28</td>
<td>579 ± 119</td>
</tr>
</tbody>
</table>

In the first phase animals in the treatment group had an average weight gain of 521g/day compared to 332g/day in the control group. The difference was significant (p<0.5). Whereas in the second phase, animals in the treatment group had a weight gain of 753g/day, compared to 579g/day in the control group. The difference was again significant (p<0.5).

DISCUSSION

The results indicate that incorporating bagasse in a mixed ration increases its palatability as evident from the voluntary intake of the ration. Hassoun et al. (1990) and Khan et al. (1992) obtained similar results with feed containing sugar cane bagasse. The inclusion of 3 % urea in the feed has been beneficial as it enhances rumen fermentation by providing a non-protein nitrogen source and maintains the rumen ammonia level to the optimum (Owen and Jayasuriya, 1989). It also increased the protein content of the mixture (Table 2).

CONCLUSION

The data show that the bagasse-based ration has a good nutritive value and can be used as roughage for ruminants in periods of scarcity. However the availability of bagasse to small livestock farmers has to be looked into.

ACKNOWLEDGEMENTS

The author gratefully acknowledges all those who have contributed directly or indirectly to the success of the feeding trial.
REFERENCES


HEIFER LIVE WEIGHT AND REPRODUCTIVE PERFORMANCE ON SMALLHOLDER FARMS

P. Toolsee and G. Saraye
Agricultural Research and Extension Unit

ABSTRACT
The study was carried out in 2 phases to determine the age at which heifers were first inseminated at village level, the concentrate-feeding regime of heifers and to estimate the body weight of heifers in the age range of 18 to 24 months. In phase 1 data on 441 first AI’s were collected from veterinary sub-centres for the years 1998-2000. Phase 2 was a survey of 62 farms in 2003 and the body weight was estimated by a standard tape. Results show that the average ages of heifers at which first artificial insemination was done are 25.5, 25.0 and 25.4 months for the North, East and Centre regions respectively. Dairy farmers do give concentrate (cowfeed), as supplement to the basal diet. The average body weight of heifers as estimated by tape was 320 ± 92 kg (n=50) at the age of 20.5 months. The farmers observed first heat signs at the age of 18.2 ± 3.5 months (n = 64). At the age of 20.5 months heifers had already reached their breedable weight. However, first insemination was done around 21.5 months and the fall of temporary incisors is no longer used as a guide to decide when to inseminate the heifers.

Keywords: Heifers, bodyweight, supplementation, first insemination, increased income.

INTRODUCTION
Breeding replacement heifers is an important operation in a dairy farm. A traditional practice adopted by smallholder dairy farmers in Mauritius was that heifers were inseminated only when the temporary incisors had fallen (le dent tombé), normally occurring at about 24 months of age. This resulted in the heifer calving at about 36 months of age. This contrasts sharply with practices in South Africa where recommendations for calving age are 24-36 months (Eramus, 2000). Therefore efforts are necessary to reduce the age at which heifers calve to < 30months. This would result in increased revenue for the farmers (Toolsee, 2003).

OBJECTIVES
To determine the age at which heifers were first inseminated at village level as per records and the concentrate feeding regime of heifers from 3 months of age until insemination; and to estimate the body weight of heifers in the age range of 18 to 24 months.

PHASE 1: Age of heifers inseminated for the first time as per records at Veterinary sub-centres

MATERIALS AND METHODS
Data were collected for years 1998 to 2000 from records kept at the veterinary sub-centres- Abercombie (North region), Flacq (East region) and St Pierre (Centre and West regions). Under the project Milk Productivity Bonus Scheme (MPBS) (1994-2000), for each calf born the farmer received a cash bonus of Rs 500 provided he had registered with the Division of Veterinary Services. The objective of the MPBS was to decrease calving interval and increase milk production. At the time of registration the farmer’s address, cow data (tag number) and calf data (sex, date of birth) were recorded. At the time of artificial insemination (AI), a receipt was given to the farmer and the date of AI was recorded in the AI book kept in each veterinary sub-centre. Data on 441 AI’s done to heifers during the years 1998 to 2000 were retrieved from records.
RESULTS AND DISCUSSION

The average ages of heifers at which first artificial insemination was done are 25.5, 25.0 and 25.4 months for the North, East and Centre regions respectively, as shown in Table 1.

Table 1 Average age of heifer (months) at first artificial insemination

<table>
<thead>
<tr>
<th>Region</th>
<th>North</th>
<th>East</th>
<th>Centre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifer age at first AI</td>
<td>25.5 ± 5.2* (174)</td>
<td>25.0 ± 5.5 (181)</td>
<td>25.4 ± 5.3 (86)</td>
</tr>
</tbody>
</table>

* = Mean ±SD
Parenthesis: Number of observations

Table 2 Cumulative percentage – age of heifers at first insemination

<table>
<thead>
<tr>
<th>Regions Age at first AI</th>
<th>North</th>
<th>East</th>
<th>Centre</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 months</td>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>&lt;=20 months</td>
<td>15</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>&lt;=22 months</td>
<td>26</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>&lt;=24 months</td>
<td>42</td>
<td>51</td>
<td>45</td>
</tr>
<tr>
<td>&lt;=25 months</td>
<td>55</td>
<td>62</td>
<td>50</td>
</tr>
<tr>
<td>&gt;25 months</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

During the period 18 to 24 months of age, only 42, 51 and 45% of heifers were inseminated in the North, East and Centre regions respectively. A very low percentage of heifers (around 30%) were inseminated by 22 months whereas the figure increased to about 55% by 25 months.

The lifetime productivity of a cow is influenced by age at puberty, age at first calving and subsequently calving intervals. Factors affecting the age at first artificial insemination are nutrition, genotype, management, environmental factors and health status (Urgate, J, 1999). Major factors controlling the onset of puberty are growth rate and bodyweight rather than age (Morton, J. 1997). It was difficult to identify which were the factors affecting the age at first artificial insemination of heifers as no records (body weight, body condition score, health status) were available. It appeared that under smallholder management conditions, heifers received little attention in terms of nutrition and management, resulting in late age at puberty and therefore delay in first calving. Thus it was proposed to look into the feeding regime and the weight of heifers in the age range of 18 to 22 months at the smallholder level to get more insight in the matter.

PHASE II. On-farm appraisal of heifer live weight and reproductive performance

MATERIALS AND METHODS

A structured questionnaire was designed to collect on-farm data. 62 farms were surveyed in the following regions: North (Triolet, Fond du Sac, St Andre, Point aux Piments, Bois Pignolet, Terre Rouge, Depinay), Centre (Vacoas, St Pierre, Novelle Decouvert), West (Bambous, Cannot, St Martin). The survey started in October 2003 and ended in November 2003. The body weight of heifer was estimated by using a standardised tape placed around the girth.
RESULTS AND DISCUSSION

Dairy farmers do give concentrate (cowfeed), as supplement to the basal diet, to their calves as from 15 days of age up to three months when they are weaned. The amount of concentrate given increased gradually from weaning till the age of 20 months. At 20 months of age, concentrate feeding varied among the farms and it ranged from 0.5 to 5 kg/h/d. The average body weight of heifers as estimated by tape was 320 ± 92 kg (n=50) at the age of 20.5 months. The farmers observed first heat signs at the age of 18.2 ± 3.5 months (n = 64). However the heifers were not inseminated on first observed heat signs. The farmers traditionally preferred to skip at least 2 heats before inseminating the animals. This on-farm study has shown that first insemination was done at the age of 21.4 ± 2.9 months (n= 64). Liveweight is more important than age in determining the onset of oestrus and this study has shown that the critical weight of about 300 kg for heifers to be inseminated was already achieved on smallholder farms by 20 months of age. 95 % of the farmers surveyed no longer waited for the temporary incisors to fall (le dent tombé) before inseminating heifers for the first time. The farmers now pay more attention to the body condition of the animals which is closely related to the body weight.

Results from the first phase show that heifers were 25 months old at first insemination (period 1998 to 2000). However, in 2003 farmers were inseminating their heifers by 20 months of age at the appropriate liveweight. This clearly shows that the farmers are improving their reproductive management.

CONCLUSION

Heifers reach the breedable weight of 320 kg at the age of 20.5 months. First insemination is done around 21.5 months and the fall of temporary incisors is no longer used as a guide to decide when to inseminate the heifers. The age at first AI of heifers has decreased from 25.5 months in the early 2000 to about 21.5 months in 2003. With continuous good management this gain in time will certainly result in an increase in the farmer’s income.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the collaboration of the Division of Veterinary services of the Ministry of Agriculture for access to the records and to the cowkeepers at the village level for having participated in the study.

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SUPPORTING GOAT LIVESTOCK PRODUCTION IN THE ISLAND OF REUNION: WHICH PRODUCT FOR WHICH MARKET?

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CONTEXT

In the French island of Reunion (Indian ocean, 706,300 inhabitants, 37,600 goats) goat is reared mainly for meat production (Figure 1). Two production systems co-exist: numerous smallholders scattered throughout the island and emerging goat units within mixed farms where goat production represents a diversification form and a secondary source of income. Meat consumption is free of any religious taboo and is highly popular among all ethnic groups. Annual consumption ranges between 600 - 800 tons yr⁻¹. 10% only is being supplied by local production mainly by the way of direct farm selling (informal sector). The formal sector (butcher's shops and supermarkets) regularly supplied with frozen and vacuum sealed fresh imported meat and in addition with a little part of local fresh product suffers from this competition.

Figure 1 Rearing of goat for meat production

Since 1997, a goat farmers cooperative has been trying to reorganise local production in order to regularly supply the formal market demand with local product, named cabri pays. Therefore, a study has been initiated in order to better define the existing marketing channels in the formal sector and to identify the types of products the cooperative should offer. An enquiry based on 40 interviews has been implemented within a representative subset of butcher's shops (●) and at least one of every supermarket (○) (Figure 2).
RESULTS AND DISCUSSION

The *cabri pays* is sold mainly by the channel of butcher's stores which get their supply directly from breeders (Table 1). The study revealed the absence of a real commercial strategy. *Cabri pays* remains an appealing product even though it is expensive.

<table>
<thead>
<tr>
<th>Part of enterprises</th>
<th>Butcher's stores</th>
<th>Supermarkets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcasses month-1</td>
<td>70%</td>
<td>17%</td>
</tr>
<tr>
<td>Mean weight</td>
<td>17.3 kg</td>
<td>16.2 kg</td>
</tr>
<tr>
<td>Supply</td>
<td>breeders (ind &amp; coop)</td>
<td>wholesalers</td>
</tr>
</tbody>
</table>

The mean prices of meat for purchase and sale to consumers are respectively 12.2 & 16 € kg⁻¹. In comparison the mean prices for purchase of fresh and frozen imported meat are respectively 8.8 & 4.4 € kg⁻¹.

The standard carcass description differs noticeably between butchers and supermarkets. The ideal carcass that butchers would like to have is described in the Table 2.
Each retailer voices a general complaint about the fluctuating quality of the product. 62% of the butchers would prefer leaner and better-shaped carcasses. In addition to these criterion supermarkets also prefer younger animals. All retailers wish for an increase in the marketable volume to better respond to the demand. 75% of the butchers would be interested in a partnership with farmers to regulate the supply with an even quality home product.

CONCLUSION

The demand for cabri pays meat is high in spite of the large availability of imported products that are cheaper and more adapted to the purchasing power of customers with limited budgets and who generally prefer to buy at the supermarket. The regular clientele of traditional butchers seem willing to pay a surplus for a home-grown product which guarantees quality, traceability with a regular and homogeneous supply. Local production is not limited to one particular market; the main brakes to a real development of this sector are mostly linked to the price differential with the informal market and to the legal constraints imposed on formal marketing.
A SOCIOECONOMIC, CONSUMER AND ECOLOGICAL STUDY OF THE USE OF PANDANUS UTILIS LEAVES IN MAURITIUS

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²Association des Consommateurs de L’île Maurice

ABSTRACT

This project found its origin as a consumer issue faced by ACIM regarding a perception of the increased use of plastics bags at the detriment of our nationally notorious tante bazaar produced from the vacoa leaves. At the same time MAA were involved at le Bouchon with the vacoa weaving community. This concern for consumer waste issue from ACIM and the community based approach development drive of MAA brought together this trio of the above-named interested parties to find a solution to the issue of practical plastic bags and ultimately environmentally unfriendly waste against bio-degradable and expensive tante vacoas. Little did we know, in fact, that we were embarking on a project that embraced the state of raw materials, the vacoa plant – the scientifically termed Pandanus utilis and its leaves, the state of the workforce in this sector and the consumer’s preferred choice as regards to the vacoa and plastic products. A cradle to grave set of studies hence emerged that carried us into an ecological, socio economic and consumer study. We found ourselves dealing with the idea of the plastic waste with the concepts of biodiversity, female labour and handicraft; environmental awareness, and sustainable consumption. The project raised important questions about a weak sector, female environmental managers, mainly, are trying to make a living in the face of market forces and consumer attitudes that do not necessarily support the idea of sustainable consumption. It raises some important questions about the future of this sector, the need for government and other support if the vacoa handicraft sector is to prevail, the need for replenishing raw material and the need for consumer awareness.

Keywords: Pandanus utilis leaves, Vacoas plant, Plastic waste, Plastic bag
ABSTRACT

One of the most marked changes in the food industry has been the increasing consumer demand for food quality. This awareness has been brought about by an increased information flow from various sources, including third parties. The surge in demand is more common in high-income countries, given that consumers are more akin to the relationship between diet and health, and also more conscious of the food quality attributes. Food producers are therefore compelled to respond to this food quality demand. They are modelling their marketing strategy accordingly, and providing information to show that they are responding to consumers’ demand. One of the ways to satisfy this demand for food quality information is through food labelling.

This paper looks at food labels as a way to providing food quality information and explores its underlying theory. It builds on an extensive literature search and application of economic theory. Aspects looked into are: importance of food labelling and demand and supply of food labelling. Then we relate the topic to the Mauritian context by taking the economic theory as basis and layering it with realities that pertain to Mauritius, to give an overall economic and legal picture of the current situation. The main finding is that Mauritius has a legal framework, which is being used to a certain extent to provide a degree of information symmetry in the market, but there remain lots to do, to consistently implement and improve the existing situation.

Keywords: food labelling, food quality, economic theory
CONTROL STRATEGIES OF THE MELON FLY BACTROCERA CUCURBITAE (COQUILLETT) (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The melon fly Bactrocera cucurbitae (Coquillett) is the most destructive pest of vegetable crops, particularly the cucurbits: cucumber Cucumis sativus L., melon Cucumis melo L., watermelon Citrullus lanatus (Thunb.) Mat., squash Cucurbita pepo L., pumpkin Cucurbita maxima Duch, calabash Lagenaria siceraria (Mol.) Stand, ridgegourd Luffa acutangula (L.) Roxb., bitergourd Momordica charantia L., snakegourd Trichosanthes cucumerina L. and chayote Sechium edule (Jacq.) Sw. This insect commonly infests immature fruits by laying eggs under the skin. The eggs hatch into larvae that feed in the decaying flesh of the vegetable. Infested vegetables quickly become rotten and inedible or drop to the ground prematurely, thus causing considerable losses in production. If uncontrolled, this pest can destroy the whole harvest. An integrated melon fly management has been developed and recommended to cucurbit growers. This package includes regular application of protein baits, placement of plywood blocks impregnated with Cuelure and malathion along the field border, collection and placement of infested fruits in a fruit cage and insecticidal treatments.

Keywords: Melon fly, Bactrocera cucurbitae, control, integrated pest management, bait, insecticide
IMPROVING THE QUALITY OF HORTICULTURE PROJECT

APEXHOM¹ / NRI²

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ABSTRACT

In response to heightened consumer concerns over food safety and quality, codes of good agricultural practices are being adopted throughout the world. In order to retain market share in the global market place, growers and exporters have to adhere to accredited codes of practice. In a collaborative EU-funded project between APEXHOM and NRI, codes of practice have been drafted for the Mauritian horticultural sector so as to maintain consumer confidence in food quality and safety by improving the efficiency of natural resources use; applying due diligence when using pesticides; protecting the environment, and ensuring worker health and safety. As part of the elaboration and implementation of a National Code of Practice, three levels of Code have been drafted, set at various levels of quality control measures, the highest level being compatible with the level required for export. Validation of the codes is currently under way, through trials with volunteer growers in the litchi, vegetable, pineapple and anthurium sub-sectors, with the assistance of the Agricultural Research & Extension Unit.

Keywords: codes of practice, horticulture, food quality, health and safety, control measures
PINEAPPLE: EFFECT OF DIFFERENT SUCKER WEIGHT ON CROP CYCLE LENGTH AND YIELD

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ABSTRACT

Pineapple (cv. Queen Victoria) planted at three sites: Camp de Masque Pavé, Camp La Boue / Congomah, Beau Vallon / Ville Noire was evaluated for effect of different sucker weights at planting on crop cycle length and yield of pineapple fruits. The treatments were three sucker weights: S1: 200 – 250 g; S2: 250 – 300 g; S3: 300 – 350 g with a complete randomised block design replicated two times. Data on length of crop cycle, total yield, and fruit weight were collected. Results showed that there was no significant difference in crop cycle length at the three sites. Sucker size influenced significantly yield and mean fruit weight. Besides sucker size, regions also had a direct effect on growth of plants and size of fruits at harvest.

Keywords: pineapple, sucker weight, crop length, pineapple fruit yield
Characterization of the phenolic profile of endemic Mauritian Chassalia species and assessment of their antioxidant activities.....13
Chellum J.............................................401
Chintaram E........................................111
Choisis JP...........................................397
Collignan A.........................................65
Control strategies of the melon fly Bactrocera cucurbitae (coquillett) (diptera: tephritidae)..........................405
Crozier A...........................................23
Cullen DC..........................................51
Cypress aphid status in Mauritius and trial releases of Pausia juniperorum (Hymenoptera: Braconidae), a promising biocontrol agent ........................................313
D’Espagnac L........................................217
Delineation of Major Drainage Basins of Mauritius.........................................................289
Determination of genetic variation among some anthurium cut-flower cultivars .........71
Dobee B..............................................323
Dookun-Saumtally A...........................................77
Dunhawoover C....................................297
Early growth and Phyllochron of eight Palm Species at six sites in a Tropical environment ........................................217
Economics of food labelling: a Mauritian perspective.........................................................403
Effect of static magnetic fields on the growth and yield of Butterhead lettuce seeds (Lactuca sativa var. Salina)..........207
Effect of wind-protection on agronomic performance of banana cv Petite naine ......198
Effects of two commercially available composts on soil properties, and yield and mineral content of bean.........................175
Evaluation and potential of biological nitrogen fixation by French bean (Phaseolus vulgaris cv Long Tom)..........167
Evaluation of a ration for ruminants using locally available by-products ...................389
Facknath S............................................351
Fakim R..............................................263
Field evaluation of synthetic sex attractants of the tomato fruit worm, Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) in Mauritius........343
Fontaine O ..........................................397
Gokool A.............................................323
Govinden N...........................................217, 187, 129
Gunagah B...........................................307
Heifer live weight and reproductive performance on smallholder farms...........393
Hossenbux AS........................................279
A Geographical marketing information system for potato...............................................119
A survey of the Mauritian endemic flora for potential prophylaxis ..........................1
Agricultural diversification under changing land use: Modelling the Riviere Des Anguilles catchment .........................269
Agroforestry - a potential system to poverty alleviation: The case of Calliandra calothyrsus on the slopes of mount Kilimanjaro, Tanzania.........................................249
Agronomic performance and tuber characteristics and quality of newly-released local potato clone Belle Isle .......129
Alleck M..............................................313
An economic analysis of medium and small dairy farms in Mauritius.............263
APEXHOM / NRI........................................407
Application of microsatellite markers to the sugar cane breeding programme......77
Arumoa Ol.............................................23, 1
Assessment on the population of Cryptoplebia Peltastica Meyrick (Lepidoptera: Tortricidae) and fruit damage in litchi orchards and backyards in Mauritius.................................323
Bahorun Dr T...........................................xi
Bahorun Dr T.....................................235, 207, 95, 71, 23, 13, 1
Beeharry GK........................................207
Beni Madhu SP.....................................369
Bhikajee Dr Mitrasen....................................xix
Bhugaloo RA..........................................409
Bhunnoo MA.........................................193
Biological control of the spiralling whitefly, Aleurodicus dispersus ...................307
Biosensor as a tool for monitoring the status of fruits and vegetables ..........51
Bodha Nandoomar ..................................xiii
Bojnaught G...........................................95
Boodoo A.............................................263
Boojhawon R.........................................279
Bunwaree M.........................................207
Cadersa Y.............................................383
Characterisation of the Creole cattle in Mauritius.......................................................255
A farmer-participatory approach for the implementation of a developed Integrated Pest Management System against Plutella xylostella in crucifer cultivation in Mauritius ..........................................................297
A socioeconomic, consumer and ecological study of the use of Pandanus utilis leaves in Mauritius ..................401
Assessment on the population of Calliandra calothyrsus on the slopes of mount Kilimanjaro, Tanzania.........................................249
Agronomic performance and tuber characteristics and quality of newly-released local potato clone Belle Isle .......129
Alleck M..............................................313
An economic analysis of medium and small dairy farms in Mauritius.............263
APEXHOM / NRI........................................407
Application of microsatellite markers to the sugar cane breeding programme......77
Arumoa Ol.............................................23, 1
Assessment on the population of Cryptoplebia Peltastica Meyrick (Lepidoptera: Tortricidae) and fruit damage in litchi orchards and backyards in Mauritius.................................323
Bahorun Dr T...........................................xi
Bahorun Dr T.....................................235, 207, 95, 71, 23, 13, 1
Beeharry GK........................................207
Beni Madhu SP.....................................369
Bhikajee Dr Mitrasen....................................xix
Bhugaloo RA..........................................409
Bhunnoo MA.........................................193
Biological control of the spiralling whitefly, Aleurodicus dispersus ...................307
Biosensor as a tool for monitoring the status of fruits and vegetables ..........51
Bodha Nandoomar ..................................xiii
Bojnaught G...........................................95
Boodoo A.............................................263
Boojhawon R.........................................279
Bunwaree M.........................................207
Cadersa Y.............................................383
Characterisation of the Creole cattle in Mauritius.......................................................255
Author and Subject Index  MAS 2005

MAS 2005. Food and Agricultural Research Council, Réduit, Mauritius. 411
Infrared spectroscopy: An analytical tool

In-vitro

Improving the quality of horticulture

(Mas 2005. Food and Agricultural Research Council, Léduit, Mauritius.)

Infrared spectroscopy: An analytical tool in food science.

In-vitro

In-matière de salaison de venaison en milieu tropical.

In-vitro mutation studies of Taro

(Colocasia esculenta var. esculenta) in Mauritius.


In-vitro and molecular studies in Asparagus officinalis.

Information Technology as a tool to improve the utilisation and management of sugarcane germplasm at the MSIRI.

Infrared spectroscopy: An analytical tool in food science.

Innovation en matière de salaison de venaison en milieu tropical.

In-vitro mutation studies of Taro

(Colocasia esculenta var. esculenta) in Mauritius.
<table>
<thead>
<tr>
<th>Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toolsee P</td>
<td>393, 263</td>
</tr>
<tr>
<td>Trystram G</td>
<td>65</td>
</tr>
<tr>
<td>Umrit G</td>
<td>161</td>
</tr>
<tr>
<td>Unmole L</td>
<td>343</td>
</tr>
<tr>
<td>Vally V</td>
<td>369</td>
</tr>
<tr>
<td>Venkatasamy S</td>
<td>375, 71</td>
</tr>
<tr>
<td>Water budget estimation for water basins of Mauritius</td>
<td>279</td>
</tr>
<tr>
<td>Welcoming address</td>
<td>xi</td>
</tr>
<tr>
<td>White SF</td>
<td>51</td>
</tr>
<tr>
<td>Wong Yen Cheong K</td>
<td>129</td>
</tr>
</tbody>
</table>

The impact of natural pollinator, *Apis mellifera* Latreille on onion seed production in Mauritius......................193