Pineapple News

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Table of Contents

News of General Interest **2**
Maui Pineapple Company Closes 2
'MD-2' Pineapple Named the American Society for Horticulture Science 2010 Outstanding Fruit Cultivar 2
7th International Pineapple Symposium 2010 3
Pineapple Working Group 4
Contribute to Pineapple News 4
News From Belgium **4**
Pre and Postharvest Metabolism of Leaves on the Pineapple **Fruit Crown** 4
News From Brazil **8**
Alternative Control of Pineapple Fusariosis on Irrigated 'MD-2' Pineapple in Brazil 8
News From Cuba **9**
Chemical Sterilization for Propagation of Pineapple Plantlets in Temporary Immersion Bioreactor 9
News From France **15**
Growth Indicators for Queen Victoria Pineapple Versus Sum of Temperatures, Basis for a Heat-unit Model of Vegetative Growth 15
A Bait and Trap Method for Sampling Symphylid Populations in Pineapple 18
News from Ghana **23**
Pineapple and Carbon Emissions 23
News from Malaysia **23**
Handling of Fresh-cut Pineapple for Fresh Consumption 23
News From Taiwan **24**
RAPD Marker Assisted Selection of EMS Induced Pineapple (Ananas comosus (L.) Merr.) Mutants 24
News From the United States **27**
Role of Proteases During Ripening of Pineapple Fruit 27
Environmental Friendly Approaches for Managing Nematodes and Weeds on Pineapple 27
Commonalities of Pineapple, Agaves, and Cacti 32
Services **33**
Commercial Services 33
Professional Services 34
Book Reviews and Web Sites **34**
Book Reviews 34
Web Sites of Possible Interest 34
New References on Pineapple **35**
Instructions to Contributors to Pineapple News **41**

News of General Interest, Pineapple Working Group News

Dear Colleagues:

The big news this year is the 7th International Pineapple Symposium, which is being held in Johor, Malaysia in July 2010. As of April 19, 2010, the Secretariat of the symposium reported they had received 32 oral and 105 poster papers. I am looking forward to attending this year's symposium and hope to meet many of you there.

Maui Pineapple Company Closes

Many of us associated with the Hawai`i pineapple industry were saddened to read on Tuesday, November 3, 2009 that Maui Pineapple Company planned to cease planting of pineapple immediately and would shut down all operations at the end of the year (http://www.hawaiimagazine.com/blogs/hawaii_today/2009/11/4/Maui_Land_Pineapple_production_ends). The last harvest was to be made on December 23, 2009. Not long after the closure was announced, a news article reported that a group of investors planned to continue growing fresh pineapples on Maui under the name Haliimaile Pineapple Company, Ltd. The new company is to lease land from Maui Land and Pineapple Company. The organizers of the new company included five former Maui Pineapple Company executives and former Maui Pineapple Company manager Darren Strand will take over as president of the new company. The news release (http://www.mauinews.com/page/content.detail/id/527256.html) indicated that the new company would concentrate on the island market with a small percentage of the fruit to be shipped to premium markets on the U.S. mainland. The company plans to grow Maui Gold® pineapple on about 1,000 acres in the Haliimaile area of Maui. The company expected to save about 65 agricultural jobs with the hope that more could be added in the future.

As of this writing, Dole continues to grow pineapple on about 3,000 acres on the island of Oahu, which is where the main market is located. Dole also ships a limited amount of pineapple to California and other west-coast markets.

'MD-2' Pineapple Named the American Society for Horticulture Science 2010 Outstanding Fruit Cultivar

On reading an announcement in 2009 about the American Society for Horticultural Science Outstanding Fruit Cultivar Award, Duane Bartholomew and Robert Paull spearheaded an effort to nominate 'MD-2' for this award. The nomination reads in part: The cultivar MD-2 resulted from a cross made in 1970 by Dr. David D. F. Williams, plant breeder and director of the Pineapple Research Institute of Hawaii (PRI), a nonprofit research institute supported by the Hawaii Pineapple Growers Association (HPGA). PRI was funded at the time the cross was made by Del Monte Hawaii Inc., Dole Food Co., Hawaiian Fruit Packers, Libby McNeil & Libby, Maui Pineapple Co. and several smaller pineapple growers. Dr. Williams' able assistants in the pineapple breeding program were Frank Bermudas and Toshio Minagawa. The nomination called attention to the outstanding contributions to pineapple breeding made by the Pineapple Research Institute of Hawaii and to David D.F. Williams. The justification for naming 'MD-2' as the outstanding fruit cultivar in 2010 was based on the cultivars overwhelming success in the international pineapple markets.

Figure 1. Photo of Dr. David D.F. Williams holding the medal recognizing 'MD-2' pineapple as the American Society for Horticulture Science 2010 Outstanding Fruit Cultivar. The medal was awarded to Dr. Williams and the inscription reads: 'MD-2' Pineapple. Dr. David D.F. Williams and Forbears, Pineapple Research Institute of Hawaii.

Dr. Williams, now in his early 80s, and his daughter Wendy flew to St. Louis, Missouri to accept the award. Photos of the award ceremony did not survive a camera accident so the photo in Figure 1 was taken at a friends home in the beautiful Rocky Mountains in Colorado. Dr. Williams has been retired for more than 10 years and he and his wife Elsa currently reside near a number of their children in Fort Collins, Colorado, U.S.A.

7th International Pineapple Symposium 2010

7TH INTERNATIONAL PINEAPPLE SYMPOSIUM 2010

Jointly organized by:

- Malaysia Agriculture Research and Development Institute (MARDI)
- Malaysian Pineapple Industry Board (MPIB)
- International Society of Horticultural Science (ISHS)

In co-operation with:

- Department of Agriculture, Malaysia (DOA)
- Federal Agricultural Marketing Authority (FAMA)
- International Tropical Fruits Network (TFNet)

Supported by:

- Ministry of Agriculture and Agro-based Industry Malaysia (MOA)
- Tourism Malaysia

For further details please visit our website at

http://www.mardi.my

Malaysia Hosts the 7th International Pineapple Symposium (7th IPS)

The Symposium Organizing Committee, Malaysia Agriculture Research and Development Institute (MARDI), Malaysian Pineapple Industry Board (MPIB) and International Society of Horticultural Science (ISHS), invites all of you to the 7th IPS to be held from 13 to 15 July 2010. The theme "GLOBAL PINEAPPLE INDUSTRY-THE WAY FORWARD" emphasizes on the latest trends and development in the global pineapple sector. All aspects relating to pineapple cultivation (industry and trade, breeding, genetics, plant physiology and cultural practices, pest and disease management, post harvest handling and product development, consumer and marketing) will be covered, taking into account the most recent scientific and technical advancement around the world.

The symposium will be held at PERSADA Johor International Convention Center in Johor Baru, Malaysia. The state of Johor is known for its pineapple cultivation on peat soil since 1960s. Being tropical and evergreen, Johor Baru with its beautiful landscape, just across the causeway from Singapore is an excellent meeting place for scientists, professional, industry players, traders, students and any one that has interest in pineapple business.

About 200 participants locally and from all over the world and international organizations will participate in the 7th IPS 2010. Interesting abstracts are being submitted by participants locally and abroad covering various disciplines.

The scientific program which encompasses oral and poster presentations, exhibition and field visit, will provide delegates with information, a forum for discussion and networking opportunities for building beneficial relations with the dynamic pineapple industry.

Important dates for 7th IPS:

- Full paper submission- before or on 13th July 2010
- Paper presenters must pay their registration fees 2 months before the Symposium i.e. 13 May 2010.

We are looking forward to meeting you at the 7th IPS

Convener,

7th IPS

For the most current information on the symposium, please point your web browser to the symposium web site at https://anjungnet.mardi.gov.my/Conference.nsf/PineApple?OpenPage.

Pineapple Working Group of the International Society for Horticulture Science

I would like to remind readers of Pineapple News that all proceedings of the six international pineapple symposia are available for sale from the ISHS web site. The ISHS also provides free web access to the abstracts of all papers presented at the six symposia as well as to the abstracts of papers in all volumes of Acta Horticulturae published by the ISHS. There are many benefits to being a member of the ISHS and one that I have enjoyed is the opportunity to download at no additional cost a limited number of copies of articles published in Acta Horticulturae. The ISHS is one of the foremost organizations promoting cooperation and communication among horticultural researchers, growers and consumers. The ISHS continues to expand its offerings to members as well as to provide the structure under which our Pineapple Working Group (http://www.ishs.org/science/T07.php) functions. Detailed information about ISHS and the benefits of membership can be found at http://www.ishs.org or you can write to the ISHS Secretariat, P.O. Box 500, 3001 Leuven, Belgium (E-Mail: info@ishs.org).

Contribute to Pineapple News

Information on how to contribute to Pineapple News can be found at the end of the newsletter. You can also contact Duane Bartholomew, the editor, at duaneb@hawaii.edu.◆

News From Belgium

Pre and Postharvest Metabolism of Leaves on the Pineapple Fruit Crown

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Abstract

Crown burn on exported pineapple fruit causes serious economical losses. In this study, crown burn occurrence was hypothesized to be related to the Crassulacean Acid Metabolism (CAM). However, the functioning of this metabolism in the crown leaves remained unexplored. Therefore, pre and post harvest bio-activity of the crown leaves of pineapple fruit were studied. With the fruit still attached to the plant, CAM activity was detected in the crown leaves. While post-transport diurnal light cycle conditions seemed to reactivate CAM in the crown leaves, no CAM activation was registered during cold-storage transport under dark conditions (simulating transport). The substantial organic acid increase in the crown leaves observed during transport is thought to originate from the pineapple fruit itself.

Introduction

As a part of the grading standard, not only pineapple fruit but also crown quality is an aesthetic characteristic of economic concern. As such, leaf damage, occurring as brown spots on the crown leaves, is causing economical losses. The authors hypothesized these spots to be the result of physiological disorders in a similar way as earlier described for the ornamental bromeliad *Aechmea*. In these ornamental plants the build-up of high organic acid concentrations causes chlorenchyma cells to rupture (Londers et al., 2005b; Londers, 2006).

Nocturnal organic acid build-up is a central feature of CAM (Crassulacean Acid Metabolism) with pineapple being the most important crop exhibiting this metabolism. Stomata in mature leaves open at night and CO_2 is assimilated into malate by the carboxylating enzyme phosphoenolpyruvatecarboxylase (PEPC). During daytime CO_2 will be released and processed via Rubisco behind closed stomata. The pineapple crown is a continuation of the vegetative stem, and the spirally arranged leaflets have a similar morphology. However, the photosynthetic activity of crown leaves remained unexplored.

Therefore the authors investigated the metabolism of the crown leaves both in the field and under post harvest transport conditions. To figure out on plant carbon metabolism, nocturnal organic acid build-up is often used as a key determinant for CAM (Winter and Smith, 1996). Furthermore, the stomata opening index gives a good indication for gas exchange patterns (Londers et al., 2005b), with nocturnal gas exchange characteristic for CAM. These measurements can further be refined by gas exchange data about leaf CO2 exchange. The results are presented and discussed in the broader context of crown leaf damage.

Materials and methods

Experimental setup and plant material

The experimental setup is summarized in Table 1. The metabolism of the pineapple crown leaves was studied at different stages in the pineapple production chain. For the preharvest stage, the metabolism was studied by determining the stomata opening index of the leaves of the pineapple crown. Therefore, *Ananas comosus* hybrid plants were grown in the greenhouse of the Katholieke Universiteit Leuven under greenhouse conditions as described in Londers et al. (2005b). For the postharvest transport analyses, fruits (*Ananas comosus* hybrid) were obtained from a commercial pineapple plantation in Ecuador. Pineapple crown leaves were sampled at harvest and at their arrival in the port of Antwerp, Belgium, after a transport period of three weeks in dark and cooled conditions (about 8 °C). Finally, storage simulation experiments were executed, by placing fruits in cooled (10 °C) climate chambers under both a continuous dark and a diurnal light cycle (12/12) regime. Under the diurnal light cycle regime, light intensity in the climate chamber averaged around 150 mol m^{-2} s^{-1}.

Stomata opening index

The stomata index was the percentage of opened stomata of hundred stomata examined. Fully developed crown leaves were sampled on the abaxial leaf side between the half leaf length and the leaf tip. After removing trichomes with adhesive tape, a thin coat of transparent nail polish was layered onto the leaf surface and allowed to dry for 15 min. Dry polish coats were carefully removed using transparent adhesive tape (Scotch crystal clear) and stuck on glass slides for microscopic observations (Londers, 2006).

Organic acid measurements

Samples for organic acid analysis were collected from fully developed leaves of the pineapple crown (n = 8 fruits). Samples were subsequently weighed, immediately frozen in liquid nitrogen to arrest enzyme activity and stored at -20 °C until required for analysis. The samples were extracted with water after grinding nitrogen frozen tissues with a tissue grinder. For the determination of total titratable acids, extracts were titrated (Metrohm, 702 SM Titrino) to an end point of pH 8.1 using 0.01 N KOH. Extraction and quantification of malic acid was accomplished as described by Londers et al. (2005b) using high performance liquid chromatography (Waters 510, Waters, Milford, MA, USA) with detection at 210 nm (Waters 484, Waters, Milford, MA, USA) using an aminex HPX87-H (300 mm x 7.8 mm) resin-based column from Bio-Rad (Hercules, CA, USA).

Table 1. Experimental setup and actions

Sampling points in production chain	Time of sampling (days)	Climate conditions	Type of measurement
Field	Before harvest		Stomata opening index
Harvest	0		Total titratable acids
Transport	21	Dark + 8°C	Total titratable acids
Storage	28	Dark + 10°C	Total titratable acids
			Net CO_2 uptake
		Diurnal light cycle + 10°C	Total titratable acids
			Net CO_2 uptake

Gas exchange measurements

Leaf CO_2 exchange data were collected on the youngest fully expanded leaf from the pineapple crown under a continuous dark and alternated light/dark (12/12) regime. A LCi Portable Photosynthesis System (ADC BioScientific Ltd., UK) was used. The top part of the leaf was enclosed in the leaf chamber. Short-term fluctuations in the CO_2 concentration of the incoming air were buffered by passing the air through a 25 L metal bottle. Gas exchange data were collected using a 10 min interval (n = 5 fruits).

Where appropriate, the data were analyzed using the statistical software package SAS Entreprise Guide 4.0. Before carrying out statistical tests normality of the data was checked by means of the Kolmogorov-Smirnoff statistic ($p > 0.05$). Means were compared by two sample t-test or by Tukey's studentized range test.

Results

Preharvest metabolism of leaves

The pattern of stomatal opening of the crown leaves (Figure 1) with the fruit still attached to the plant was typical of CAM plants. Stomata of CAM plants remain mainly closed during daytime and open at night, controlled by internal CO_2 concentrations (Salisbury and Ross, 1991).

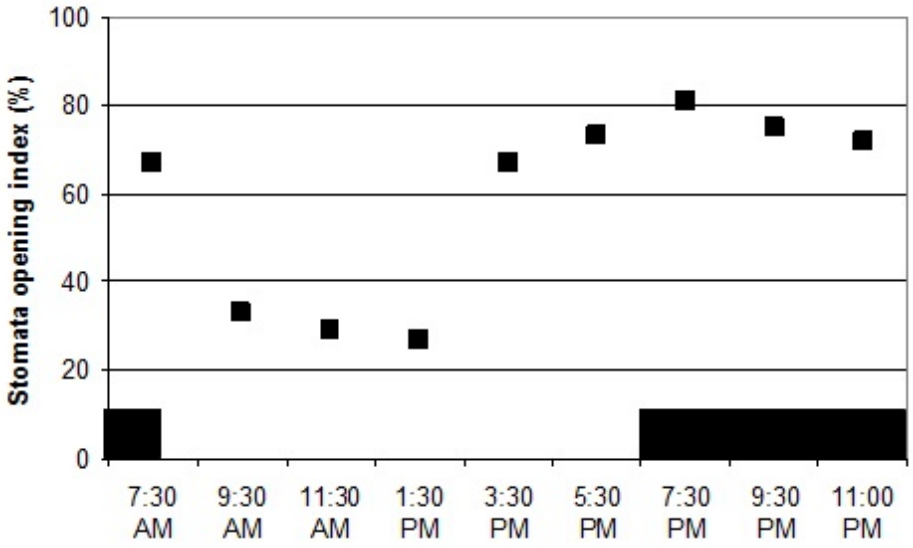

Figure 1. Stomata opening index (% open stomata) of pineapple crown leaves sampled before harvest. The curve is representative of 10 measurements with SE < 15 %.

Postharvest metabolism of leaves

Total titratable acids in the crown leaves at some critical points in the pineapple fruit production chain, starting from the time of harvest, can be seen in Table 2. Relative to leaves sampled at harvest, total titratable acid values almost doubled during dark, cold transport. The post-transport treatments evaluated did not affect the organic acid levels. There was a significant accumulation of malic acid in pineapple crown leaves after 7 days of post-transport treatment when crowns were exposed to a diurnal light cycle cold-regime (Figure 2). Under dark cold conditions, CAM seems not to be active. Net CO_2 uptake under different post-transport treatment conditions (Figure 3) supports this statement. While there is a clear pattern of CO_2 uptake during the night in a 12/12 light-dark diurnal cycle, it is absent under continuous dark conditions.

Table 2. Total titratable acids (Eq gfw^{-1}) in the crown leaves of pineapple fruit at critical points in the production chain.

Time of sampling (days after harvest)	Treatment†	Leaf total titratable acids (Eq gfw^{-1})
0	---	162
21	OT	308 ± 22 a
28	OT + Dark + 10°C	299 ± 18 a
28	OT + DLC + 10°C	288 ± 12 a

†OT = Overseas Trasport; DLC = Diurnal Light Cycle. Leaves were sampled at 8:00 AM. SE (n = 8 fruits) and Tukey's Studentized Range Test ($P < 0.05$). Treatments followed by the same letter were not significantly different from each other.

Discussion and conclusions

Crown burn during overseas transport of pineapple fruit is a problem of economic concern. Visually, crown burn seems to be identical to a specific type of leaf damage on the leaves of many ornamental *Aechmea* cultivars, which was studied earlier by the authors (Londers et al., 2005a). Microscopic analysis of damaged crown leaves (results not shown) revealed ruptured cells in the chlorenchyma of the leaves, which matches damage images obtained earlier for *Aechmea* (Londers et al., 2005a). Based on both the visual and microscopic images, the authors hypothesized crown burn to be the result of physiological disorders similar to those described earlier for *Aechmea* (Londers et al., 2005b; Londers et al., 2006; De Proft et al., 2007). More specifically, high organic acid concentrations in the leaves due to CAM might act as water pumps, resulting in (lethal) leaf turgor pressure. Especially *Aechmea* plants kept under prolonged dark conditions seem to be at high risk for this type of leaf damage, due to a disturbed CAM pattern (Ceusters, 2008). To evaluate the mechanism described above for the crown burn case, the metabolism of the crown leaves and especially leaf organic acid concentrations during overseas transport needed to be investigated.

With the pineapple crown being a continuation of the vegetative stem leaves, and the spirally arranged leaflets having a similar morphology, the authors suppose the crown to act as CAM. This was supported by diurnal stomata opening analyses (Figure 1) with the fruit still attached to the plant, which clearly indicated CAM.

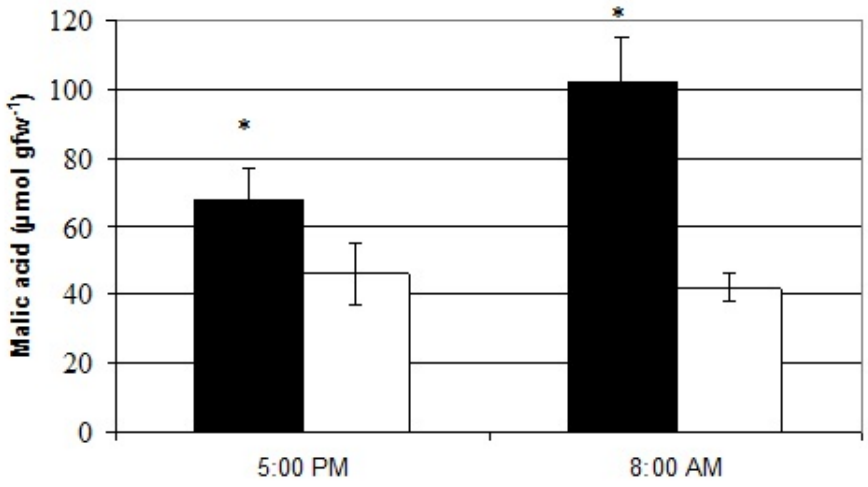

Figure 2. Nocturnal malic acid build-up in pineapple crown leaves from 5:00 p.m. to 8:00 a.m. after 7 days of post-transport treatment (n = 8 fruits). Diurnal light cycle at 10 °C (black bars); continuous dark at 10 °C (white bars). Statistically significant nocturnal malic acid build-up (*P<0.05) is indicated by *.

Concerning the organic acid concentrations in the crown leaves, total titrable acids doubled during the three weeks cold-transport period (Table 2). Nanayakkara et al. (2005) already described the increase of total titratable acids in the crown leaves during cold-storage.

Though, storage under diurnal light cycle conditions seems to activate a diurnal stomatal rhythm with a similar pattern of CO_2 gas exchange (Figure 2) and nocturnal accumulation of malate in the leaves (Figure 3).

To conclude, the increase of total titratable acids in the leaves on the crown of pineapple fruit during dark cold-transport is not believed to be caused by a disturbed CAM. Total titratable acids seem to build-up gradually during the overseas cold-transport period (results not shown). Possibly this increase in total titratable acids in the crown originates in the migration of organic acids from the fruit into the crown. Therefore, CAM interacting measures will not affect the risk of crown burn. Though, one should be aware that the availability of external water should be absolutely avoided throughout the whole postharvest chain. External water restriction limits the risk of lethal turgor pressure build-up, driven by organic acid concentrations in the leaves which are increasing during transport.

Funding

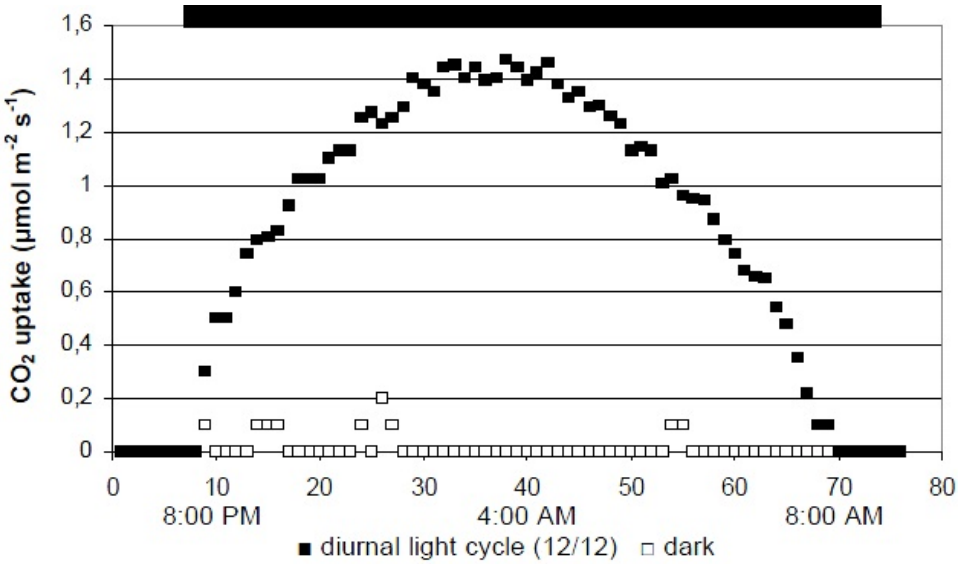

Figure 3. Postharvest net CO_2 uptake (µmol m^{-2} s^{-1}) pattern for young fully expanded pineapple crown leaves under a continuous dark (■) and diurnal light cycle (12/12) (□) cold-regime. Both curves are representative of five replicate runs with SE < 10 %.

This research was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen).

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News From Brazil

Alternative Control of Pineapple Fusariosis on Irrigated 'MD-2' Pineapple in Brazil

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Brazil is the biggest pineapple (*Ananas comosus* var. *comosus*) producer in the world with high yields and excellent fruit quality. However, despite the development of technologies for the pineapple crop, huge losses still occur due to high incidences of fusariosis disease, a devastating fruit rot caused by the fungus *Fusarium subglutinans,* which attacks not only the fruit, but also the whole plant and its slips which are used as propagating material in planting. In Brazil, 'Pérola' and 'Smooth Cayenne' plants are susceptible to fusariosis and 'MD-2' is extremely susceptible and the disease is difficult to control in irrigated commercial fields. Traditionally, the control of this disease is based on preventive applications of chemical fungicides. Weekly applications of a mixture of benzimidazole fungicides and carbamate insecticides (to control a fruit pest which occasionally opens the way for the fungus) during the 4 to 5 week period in which the pineapple flowers are open efficiently control fusariosis. Usually excessive residues of pesticides make exportation of the fruit impossible. On the other hand, there is increasing concern about the possible impact of fungicides on the environment and the onset of resistance to chemical crop protection agents together with a consumer preference for chemical-free produce, which has led to a search for agriculture products treated with natural substances. Therefore, the objective of this research was to identify an alternative control of pineapple fusariosis. Different dosages of citric extracts, food preservatives, pyroligneous acid and tannins of cultivated *Acacia mearnsii* were evaluated. The tannins are extracted and commercialized worldwide for utilization on the leather and tanning industries. Four experiments involving almost 15,000 plants were carried out in a private pineapple farm located in the semi-arid zone of the state of Bahia, Brazil in 2008. The experimental design was of randomized blocks with 8 treatments and four replicates. The tannins were applied once and twice a week at 5, 6, and 7% dosages. Most of the alternative products were inefficient against this disease except for the tannins of *A. mearnsii*. Although there was no difference among tannins treatments the difference in relation to the control was very impressive considering the favorable environment to the disease. The fruits were evaluated immediately after the harvest in search of symptoms of fusariosis rot. In this experiment the incidence of fusariosis was reduced from 72% in the control treatment (Figure 1A) to 22% in some tannin treatments (Figure 1B).

Figure 1. Fruits from untreated control infected with *Fusarium subglutinans* (A) and fruits with fusariosis from a comparable plot treated with black wattle tannins (B).

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News From Cuba

Chemical Sterilization for Propagation of Pineapple Plantlets in Temporary Immersion Bioreactor

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Summary

Vitrofural G-1 (patent no. 22676) is a relatively new, broad spectrum biocide derived from sugarcane plants, used in the medical industry and in plant tissue culture. The active ingredient is 1-(5 bromofur-2-IL)-2-bromo-a-nitroeteno. G-1 is effective against both bacteria and fungi; it is heat stable and can be used in the culture medium without any toxic effect on plant tissue. These characteristics of G-1 make it an attractive alternative for the control of contaminations during temporary immersion culture. In this context, the main objective of this study was to establish a procedure for pineapple propagation in temporary immersion bioreactors using G-1, as way of medium chemical sterilization, as well as to evaluate net photosynthesis and quality of shoots prior to acclimatization (elongation phase) using this procedure.
Key words: pineapple, Temporary Immersion, Bioreactor, photosynthesis, liquid medium.

Introduction

In order to overcome the current limitations of micropropagation, different procedures have been developed. Among them, the temporary immersion of explants in a liquid medium has been achieved by using different bioreactors, which are described and grouped into four categories according to operation. All of these systems have as advantages the avoidance of continuous immersion, which adversely affects growth and morphogenesis, provision of adequate oxygen transfer, sufficient mixing, and limited shear levels. The technique enables sequential medium changes and automation while reducing contamination and costs (Etienne and Berthouly 2002).

In vitro culture methods of pineapple plants allows for a more rapid increase of selected cultivars than traditional propagation methods. Commercial pineapple micropropagation involves sequential culturing in liquid medium for meristem and axillary shoot bud multiplication (Daquinta and Benegas, 1997). Using this approach, annual pineapple production is limited as a result of the number of pineapple plants needed annually to start up new plantations. In general, the commercial use of micropropagation is currently reduced because of high production costs. It is due primarily from high labour cost, low multiplication rate, and poor survival rates during acclimatization. .

A procedure for the mass propagation of pineapple plants (*Ananas comosus* L. Merr cv Smooth cayenne) using a temporary immersion technique was described by Escalona *et al*., (1999). This procedure involved three distinct phases in the semi-automated temporary immersion system: shooting, bud differentiation and elongation. An efficient and cost-effective method for commercial

micropropagation of 'Smooth Cayenne' pineapple using a periodic immersion bioreactor was also developed by Firoozabady and Gutterson (2003). Both methods are applicable to the micropropagation of standard cultivars but are also applicable to the rapid scale-up of planting material of a newly developed cultivar.

Microbial and fungal contamination is one of the most persistent problems in plant cell and tissue culture. Surface removal of contaminating organisms from plant tissue being prepared for culture is generally by treatment with oxidizing hypochlorite solutions, ethanol, mercuric chloride or a combination of these or other treatments. Antibiotics are used, but are not always effective, can alter plant growth, are costly, and resistant strains can result with extensive use (Niedz 1998). Plant Preservative Mixture (PPM) has been routinely added to tissue culture medium to control air and waterborne bacterial and fungal contaminants effectively (Niedz 1998; George and Tripepi 2001; Beruto *et al*., 2004).

Vitrofural G-1 (patent no. 22676) is a relatively new, broad spectrum biocide derived from sugarcane plants, which has been used in medical industry and plant tissue culture. The active ingredient is 1-(5 bromofur-2-IL)-2-bromo-a-nitroeteno. G-1 is effective against both bacteria and fungi, is heat stable and can be used in the culture medium without any toxic effect on plant tissue (Alvarado *et al*. 1997). These characteristics of G-1 make it an attractive alternative for use in temporary immersion culture. In this sense, the main objective of this study was to establish a procedure for pineapple propagation in temporary immersion bioreactor (TIB) using G-1, as way of medium chemical sterilization, as well as to evaluate some physiological parameters of pineapple shoot elongation in TIB using this procedure.

Material and Methods

Plant Material

Pineapple plantlets (*Ananas comosus* L. cv Smooth Cayenne) were obtained from established liquid cultures grown on a shooting medium, which consisted of MS (Murashige & Skoog 1962) salts supplemented with 9.32 µM benzyladenine (BA) and 1.61 µM naphthaleneacetic acid (NAA), as recommended by Daquinta and Benega (1997). The cultures were grown under a 16-h photoperiod from cool-white fluorescent lamps (80 µmol m^{-2} s^{-1}), at 25 ± 1.0 °C.

Culture conditions

The Temporary Immersion Bioreactor (TIB) used was described by Escalona *et al*. (1999). Five explants (*in vitro*-cultured plants with two small shoots) approximately 2 to 3cm in length were cultured in each container. The degree of endogenous contamination in explants was determined by culturing each explant on nutrient broth medium for 72 h at 37 °C before inoculation in the TIB. The culture vessels used for the experiment were 10.5 cm in diameter, 20 cm high and had a volume of 1000 ml; 200 mL of liquid medium was added to the culture vessel for each explant (Escalona *et al*., 1999). The basal medium consisted of MS salts with 30 g L^{-1} sucrose and 1.0 mg L^{-1} thiamine. The medium was sterilized using 28 mg L^{-1} of 100% a.i. G-1, the amount recommended to reduce or eliminate airborne contamination in liquid media using the TIB (Fundora *et al*., 2003). The control treatment was autoclaved at 121°C for 30 min. The pH of the culture medium was adjusted to 5.8 after addition of G-1 and autoclaving, respectively. All cultures were kept in a growth room at a temperature of 24 °C under cool-white fluorescent lamps as above with a 16 h photoperiod.

Concentration of plant growth regulators in the medium for shoot proliferation

Different concentrations of the main plant growth regulators that are included in the proliferation medium for pineapple, established previously by Escalona *et al*., (1999), were tested. A medium with 9.32 µM BA, 1.61 µM NAA, and 4.37 µM PB was the control. BA levels assessed were 0, 2.33, 4.66, 6.99, and 9.32 µM, all with the same composition of NAA and PB as described above. Once the best concentration of BA was determined, 4.37 µM PB was added and NAA levels of 0, 0.54, 1.07 and 1.61 µM NAA were tested. After the best concentrations of BA and NAA were determined, paclobutrazol (PB) [(2RS,3RS)-1-4-chlorofenyl 4,4-dimethyl-2-(1H-1,2,4-triazol-1-yL)pentan-3-ol] at 0.0, 1.09, 2.18, 3.28 and 4.37 µM were tested. The experimental conditions were as established by Escalona *et al*., (1999). Immersion frequency was 4 min every 3h. The duration of the proliferation phase was 30 d and 28 mg L^{-1} of G-1 was also used to sterilize culture medium. Multiplication rate was determined by dividing the final number of shoots by the initial number of explants, and was evaluated after 30 d of culture.

Immersion frequency during proliferation and elongation phases

Based on the experiments described above, a proliferation medium consisting of MS formula with 6.66 µM BA, 1.07 µM NAA and 4.37 µM PB was used. Then, different immersion frequencies of 1, 3 and 5 h were tested. For all treatments, the immersion time was 4 min. After a shoot-multiplication period (30 d), the medium was replaced with MS medium supplemented with 2.22 µM BA and 4.06 µM GA3. After seven days, the medium was replaced again with a MS medium supplemented with 4.06 µM GA3 and shoots were grown for 21 d to promote shoot elongation. All of these steps were previously described by Escalona (1999), but in this experiment, were modified through the addition of 28 mg L^{-1} G-1 to sterilize the culture medium. Multiplication rate, length of shoots, leaf area, fresh and dry mass and chlorophyll content were determined after the 21-day elongation phase. Leaf area was determined by a photogravimetric method (Sestak *et al*., 1971). Chlorophyll a + b were extracted in acetone (80%) and chlorophyll contents were determined using the equations of Porras *et al*. (1991). Dry weight was measured after drying the shoots for 72 h at 70°C.

Determination of photosynthetic parameters

For measurements of the maximum photosynthetic capacity (Pn), fully expanded leaves from shoots were used between 4-5 h after the beginning of the photoperiod. A maximum photosynthetic rate was measured using a portable CIRAS-2 photosynthesis System (Europe, PP Systems, UK). The whole area of the cuvette (PLC6) was covered with completely expanded young 2.5 cm^2 pineapple leaf. The carbon dioxide concentration and the humidity of the air entering the leaf chamber was 375µmol mol^{-1} and 80%, respectively, under a photosynthetic photon flux of 2000 µmol m^{-2} s^{-1}. The measurements were done on five plants with 10 replicates for a total of 50 values. Prior to light measurements the maximal and stable photosynthesis for the pineapple plants was determined. At 600 µmol m^{-2} s^{-1}, the photosynthesis was maximal and stable. Measurements were performed after 21d of culture prior to acclimatization (elongation phase).

Statistics

All statistical analyses were done using SPSS (Chicago, IL, USA, version 9.0). Differences due to hormone concentration, immersion frequency and shoot morphological parameters were analyzed using one-way ANOVA, followed by a Tukey comparison at p 0.05.

Results

Concentration of plant growth regulators in the medium for shoot proliferation

Three experiments were conducted to determinate the effect of different concentrations of the main plant growth regulators of pineapple in the presence of G-1 on multiplication rate and shoot quality. When different concentrations of BA were tested, the highest multiplication rate was achieved at 6.99 µM, which was not different from the autoclaved treatment. At the highest concentration of BA, shoot multiplication rate was decreased relative to the autoclaved treatment in the presence of G-1. Shoot multiplication rate in media with the best concentration of BA and without NAA, but with 4.37 µM PB increased significantly. However, there was no difference in the multiplication rate achieved at 1.07 µM NAA in presence of G-1, and the control treatment (autoclaved). When different concentration of PB were assayed together, at the best BA (6.99 µM) and NAA (1.07 µM) concentrations, the highest multiplication rate was achieved at the maximal PB concentration in presence of G-1 (Figure 1).

Immersion frequency in the proliferation and elongation phases

A 3-h immersion frequency significantly increased the multiplication rate relative to lower frequencies (Figure 2). Shoots grown in the TIB with a 1-h immersion frequency were taller and had a higher number of leaves, leaf area, and fresh and dry mass and total chlorophyll content than did shoots grown at longer immersion frequencies. All parameters above except the number of leaves per shoot were reduced for shoots grown at a 5-h immersion frequency (Table 1). Photosynthetic CO_2 assimilation of shoots in the 1 and 3-h immersion frequency treatments were not significantly different but were significantly higher than results for shoots grown in the TIB under the 5-h frequency (Table 1).

Table 1. Effect of immersion frequency on the growth parameters, net photosynthesis and chlorophyll content of pineapple shoots after 21 days in the culture previous acclimatization phase.

Frequency	Shoot mass, (g)		Shoot leaf	Total Chlorophyll	Net Photosynthesis	Shoot length	Leaves/shoot
	fresh	dry	area (m^2)	(mg g^{-1} FW)	(µmol CO_2 m^{-2} s^{-1})	(cm)	
1h	0.66 a	0.037 a	0.0118 a	160 a	8.07 a	9.37 a	6.66 ab
3h	0.49 b	0.026 b	0.0080 b	145 b	8.99 a	6.83 b	6.03 b
5h	0.18 c	0.015 c	0.0038 c	105 c	4.56 c	4.52 c	7.20 a

Values represent the means of three replicated samples. Values indicated by different letters within columns are significantly different at the 5 % level by Tukey´s Multiple Range Test.

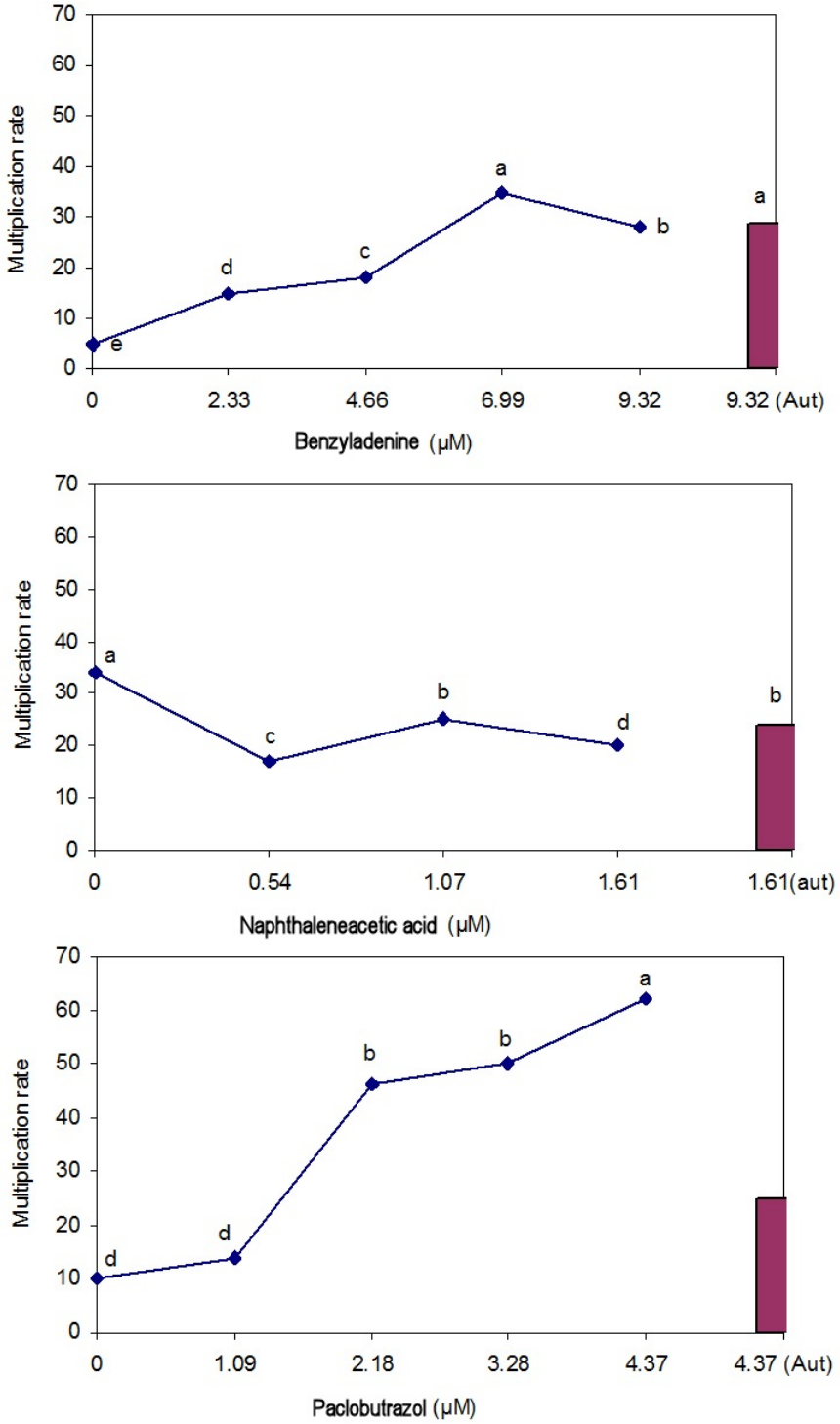

Figure 1. Effect of plant growth regulator (PGR) concentration with Vitrofural G-1 sterilant on pineapple shoot multiplication rate after 30 days of proliferation in a temporary immersion bioreactor (TIB). The bar indicates the rate for autoclaved samples at the optimum PGR concentration. Data points (n=3 TIB samples) associated with the same letter were not significantly different as determined by the Tukey Test ($p < 0.05$).

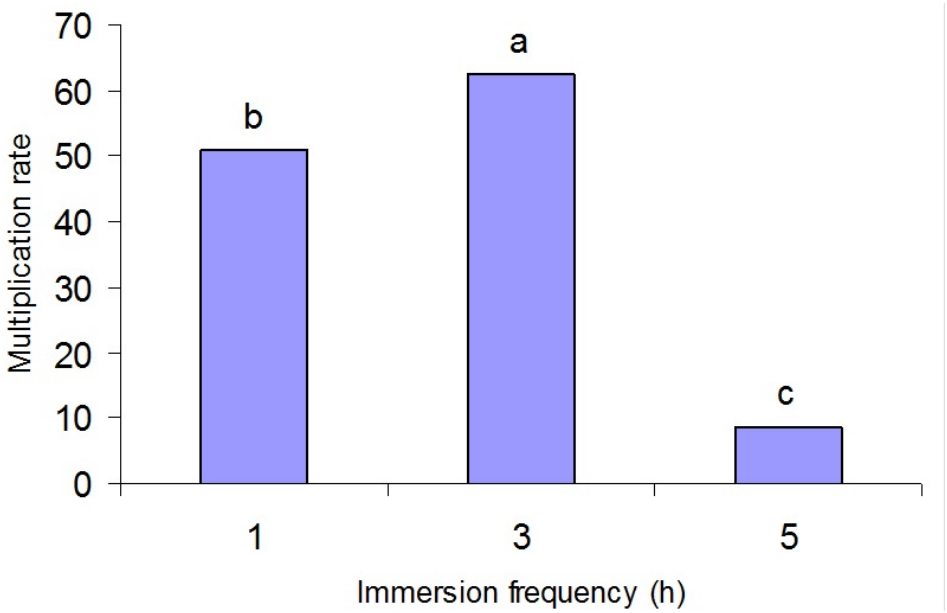

Figure 2. The effect of immersion frequency in a temporary immersion bioreactor (TIB) on pineapple shoot multiplication rate. Bars having associated with the same letter were not significantly different ($p<0.05$) as determined with the Tukey Test. Data are means of 3 TIB samples.

Discussion

Among the components of a culture medium, plant growth regulators are the best documented, although probably the least understood, elements controlling the morphogenetic response in micropropagation. The composition of plant growth regulators in the pineapple culture medium had as precedent the results obtained by Daquinta and Benegas (1997) in the propagation of 'Smooth Cayenne' using liquid medium. Later, Escalona *et al.* (1999) established that a supplement of 4.44 µM PB to this medium was optimal for pineapple proliferation in TIB.

Protocols for pineapple micropropagation generally use BA as the cytokinin, at concentrations between 2 and 5 mg L^{-1}. Using these concentrations, ten to 15 plants can be produced per month (Smith 2003). The role of BA to induce axillary proliferation in pineapple micropropagation is well documented (Smith 2003). During pineapple proliferation in TIB, when BA is reduced (6.99 µM) in the presence of PB (4.44 µM) the higher multiplication rate is achieved when NAA is omitted. However the use of 1.07 µM NAA permitted similar multiplication rate to control treatment and improved the length and shoot quality (Daquinta and Benegas, 1997).

When BA concentrations were tested in the presence of G-1, it was possible to achieve a higher multiplication rate at a lower BA concentration than was used when the medium was autoclaved. This result indicates that high temperatures during autoclaving affected the morphogenetic response of pineapple shoots in TIB. In this case, high temperatures could be denaturing proteins that can act as receptor sites at the membrane level.

The positive effect of paclobutrazol on pineapple micropropagation was previously described by Escalona *et al.* (1995) and was once again confirmed using TIB (Escalona *et al.*, 1999). Paclobutrazol controlled shoot growth and induced axillary bud proliferation. In the TIB, the use of paclobutrazol for pineapple micropropagation promoted formation of compact bud clusters with limited leaf development, avoiding unnecessary leaf growth during the shoot formation stage. It has been demonstrated that the use of inhibitors of gibberellin biosynthesis in tissue cultures containing a cytokinin increases the number of buds, especially with monocots. This phenomenon is not observed when media are supplemented with growth retardants only, indicating that they are not cytokinins per se. The interaction between gibberellins and growth retardants such as PB, is well documented. In Spathiphylum plants, endogenous gibberellins seem to alleviate the shoot induction capacity of exogenous cytokinin. Imizadoles such as prochloraz inhibit the biosynthesis of gibberellins and as a consequence cytokinins can manifest their full shoot induction potential (Werbrouck *et al.*, 1996). The high multiplication rate we achieved when PB was used with reduced levels of BA and NAA in presence of G-1 could corroborate this statement.

In this study, we demonstrated that G-1 could be present in the medium continuously without seriously affecting pineapple shoot proliferation when BA and NAA levels present in the traditional medium established by Escalona (1999) are reduced. The main reason for this is probably that it combines ventilation of the plant tissues and intermittent contact between the entire surface of the tissue and the liquid medium (Etienne & Berthouly, 2002). The immersion frequency determined in this paper varies considerably between shooting and elongation phase. A high multiplication rate was achieved with an immersion frequency of 3h, while a one-hour immersion frequency gave shoots of a better quality, indicating that nutrient uptake is increased in this phase. The 1 h immersion

frequency using G-1 during the elongation phase increased not only morphological parameters of the shoots, but also the total chlorophyll contents and net photosynthesis. The photosynthetic capacity of shoots measured on day 21 of the elongation phase was higher than results obtained by Escalona *et al.*, (2003) at a 3 h immersion frequency without G-1. This factor constitutes an innovative step to take account during pineapple propagation in TIB.

The new procedure of pineapple propagation in the Temporary Immersion Bioreactor using G-1 introduces significant changes to the pineapple propagation scheme established by Escalona *et al*. (1999). First, it was possible to achieve a high multiplication rate at reduced concentrations of BA and NAA in the proliferation medium. Second, shoot elongation was most rapid at an immersion frequency of only 1h, which means the immersion frequency was increased to 24 times per day.

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News From France

Growth Indicators for Queen Victoria Pineapple Versus Sum of Temperatures, Basis for a Heat-unit Model of Vegetative Growth

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List of abbreviations : SC : Smooth Cayenne, WI : plant Weight Increase, WpW : Whole plant Weight, PlW : Weight of Planting material, DaP : number of Day after Planting.

Introduction

Queen Victoria is the main pineapple variety grown in Reunion Island which shows very clear-cut natural conditions (meaning of foregoing not clear). The pineapples are usually grown from sea level to an elevation of 900 m, with average annual temperatures varying from 19°C to 25°C, and annual rainfall from 1,500 mm to 5,000 mm. In these conditions, it is relatively easy to set up experiments to study the relationships between climatic parameters as temperatures and the growth of the plants.

Plant weight and D leaf weight are classical growth indicators for pineapple. The date of forced induction of flowering is usually determined in relation to the weight of the 'D' leaf (which is closely linked to plant weight, and therefore to fruit weight. Fleisch and Bartholomew (1987) and Malezieux et al. (1994 and 2003) built a heat-unit model able to predict the date of harvest. Heat-unit models have been developed to explained the impact of temperature on many aspects of plant growth and quality (Ganry, 1978; N'Da Adopo et al., 1996; Roman et al., 2000; Marzurczyk et al., 2003; Borreani et al, 2003; Tixier et al., 2007).

The aim of this study was to analyze the growth rate of Queen Victoria, and then to establish the basis to build a growth heat-unit model. First, changes in plant weight were monitored from planting to forcing, then changes in 'D' leaf weight were compared with the plant weights to determine the optimal value to be reached before forcing. Finally we looked for a relationship between growth of Queen Victoria and sum of temperatures.

Materials and methods

The trials were located at 21°19'21" E and 55°29'20" S, at an altitude of 150 m and were conducted on brown andic soils (pH=5.5). The pineapple was planted through plastic mulch and was provided with drip irrigation. For modelling purpose, two series of trials was set up in Reunion Island in 2007-2009 with Queen Victoria suckers. Suckers of comparable weight were planted in 2 different seasons, September (250 g) and February (225 g). Average temperature was lower, 21.8 °C, for the February planting, compared to 24.1 °C for September. Temperatures were recorded every 15 minutes with a data logger and the growth indicators (WI : plant Weight Increase, WpW : Whole plant Weight, D-leaf weight), were monitored on samples of 15 plants per observation.

Results - discussion

Increase in plant weight

The curve representing the increase in whole pineapple plant weight traditionally shows a sigmoid pattern (Py et al., 1984). However, in our case and during the observation periods (up to 230 days after planting), the curve showed a parabola pattern (Figure 1). The plant weight increase (WI) could be calculated as: WI = WpW - PlW (where WpW = Whole plant weight, and PlW = Weight of Planting material). Then the plant weight increase (WI) could be expressed as a function of time (number of days after planting) : $WI = a \times DaP^2$ (where DaP = number of Days after Planting, and a is a calculated coefficient of the parabola curve).

'D' leaf weight

As previously observed for 'Smooth Cayenne' (Py et al., 1984), the 'D' leaf growth showed a sigmoid pattern with the following peculiarity (Figure 2): Weight increase of the 'D' leaf could be estimated by the equation (Verhulst, 1845): $y = y_{max}/(1+e^{-\alpha-\beta x})$ (where y_{max} represents the maximum weight reached by the 'D' leaf and α and β are calculated coefficients of the sigmoid curves). The maximum D leaf weight reached in our experimental conditions was 70 g for this variety. Exportable-size fruits (0.7kg) were obtained with a plant weight of about 1.2 kg at forcing with a 'D' leaf weight of 45 to 50 g. The plant weight for forcing (1.2 kg) was reached 210 to 230 days after planting under our experimental conditions.

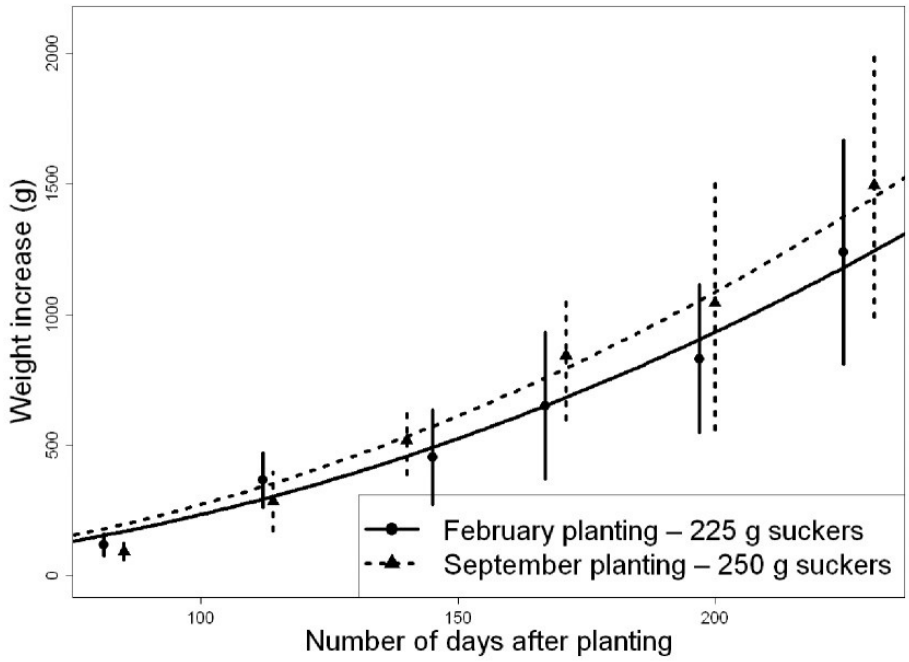

Figure 1. Queen Victoria plants were planted in February and September and 15 plants were harvested, washed, dried and weighed each month. Weight increases over time were fitted and for February, WI = 0.0233 x DaP^2 (R^2 = 0.89) and for September, WI = 0.0272 x DaP2 (R2 = 0.89). Weight increases for the two dates were significantly different (P<0.05).

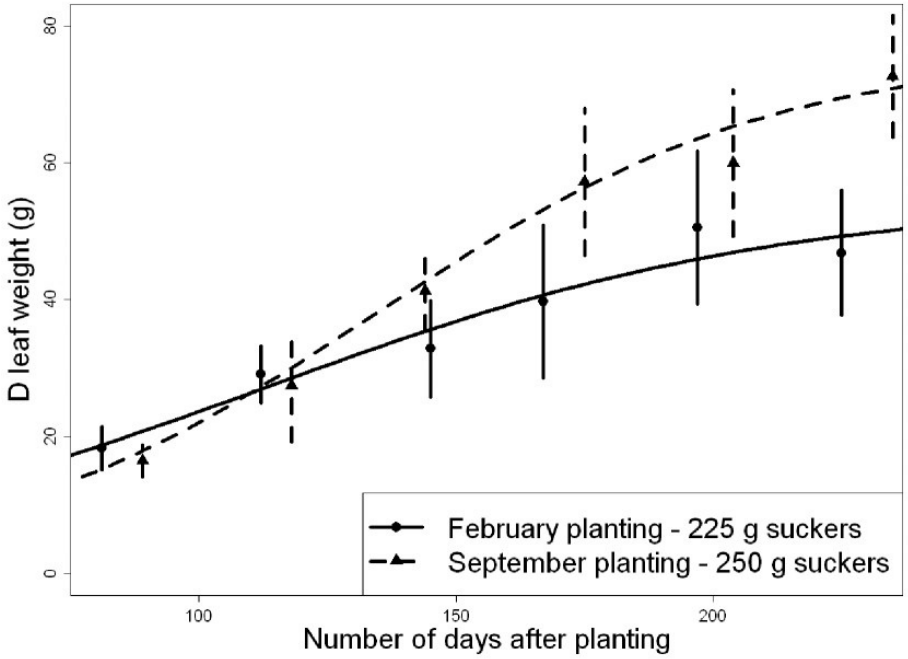

Figure 2. Increase in D leaf weights of Queen Victoria pineapple plants. The data for February were fitted by the equation D leaf weight = 54.51/(1+exp(2.27-0.02 x DaP) - R^2 = 0.61; weights for September were fitted by the equation D leaf weight = 76.13/(1+exp(3.48-0.0259 x DaP) - R^2 = 0.84.

Basis for a Heat-unit model of vegetative growth

The growth (WI) of Queen Victoria was then related to the sum of temperatures and compared in each situation. For this preliminary stage, we added the daily averages of temperature above a threshold temperature for plant growth set at 8.34°C (Determined by successive approaches to maximize the coefficient of correlation).

During the 2 periods, it appeared that growth was linearly linked to temperature (Figure 3), and was also statistically identical, revealing the possibility of designing an original and relatively simple model in which pineapple growth is a function of temperature.

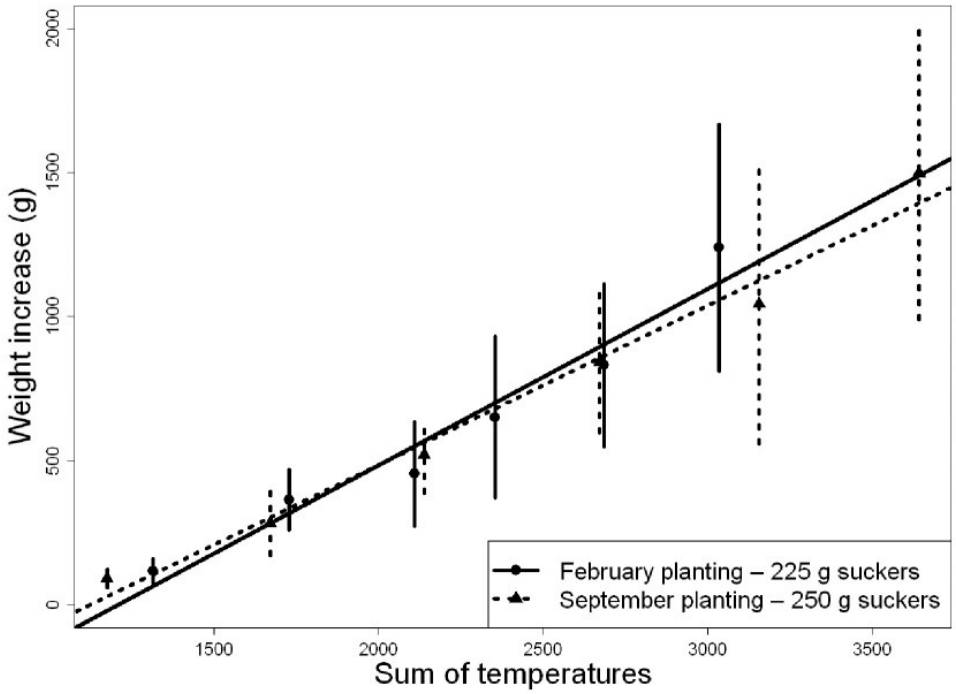

Figure 3. Growth in fresh weight of Queen Victoria as a function of sum of temperatures. Fifteen suckers were harvested monthly and were washed, dried and weighed. Temperature sums were accumulated by subtracting the threshold temperature of 8.34 °C from the daily mean temperature and summing. Equations for the lines were, for February, WI = 0.6161 x TTS - 742.7648 - R^2 = 0.65, and for September, September, WI = 0.5561 x TTS - 622.6384 - R^2 = 0.70 (differences not significant at p>0.05).

Conclusion

At the current state of our observations, it appears that an increase in Queen Victoria plant weight is highly correlated with the sum of temperatures (linear relationship) in no-restrictive growth conditions, thus allowing us to establish the basis of a thermal time model of pineapple growth. Further data analysis for other varieties would indicate if this thermal time model may be a general model for all cultivated pineapples. Such a model is the basis for the development of more sophisticated models to design new sustainable cropping systems (Tixier et al., 2008).

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A Bait and Trap Method for Sampling Symphylid Populations in Pineapple

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Symphylids (*Hanseniella* sp) are polyphagous soil-borne parasites. On pineapple, they feed on roots tips (meristem tissues) producing either short roots with irregular swelling or roots with multiple branching resulting in the typical " witches brooms ", (Kéhé, 1988; Py et al.1984; Petty et al., 2002). This behavior alters the root efficiency. The reddish plants look wilted with poor anchorage and growth. At field level, the crop appears as an irregular patchwork of diseased and normal plants, similar in appearance to nematode-induced symptoms (Py et al.1984; Kéhé, 1995; Soler, 1998; Perrier et al., 1993). Actually, diagnosing this disease in pineapple fields is rendered difficult due to possible confusion with other problems as water or nutrient deficiencies, nematodes, wilt (Kéhé, 1988; Py et al.1984).

As a consequence of the reduction in the use of pesticides due to environmental concerns, the control of symphylid populations in pineapple now requires particular and novel attention by researchers looking for an alternative to the chemical control of symphylid populations. Symphylids can be trapped and counted using appropriate bait or soil samples (Umble and Fischer, 2003a and Umble et al., 2006). We developed a reliable method to monitor symphylids in pineapple that combines bait and soil sample.

Material and methods

In a preliminary experiment we found that the trapping of symphylids was strongly improved by adding potato bait to the soil sample, compared to the soil sample alone or bait trapping alone. The bait and trap devices (Figure 1) are placed into the soil, carefully geolocalized and collected 3 days later for extracting and counting. Two methods for extracting and counting the symphylids were compared. In the first one (Figure 2A), soil aggregates were carefully broken apart before collecting the symphylids following the procedure of Umble (2006). In the second “floating” method (Figure 2B), the soil sample was carefully disaggregated in a 10 L bucket of water. Then floating symphylids were picked up with a small soft brush. The vertical distribution of the symphylid population was evaluated in pineapple and grass fallow rhizospheres (five replicates at four different depths from 4cm to 50cm).

Statistical analysis developed for nematode populations (Ferris, 1984, Perry et al., 2006) could, to some extent, be applied to symphylid populations, given that both are soil borne parasites developing in similar conditions and having many common traits (host-parasite relationship through the root system, pattern of development at field level, sensitivity to agro-climatic conditions). The spatial distribution of the populations in the field plots was evaluated using the "variance/mean" ratios (for details on calculations see Ferris, 1984, Perry et al., 2006) and spatial analyses based on Moran's and Geary's indices (for detailed calculations see Fortin, 1999 and Judas, 2002).

The method was tested in field plot of *Mucuna pruriens* cv *utilis* (a rotation crop) and in a grass fallow with different levels of infestation (plot aeras 800m² with 60 samples randomly chosen). Then, the method was also tested on pineapple (cv MD-2) at different stages of development of the crop (plot of 50m² each with 5 samples randomly chosen and repeated several times over different seasons). Finally, the method can be used to evaluate rotation crop for pineapple (experiment in course).

Results and Discussion

Extraction methods

The floating method was more efficient in recovering juveniles (12.9 versus 7; $p<0.001$) while recovery of total symphylids did not significantly differ between the two methods (Figure 3). Extraction durations were about six minutes per sample with both methods. The classical manual method allowed the recovery of symphylids in much better conditions for subsequent use (rearing for example) as the mortality was only 7% compared to 59% with the floating method. Nevertheless, as the adults/juveniles ratio can be used to determine if the population is growing or stagnant, the floating method appears to give a better picture of the evolution of the population.

Distribution of symphylids in soil profiles

Both tested sites, a grass fallow and a pineapple field, proved to be highly infested with an average of 47.2 and 44.6 symphylids respectively trapped in the grass fallow top soil and in the pineapple field. The number of symphylids did not significantly differ whatever the depth in both fields, implying that the infestation can spread not only at the surface but also deeper in the soil. Trapping symphylids only in the top soil (15cm) may be sufficient to monitor the population providing that climatic conditions at the time of sampling do not lead to a downward movement of the symphylids to find suitable conditions for their survival, for example during dry periods (Kéhé, 1988; Py et al.1984).

Examples of experiments and results allowed by the method

1) Maps of smoothed abundance data of symphylids observed at irregular locations (Figure 4) allowed a good visual evaluation of the distribution of the populations of symphylids in the field plots. Spatial analyses were made with the data obtained with different types of sampling (number of samples and number of traps per sample). The results showed that the symphylid populations are highly aggregated and that the range area for the development of symphylid populations appeared to be 4 to 6 meters wide, suggesting an optimized sampling with a regular spacing of about 4 meters between the traps ("variance / mean" ratios and Moran's and Geary's indices, Soler et al., submitted to Plant Dis.).

2) We used the bait and trap devices to monitor symphylid populations on a pineapple plot. Figure 5 shows that under pineapple, there were no symphylid up to the 4th monfh, then the populations increased up to forcing then declined after harvest. This probably reflects first, the efficiency of ethoprophos applied at planting and second, the reduced rhizogenic activity after harvest with no treatment since planting. The large standard deviations observed reflect the difficulty involved in obtaining a 'correct' sample in our small pineapple plots (50m²). But it also shows that the usual statistics (mean and standard deviation on raw data) are not very convenient for the description of aggregated populations of symphylids, as is also the case for nematode populations (Quénéhervé and Ferris, 1989).

3) The method is presently used to evaluate possible rotation crops as potential hosts of pineapple pests. Non-host crops could be used as rotation crops in new pineapple cropping systems (Figure 6). This experiment is presently in course.

Conclusion

Symphylids are one of the major pineapple pests in many production areas and their control has mainly been based on pesticide applications. With the reduction in the use of such pesticides due to environmental concerns, the control of this pineapple pest requires alternative to chemical applications. These alternative solutions will use ecologically-based integrated pest management (IPM). The method for trapping and counting symphylids described in this paper is an efficient tool to monitor symphylid populations in pineapple field plots and may help in validating new solutions for their control. Using this method we demonstrated symphylid populations are strongly aggregated, showing a range area for their development of about 4 to 6 meters and that they may be distributed homogeneously deep in the soil. Nevertheless, researchers need to take into account some basic facts concerning symphylid ecology, particularly their sensitivity to short-term climate variations such as drought or very wet conditions, which may temporarily affect the presence of symphylids in the upper part of a soil profile.

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Figure 1. Bait and trap device : how to use it? Black PVC "trapping" pots (250 ml volume, 9 cm high x 11 cm wide) with 76 holes 1.3 cm in diameter, 16 of which were at the bottom were buried 15 cm in the soil (entire pot was covered with soil). Prior to burial, the pots were gently filled to a depth of 3 cm with soil removed from hole in which the pot was to be buried. Bait consisting of three potato slices 2.5 cm in diameter and 2.5 cm thick was placed on the soil, the pot was filled to the top with additional soil and then buried.

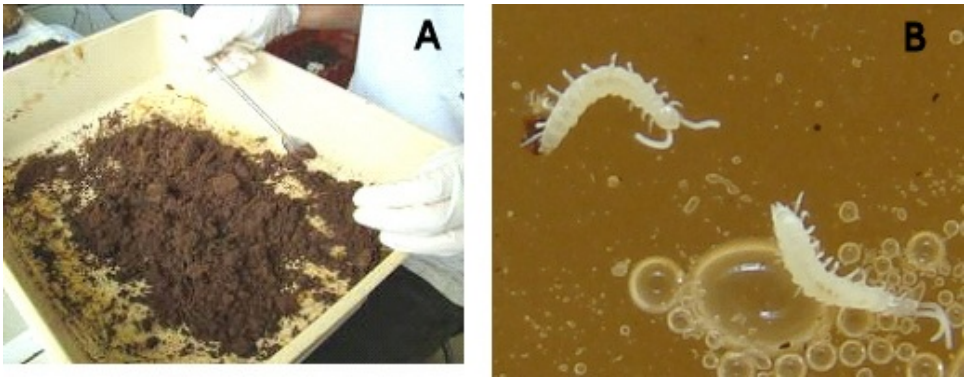

Figure 2. “Manual” (A) and “floating” (B) methods of sampling symphyllids. In the manual method, soil aggregates are carefully broken apart before collecting the symphylids. In the floating method, the soil sample was carefully disaggregated in a 10 L bucket of water and floating symphylids were picked up with a small soft brush.

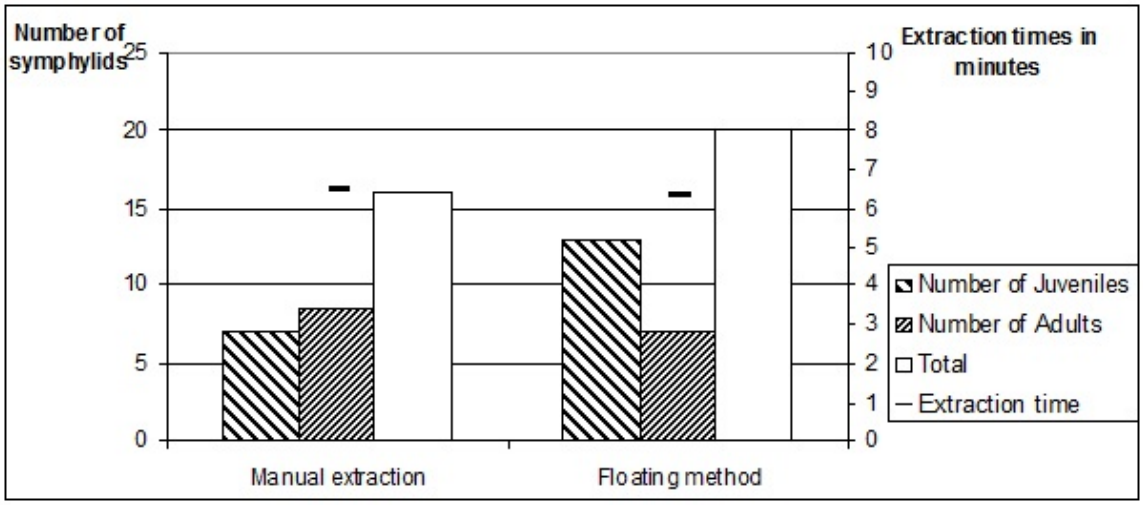

Figure 3. Comparitive efficiency of the "Manual" and "Floating" methods of symphylid extraction. Fifteen symphylids were inoculated in 250 ml of soil with organic matter. Samples were stored for 2 months at 29 °C with distilled water added daily to control humidity. Samples were counted after two months. There were ten replicates for each method.

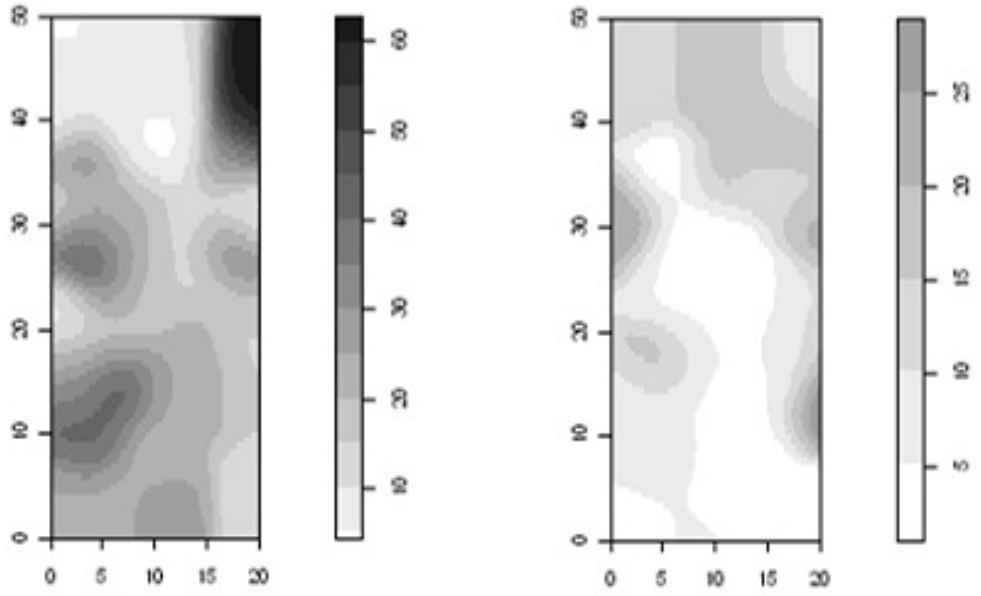

Figure 4. Maps of smoothed symphylid abundance data for plots planted to *Mucuna pruriens* cv *utilis*, (left) and grass fallow (right). Map scales in metres and the legends show numbers of symphylids per sample.

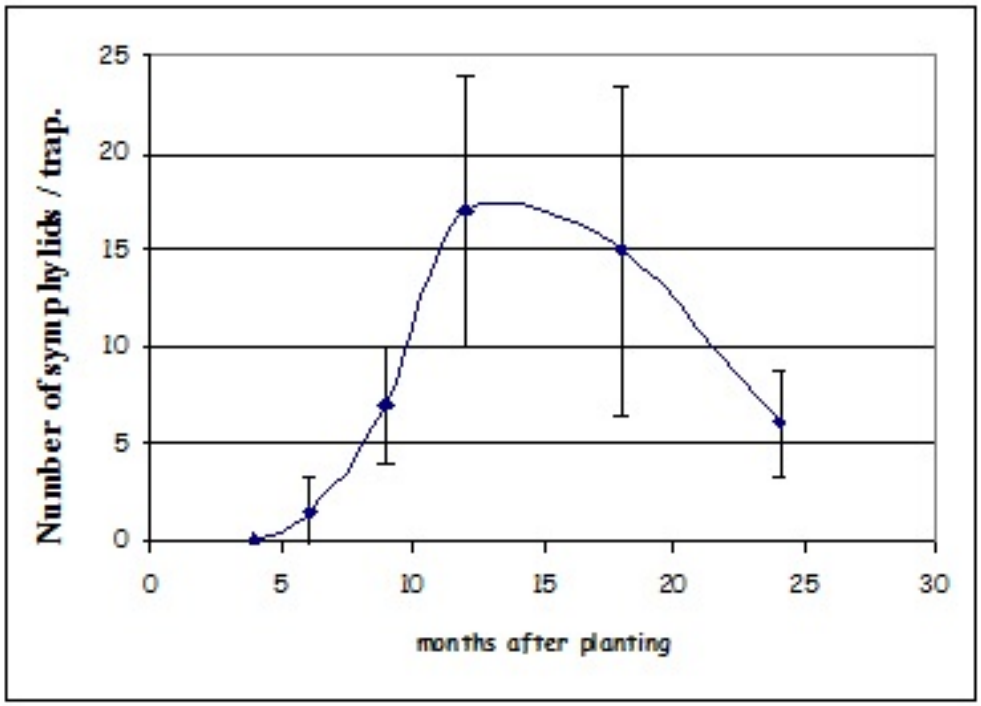

Figure 5. Variations in symphylid populations under MD-2 pineapple. Plots included 300 plants on 6 ridges giving 50m² plots. Five traps with bait were placed with a minimum space of 4 m between 2 traps. Simple samplings were made 6 times at different periods of the year for each stage of development of the pineapple plants. Forcing at 12 months and Post harvest at 18 and 24 months.

Figure 6. Evaluation of rotation crop for pineapple. Left : the trap with the potato baits is placed inside the plot. The traps are collected 3 days later and the operation is repeated during several times during the growth of the crop. Right : partial view of the experimental site with *Crotalaria spectabilis* in front and *Crotalaria juncea* behind.

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News from Ghana

Pineapple and Carbon Emissions

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Our organizations, West Africa Fair Fruit and the Dutch importer Agrofair, are undertaking a project to estimate the carbon footprint of pineapple in a commercial supply chain from Ghana to Europe using the recently released international standard PAS2050. We are doing this with consultants from the British science and agriculture consulting organization, ADAS. The end point will be in a verifiable carbon footprint (to be certified by the UK's Carbon Trust) and a plan for experimenting on how to reduce emissions.

Soils emit greenhouse gases as a consequence of fertilizer application and of soil processes. Soil emissions of nitrous oxide can be a significant contributor to the overall emissions from a product life cycle. Therefore to be able to make our footprint calculations complete we need to understand how soils used for pineapple production behave with respect to fluxes of nitrous oxide. Commercial pineapple grown in West Africa for the European market is planted through plastic mulch to conserve soil water. Farms growing pineapples tend to use very large amounts of nitrogen fertilizer, but some of this is applied to foliage above the plastic mulch. However, understanding of the source and sink strength of African soils for greenhouse gases is based very few relevant data. As part of our foot-printing project for pineapple we are looking for any data on how soils behave in conditions comparable to those in the pineapple zone of Ghana and in a high fertilizer input system with plastic mulch covered ridges. There is a need to understand the fate of the applied nitrogen fertilizer (partitioning between plant, soil, losses to atmosphere and by soil water drainage), the moisture status of the soil, the degree to which there are anaerobic conditions under the plastic mulch, soil temperature under the plastic mulch, and how these conditions favour nitrous oxide emissions. The rainfall is about 1000 mm per year in the production zone we are working in. We would be very grateful if any researcher or organization that has any insights on this could contact either Rob Moss (robmoss@waffco.org) or Jeremy Wiltshire (Jeremy.wiltshire@adas.co.uk).

Ed. Note: Rob Moss contacted me by email to ask if I had information they were seeking. I did not and invited them to contact the "pineapple community" directly for assistance. Please help if you can. D.B.◆

News from Malaysia

Handling of Fresh-cut Pineapple for Fresh Consumption

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ABSTRACT

Pineapple is one of the popular fruits served in fresh-cut form. At ambient temperature and without protective treatments, cut pineapple deteriorates rapidly, resulting in the development off-flavour and off-odour within a day. Fresh-cut pineapple sustains substantial tissue injury during processing; the disruption of tissue and cell integrity often increases respiration rate, ethylene synthesis, enzymatic browning and development of physiological disorders with associated increases in rates of other biochemical reactions responsible for changes in colour (including browning), flavor, texture and nutritional quality (sugar, acid and vitamin contents). The damaged plant tissues also provide a nourishing medium for microbial survival and growth. Chemical treatments such as sodium chloride, calcium chloride and ascorbic acid were used to improve the taste, flesh firmness and overcome the browning problem that occurred in 'Josapine' cut pineapple. The pineapple was cut into spears by machine (Fig 1A, 1B) and packed into rigid polypropylene containers (Fig 2C, 2D). Oxygen absorbent was inserted into the package for quality enhancement, which allows fresh-cut pineapple to be stored for 2 weeks at 2°C, 1 week at 10ºC and 2 days at 25ºC. Such storage periods provide sufficient time for fresh consumption of fresh-cut pineapple at the market shelf. A successful export trial was conducted in 2006 by MARDI by air shipping fresh-cut pineapple to the Netherlands. The quality of the fresh-cut pineapple remained good even after 6 days on the retail shelf. In 2009, shipment by refrigerated truck to Singapore was also successful. During both export trials, good collaboration was obtained from the Malaysian Pineapple Industrial Board (MPIB), Department of Agriculture (DOA), Federal Agricultural Marketing Authority (FAMA), local fruit exporters and fruit importers at the importing countries (Netherlands and Singapore).

Figure 1A, machine for cutting pineapple spears; 1B, fresh cut spears ready for packing; 1C and 1D, polypropylene containers packed with fresh-cut pineapple.

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News From Taiwan

RAPD Marker Assisted Selection of EMS Induced Pineapple (Ananas comosus (L.) Merr.) Mutants

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Introduction

Chemical mutagens have become more important for crop breeding and gene functions studies in recent years. Cross-breeding generally is the first choice in plant breeding programs but in some cases mutations induced on in vitro shoots, followed by clonal propagation and field selection procedures have higher probability of success (Predieri, 2001). Induced mutations, which change only one or a few specific traits of a cultivar but still meet the requirements of both the fruit industry and consumers, can offer a vast potential for fruit breeding (Predieri, 2001).

Molecular markers are detectable in all tissues regardless of the developmental stages and are not affected by the environment, pleiotropic and epistatic effects (Agarwal et al., 2008). The objective of the present study was to test the potential of using RAPD markers to analyze the putative mutants of pineapple that were produced by treatment with ethyl methane sulfonate (EMS). The effect of pH values of EMS solutions on germination rate of dormant axillary buds excised from pineapple crowns was also tested, which could be used as an alternative method for mutation induction in pineapple.

Material and methods

Fresh crowns of 'Tainung 17' pineapple were used in the present study. After the leaves were removed, crown stems were soaked in solutions containing 0.4% (v/v) EMS and 2% (v/v) dimethyl sulfoxide (DMSO), which was added as a carrier agent (Omar et al.,

1989), at different pH values for 8 hours. The pH was adjusted to 6.0, 7.0, or 8.0 with citrate-phosphate buffer (0.2M Na_2HPO4 mixed with 0.1M citrate). The pH value of non-buffered 0.4% EMS solution was 3.75. Untreated crowns were used as the control. The stems of four crowns were the replicates for each treatment. After soaking, stems were washed three times with distilled water followed by sterilization with 1% sodium hypochlorite solution for 20 minutes. About 20 dormant axillary buds from each stem were aseptically excised and then incubated in half strength MS (Murashige and Skoog, 1962) medium supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar, 2 ppm 6-benzyladenine (BA) and 0.2 ppm 1-naphthalene acetic acid (NAA). The pH value of the medium was adjusted to 5.8 before autoclaving. Germination rates of dormant axillary buds were recorded four weeks after incubation.

In another set of experiments, solutions containing 0, 0.02, 0.04, 0.06, 0.08, 0.1% (v/v) EMS were micro-filtered (0.22 um; Minisart, Sartorius) and added into sterilized half strength MS medium. 'Tainung 17' *in vitro* plantlets (5mm-10mm in length) were cultured on the EMS mutation induction medium for about two months. The plants were then transplanted into pots and hardened in a net house. Three plants were randomly selected from each treatment and the genetic dissimilarity of those plants was analyzed by RAPD-PCR with ten Operon RAPD primers, OPA-03, OPA-17, OPG-03, OPG-08, OPG-16, OPG-18, OPJ-04, OPJ-11, OPJ-13 and OPJ-19. Based on the RAPD results, the proximity of the selected plants was analyzed and a dendrogram was generated by UPGMA (unweighted pair group method with arithmetic averages).

Results and discussion

In the EMS-DMSO study at different pH values, the bud germination rate in the pH 6.0 treatment was 36.5% after 4 weeks of incubation, which was significantly higher than that in the un-buffered treatment (Table 1). Apparently, adjusting the pH of EMS solution to near neutral reduced the phytotoxic effects of EMS (Table 1). Mhatre and Rao (2002) reported that the number of multiple shoots of pineapple formed decreased as the treatment duration and concentration of EMS solution increased. As a result, to be effective and efficient, concentration and pH of the mutagen solution, adding a carrier agent such as DMSO, and duration of treatment, all should be taken into consideration. In addition, treating dormant buds of pineapple crowns has a benefit over *in vitro* shoots because the shoots generated from the apex of the dormant buds can become new plants with a different genetic composition if mutations occur.

Molecular marker-assisted selection is a breeding method which provides the potential for increasing selection efficiency by allowing for earlier selection and reducing the plant population size required for field selection (Khawaleet al., 2007). RAPD is one type of molecular marker and the basis of this technique is using short random oligonucleotide sequences for differential PCR amplification of genomic DNA (Williams et al. 1991; Agarwal et al., 2008). The main advantages of RAPD are that it is simple, cheap and there is no requirement for DNA sequence information. However, its reproducibility is lower and it is also difficult to analyze approximate fragments (Tingey and Tufo, 1993; Schlötterer, 2004). In the present study, the average genetic similarities between EMS treated plants and the control decreased as the concentration of EMS increased (Table 2). The proximity analysis (Figure 1) also showed that higher concentrations of EMS induced plants that were more distinct from the control. It seems that the genetic dissimilarities of EMS treated plants gradually increased when the EMS concentration increased. This indicates that EMS concentration plays a critical role in the efficiency of mutation induction in pineapples and RAPD is able to detect the difference in EMS induced mutants.

Some induced morphological changes of EMS treated pineapple progenies were found in the present study such as changes in chlorophyll and anthocyanin contents in leaves, elongation and swelling of roots, leaf length and width, and spine patterns on leaf margin, etc.. These mutagen-derived morphologic traits might be useful for further gene function studies. In addition, those EMS treated plants produced in this study have been transplanted in the field for further evaluation and possible selection. Flowering and fruit characteristics will be examined.

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Table 1. Regeneration of axillary buds of 'Tainung 17' pineapple crowns after an 8 h soak in 0.4% EMS-2% DMSO solutions having different pH values.[†]

EMS-DMSO solution pH	Regeneration (%)
Check (no EMS treatment)	49.3±3.5
3.75	9.5±10.3
6.0	36.5±20.9
7.0	21.2±10.8
8.0	28.3±25.0

[†]Crowns were treated with the EMS-DMSO solutions for 8 hrs before the dormant buds were excised and cultured in vitro for four weeks. Data represents the results of buds from stems of four crowns.

Table 2. Average genetic similarities between pineapple plantlets grown in media containing various concentrations of EMS and the control based on the RAPD analysis.[†]

Concentration (%)	Genetic similarity
0.02	0.948
0.04	0.928
0.06	0.923
0.08	0.896
0.10	0.896

[†] Three plantlets were incubated in medium containing each of the different concentrations of EMS for about two months.

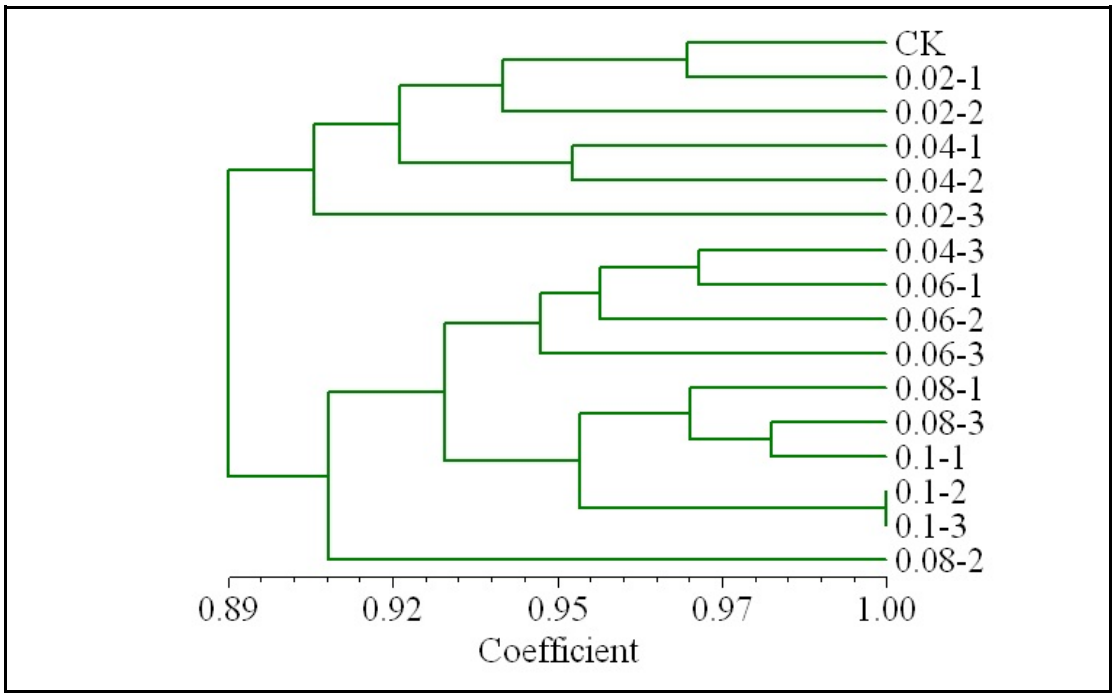

Figure 1. Dendrogram of EMS treated 'Tainung No.17' pineapple plantlets generated by UPGMA cluster analysis based on similarity matrix of RAPD analysis. CK represents the control plantlet while 0.02-1~3, 0.04-1~3, 0.06-1~3, 0.08-1~3 and 0.1-1~3 represent plantlets from media containing 0.02% ~0.1% EMS.

News From the United States

Role of Proteases During Ripening of Pineapple Fruit

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In a paper recently published in Plant Physiology, Neuteboom, et al. (http://www.plantphysiol.org/cgi/content/abstract/151/2/515/) describe a new process for the regulation of a protease and its inhibitor during pineapple fruit ripening. Fruit ripening and senescence involve hydrolysis and softening of tissues and a dramatic loss of chlorophyll. Various cellulases and proteases participate in the process. The most abundant cysteine protease in green and ripe pineapple fruit is bromelain, which accumulates without an inhibitory propeptide. How is bromelain regulated over time to avoid damaging cells until the stage of fruit development when it is needed for proteolysis? Cystatins are potent inhibitors of cysteine proteases, however the bromelains are enigmatically recalcitrant to inhibition by plant and animal cystatins. The researchers examined a novel secreted pineapple cystatin (AcCYS1), which has a uniquely long Alanine and Glutamate-rich N-terminus absent in other cystatins. They show the N-terminus is required for complete inhibition of stem and fruit bromelain. After the pineapple cystatin is synthesized, it inhibits the bromelain until ripening begins. Cleavage of the N-terminus from AcCYS1, a process which is specific to ripening fruit, renders the AcCYS1 inactive as an inhibitor against fruit bromelain, but not stem bromelain, thereby significantly enhancing bromelain activity in ripe fruit.

Environmental Friendly Approaches for Managing Nematodes and Weeds on Pineapple

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Introduction

Reniform nematodes (*Rotylenchulus reniformis*) can reduce pineapple yields by up to 38% in the plant crop and by 60% in the ratoon crop. Currently, pre-plant fumigation with 1,3-dichloropropene (Telone®) or metam sodium (Vapam®) is practiced commonly in Hawaii to manage plant-parasitic nematodes. Because of environmental health concerns, increasing fuel costs, and the risk of losing soil fumigants, alternatives to soil fumigants are needed. Soil solarization (Fig. 1) and cover cropping are viable non-chemical alternatives for managing soil-borne plant-parasitic nematodes. Previously, it was determined that sunn hemp (*Crotalaria juncea*) (Fig. 2) possesses multiple mechanisms in suppressing reniform nematodes (Wang et al., 2001). These mechanisms include being a poor host of reniform nematodes, delaying the development of female reniform nematodes, producing an allelopathic compound that is toxic to the nematode, and enhancing nematode-trapping fungi that could prey on the nematode (Wang et al., 2001). However, suppressive effects of a sunn hemp cover crop against renifrom nematodes are not consistent (Wang et al., 2002). Therefore, experiments are being conducted to determine if integrating sunn hemp cover cropping with soil solarization could improve reniform nematode suppression.

One challenge in managing reniform nematodes is their capability to survive in dry conditions through "anhydrobiosis." Anhydrobiosis is a survival strategy whereby the nematode coils and survives months to years without feeding (Tsai, 1978). Nematodes in their active stage are generally easier to kill by various nematode control strategies than when they are in their quiescent stage. Common pineapple plantation practice includes deep sub-soiling and fallowing the field for a year before the next crop planting. This may allow nematodes to enter an anhydrobiotic stage, making them more difficult to be killed. In addition, solarization only heats the top 10 to 20 cm of the soil (Chellemi, 1997; Wang et al., 2006). Thus, solarization might not be efficient in suppressing reniform nematodes that move deeper in the soil. Therefore, the objectives of the current research were to examine the potential of integrating sunn hemp cover cropping and solarization: 1) as alternatives to soil fumigation for nematodes and weed management; and 2) in improving soil health conditions as compared to fumigation.

Materials and Methods

A field trial was conducted at the Whitmore Experiment Station to compare the impacts of preplant treatments: 1) sunn hemp (SH) planted for 6 months, 2) solarized (Sol) for 2 months between March 27 and May 20, 2009, 3) SH planted for 4 months followed by 2 months of solarization (SH+Sol), and 4) fallow with weeds (control, C). Pineapple slips were planted into the prepared plots. At 3 months after pineapple planting (MAP), soil samples were taken from a nearby commercial pineapple field planted on the same date but fumigated with Telone® (1,3-d) followed by intensive herbicide application (Sipes, 2000). The purpose of this was to compare the above treatments with standard plantation practices. Soils were sampled prior to treatment installment (Pi1), after sunn hemp incorporation (Pi2), at the end of solarization (Pi3), and at 3 months after pineapple planting (MAP), which was done on July 9, 2009. Ten 20-cm deep soil cores were sampled per plot and composited. Nematodes were extracted from 250 cm^3 of soil by elutriation (Byrd

et al., 1976) and counted. Number of pineapple planting holes with weeds present was recorded at 2 and 3 MAP. Soil health was compared among treatments and plantation practice at 3 MAP using nematode community analysis as described in Wang et al. (2006).

Results and Discussion

Solarization performed during spring-early summer in Hawaii generated significant amounts of heat, achieving a maximum temperature of 55 °C for both Sol and SH+Sol in the 0-10 cm soil layer. However, maximum temperature in the 11-20 cm soil layer only reached 37 °C in the Sol treatment and 41 °C in the SH+Sol treatment. Sol accumulated 174 hours above 42 °C, the lethal temperature for reniform nematodes, in the top soil layer, while the SH+Sol treatment accumulated 135 hours. Lethal temperatures were not reached in the deeper layer. Fourteen hours at 42 °C is required to kill reniform nematodes (Wang and McSorley, 2008). At 3 MAP, Sol did not significantly suppress reniform nematodes (Fig. 3). Numbers of reniform nematodes were reduced below the economic threshold level (1 reniform/g soil, i.e. ~250 reniform/250 cm^3 soil) in the SH and SH+Sol treatments as compared to the control (Fig. 3). However, SH+Sol did not improve the reniform nematode suppressive effect as compared to SH. On the other hand, SH+Sol suppressed weeds more efficiently than SH or Sol alone (Table 1), and reduced weed pressure to one-third that of the control.

Free-living nematodes are good bioindicators of soil health because they are ubiquitous and have diverse feeding behaviors and life strategies (Bongers, 1990; Ferris et al., 2001; Neher, 2001; Yeates et al., 1990) and respond quickly to environmental soil disturbances (such as tillage, chemicals, pollutants, etc). Abundance or ratios of nematode trophic groups (Neher, 2001), diversity and richness and nematode faunal analysis (Ferris et al., 2001) are good indicators of soil health conditions. A comprehensive nematode community analysis was performed to evaluate soil health. Only nematode richness (number of genera), diversity, ratio of fungivores to bacterivores (F/B) and enrichment index (EI) are presented. EI is obtained by calculating the weight abundance of opportunistic bacterivores that responded quickly to nutrient enrichment and, therefore, is an indicator of soil rich in nutrients. Soil collected from the commercial pineapple field (T) had much lower richness than any of the treatments (Fig 4A). Although diversity was not different among treatments at 3 MAP, the commercial field and control treatment ranked lowest in diversity, another indication of less healthy soil conditions (Fig 4B). Higher F/B ratios usually indicate soil communities that are dominated by fungal decomposition rather than bacterial decomposition. Domination of fungal decomposition often is associated with environmental stress conditions (Ferris et al., 2001; Neher, 2001). At termination of solarization (Pi3), the F/B ratios indicated that Sol temporarily created a stressful condition, but this disturbance dissipated at 3 MAP (Fig. 4C). Integrating SH+Sol slightly reduced F/B at Pi3, indicating sunn hemp planting reduced the stress condition created by Sol (Fig. 4C). Although not significant, the highest F/B ratios were in the commercial field and in the control treatment.

Table 1. Pre-planting treatment effects on percent of pineapple planting holes with weeds present after planting on July 9, 2009.

Treatment	6 Aug 09	3 Sept 09
Control (fallow)	9.22 a	14.50 a
Sunn hemp	4.76 ab	9.08 a
Sunnhemp+Solarization	2.62 b	4.39 b
Solarization	5.54 ab	8.74 ab

Means are averages of 4 replications. Means in a column followed by same letters are not significantly different as determined by the Waller-Duncan (k-ratio) t-test. Data were transformed by the expression log (x + 1) prior to analysis.

Based on EI, SH significantly enriched soil nutrients at Pi2 (Fig 5). Although EI was temporarily reduced at termination of Sol (Pi3), this disturbance dissipated at 3 MAP. The commercial field had a lower EI than all treatments in the study area at 3 MAP, indicating a nutrient depleted condition. D-leaf weight was collected at 6 MAP, but no difference was observed among treatments.

In summary, planting sunn hemp without solarization significantly reduced the reniform nematode population below the economic threshold level at 3 MAP. Solarization only increased soil temperature at the top 10 cm of the soil and did not suppress reniform nematodes as compared to the control. Sunn hemp followed by a two-month solarization slightly increased soil temperature in the deeper soil layer as compared to Sol alone. Sol results could be better if Sol was performed during mid-summer. However, SH+Sol suppressed weed densities better than SH or Sol alone. Planting of sunn hemp enhanced nematodes involved in soil nutrient enrichment while the commercial field had the lowest enrichment index. On the basis of this index, the SH+Sol treatment is a more environmentally friendly option than standard pineapple plantation practices in Hawaii.

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Figure 1. Soil solarization accomplished using transparent, 25-µm-thick, uv-stabilized, low-density polyethylene mulch for two months.

Figure 2. Sunn hemp, *Crotalaria juncea*, is a tropical leguminous cover crop that has nematicidal properties and is commonly used as green manure.

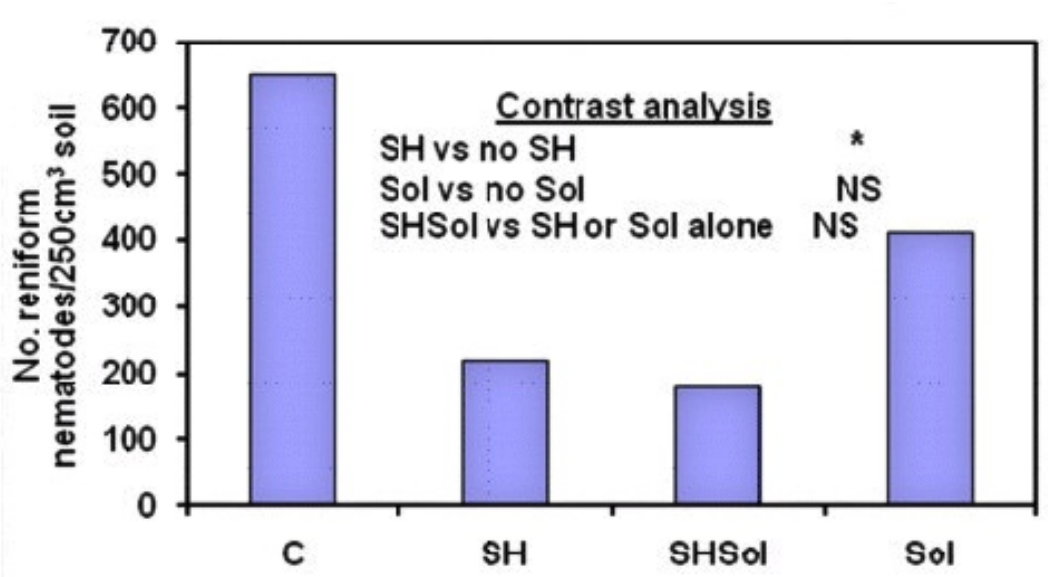

Figure 3. Number of *Rotylenchulus reniformis* nematodes recovered from soil 3 months after planting pineapple at Whitmore, Hawaii. Treatments are fallow control (C), sunn hemp (SH), SH + solarization (SHSol) and solarization (Sol). Data are means of 4 replications. No differences were found after analysis by one-way ANOVA but significant differences between SH or no SH ($P < 0.05$) were found using contrast analysis. The data were transformed by the expression $\log(x + 1)$ prior to analysis.

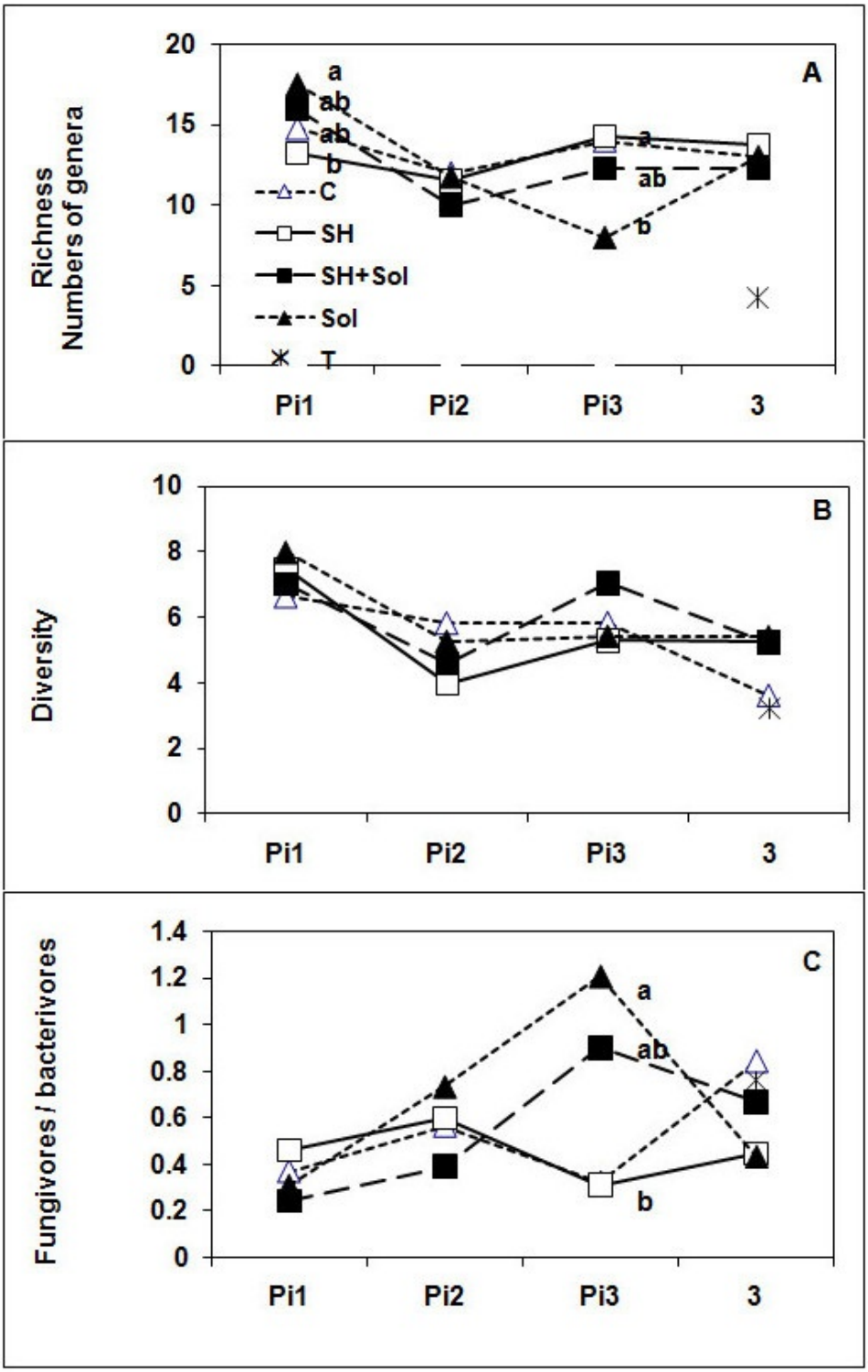

Figure 4. Nematode richness (A), nematode diversity (B), and the fungivores:bacterivores ratio (C) at the initiation of the experiment (Pi1), termination of sunn hemp cover cropping (Pi2), termination of soil solarization (Sol) (Pi3) and 3 months after planting of pineapple (3). Symbols represent fallow control (C), sunn hemp (SH), two month solarization (Sol), SH followed by Sol (SH+Sol) and samples from a commercial field treated with 1,3-d and herbicide (T). Treatment means followed by the same letter are not significantly different, $P \leq 0.05$, n=4.

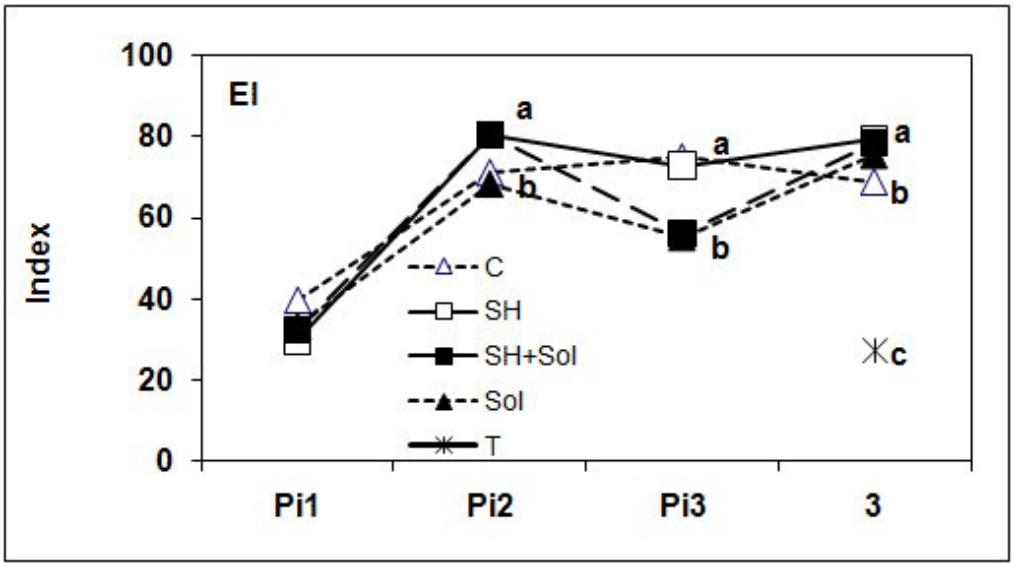

Figure 5. Enrichment index (EI) at the initiation of the experiment (Pi1), termination of sunn hemp cover cropping (Pi2), termination of soil solarization (Pi3) and 3 months after planting pineapple planting (3). Symbols represent fallow control (C), sunn hemp (SH), two-month solarization (Sol), SH followed by Sol (SH+Sol) and samples from a commercial field treated with 1,3-d and herbicide (T). Treatment means followed by the same letter are not significantly different, $P \leq 0.05$, n=4.

Commonalities of Pineapple, Agaves, and Cacti

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It all began with a taste test! Neimiah Grew reported in 1682 on the special acidic (bitter) taste of succulent plants in the morning. The taste test was expanded by Benjamin Heyne, who reported in 1815 that various succulents tasted much more acidic at dawn than at dusk. This nocturnal acidity increase was further studied in members of the Crassulaceae, becoming known as Crassulacean acid metabolism (CAM). As you all know, the most famous member of the family is pineapple, *Ananas comosus*. This bromeliad is cultivated on approximately 900,000 hectares worldwide for its fruit.

The biochemical details of CAM took a while to be elucidated. World War II stimulated advances in experimental methodology and instruments. Isotopes, especially ^{14}C, became available for tracing metabolic pathways, and chromatographic techniques were greatly improved. Based on work by Harry Beevers, Stanley Ransom, and Meirion Thomas in England and Hubert Vickery in the United States, much of the biochemistry of CAM was understood by the mid-1950s. Yet gas exchange studies indicating the daily pattern of CO_2 uptake and water vapor release by CAM plants were not interpreted with respect to the acidity studies until the 1960s.

The key feature of CAM shared by pineapple, agaves, and about 99% of cacti is the nocturnal uptake of CO_2. At night, CO_2 from the atmosphere is joined onto the three-carbon compound phosphoenolpyruvate (PEP), a step catalyzed by PEP carboxylase in the cytosol of mesophyll cells. This leads to malate, oxaloacetate, and other acids that are responsible for the aforementioned nocturnal increase in acidity. These acids are stored during the night in the large central vacuoles of mesophyll cells. During the next daytime, the C_4 acids are released from the vacuoles, decarboxylated, and the CO_2 thus produced intracellularly is fixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in the chloroplasts of the mesophyll cells. This latter pathway is the same as that used by C_3 plants, which comprise about 92% of plant species including crops such as wheat, rice and most fruits and vegetables. About 1% of plant species (e.g., maize [corn], sugarcane, sorghum, tropical grasses) use the C_4 pathway, while 7% use the CAM pathway, our primary focus here. Of the three photosynthetic pathways, only CAM plants have nocturnal stomatal opening and the accompanying nocturnal uptake of CO_2.

To understand the benefits of the CAM pathway, we shift our attention from CO_2 to water vapor. In particular, the much lower temperatures prevailing at night when the stomata of CAM plants open lead to much less water vapor diffusing out of them compared with C_3 plants and C_4 plants, whose stomata open during the daytime. A useful analogy is the rapid drying of a wet towel during the daytime versus the slow drying of a wet towel at night. This is because the water vapor concentration at saturation rises nearly

exponentially with temperature. And the inside of a pineapple or agave leaf (or a cactus stem) is essentially saturated with water vapor. The much lower force driving water vapor out of the leaves for the lower temperatures at night compared with the daytime leads to a higher water-use efficiency (WUE), the ratio of CO_2 fixed to water transpired. Indeed, the WUE of CAM plants averages about four-fold higher than for C_4 plants and eight-fold higher than for C_3 plants under the same environmental conditions.

Another interesting parameter is biomass productivity, which varies with plant species and photosynthetic pathway. Using measurements up to 1990, when such studies were fairly common, the five highest biomass-producing species averaged 41 tonnes of dry mass hectare^{-1} year^{-1} for C_3 plants, 52 for C_4 plants, and 42 tonnes hectare^{-1} year^{-1} for CAM plants. Pineapple was among the five highest-producing CAM species, with 35 tonnes hectare^{-1} year^{-1}, while two agave species averaged 40 and two cactus species averaged 47 tonnes hectare^{-1} year^{-1}. Thus, despite having the extra steps involved in nocturnal uptake and processing of CO_2, CAM plants can still have substantial biomass productivity.

The CAM species cultivated on the most area worldwide is the prickly pear cactus, *Opuntia ficus-indica*. More than 2.1 million hectares are cultivated in over 20 countries, mostly for forage and fodder. The area for the cultivation of its fruits, which are increasing called cactus pears to avoid drawing attention to the nasty spines, is about 140,000 hectares. The country currently with the largest cultivation of *O. ficus-indica* is Brazil, with 600,000 hectares, with nearly as much cultivated in Tunisia, in both cases mainly for forage and fodder . The most extensively cultivated agave is *Agave tequilana*, with 80,000 hectares used for tequila production in Mexico, while about half as much area is devoted to other agave species for other beverages.

How will net CO_2 uptake and biomass productivity of pineapple, agaves, and cacti be affected by global climate change? With regard to increasing atmospheric CO_2 levels, the news is good, as productivity increases by about 1% for each 10 ppm increase in atmospheric CO_2 level (currently, the CO_2 level is increasing at just over 2 ppm per year). None of these three taxa love freezing temperatures, so the generally increasing global temperatures are also good news. In addition, CAM plants cope well with changes in rainfall patterns. All of these influences on net CO_2 uptake and productivity can be quantified using an Environmental Productivity Index (EPI).

EPI indicates the fraction of maximal net CO_2 uptake over 24-hour periods under any set of environmental conditions. It incorporates individual multiplicative indices for the effects of light, temperature, water status, nutrients, and atmospheric CO_2 level. EPI has been used to interpret and predict the productivity of agaves and cacti, and it is ripe for application to pineapple. More detail on EPI and the other topics presented above are described in P.S. Nobel, "DESERT WISDOM/AGAVES and CACTI: CO_2, Water, Climate Change" published by the author in 2010 and available through iuniverse.com, amazon.com, and barnesandnoble.com for $16.95 ($6.00 for the ebook available through iuniverse.com).

Ed. Note: Distinguished Professor Park Nobel is one of a few people who has spent much of a career investigating the productivity of plants with CAM. I had not thought of cacti as being important agricultural plants until I began reading his interesting book and found it to be most interesting. I though readers of **Pineapple News** would be interested to know of his work. I believe there are principles in the book that we “pineapple people” can learn from. ◆

Services

The listings under Commercial Services and Directory of Professionals is maintained as a convenience to readers and should in no way be construed as an endorsement of those providing commercial or professional services. Those offering specialized services to pineapple growers or researchers are invited to contact the editor for possible inclusion in the listings below.

Commercial Services

Maintain CF 125 continues to be available for use in pineapple plant propagation. A renewal letter for registration of the product was received in 2003. For further information, contact Bhushan Mandava, Repar Corporation, P.O. Box 4321, Silver Spring, MD 20914 Tel: 202-223-1424 Fax: 202-223-0141; E-Mail: mandava@compuserve.com

Centro de Bioplantas. Dr. Justo L. Gonzalez Olmedo, Director of Foreign Affairs Office, Centro De Bioplantas. Universidad De Ciego De Avila, Carretera a Moron Km 9. Cp69450. Cuba. Centro De Bioplantas offers certificates of authenticity for pineapple material propagated in their tissue culture facility. Web site: http://www.Bioplantas.cu

LAMERSA, Dole's meristem laboratory in Honduras. Contact John T. Mirenda PhD, Dole Fresh Fruit International Ltd., San Jose, Costa Rica. Phone: 506 287 2175. Fax: 506 287 2675. E-mail: Jmirenda@la.dole.com. The laboratory can produce meristematically-derived plants of pineapple as well as banana and other crops.

Thai Orchids Lab, Dr. Paiboolya Gavinlertvatana. Horticulture/ agriculture/ forestry tissue culture laboratory with exports to Australia, U.S.A., Africa, and Asia. MD2 pineapple available (open to acquiring additional varieties) or confidential exclusive contract propagation. Phone: +1 510 931 7865 Fax: +66 2510 9452 Website: http://www.tolusa.com/ E-mail: info@tolusa.com.

Vitropic, Zone d'Activités Economiques des Avants, 34270 Saint Mathieu de Tréviers France; Tel: + 33 (0)4 67 55 34 58; Fax: + 33 (0)4 67 55 23 05. E-mail : vitropic@vitropic.fr. Web site: www.vitropic.fr. Vitropic proposes the best individuals from the CIRAD FHLOR selected clones including: Cayenne Group, Queen Group, Perolera Group, MD2, Ornamentals pineapples. The range is continuously extending, do not hesitate to ask for more information.

Professional Services

Mr. Wilbert Campos Alvarado. M.Sc. Tropical Soils & Crop Mgmt. E-mail. wcamposa@gmail.com. Phone: (506) 8815-7271. Apdo. Postal 536-7210, Guapiles, Costa Rica. Experience in all stages of production (soil preparation, plant nutrition, diseases & pest control, PGR use, etc) of pineapple for the fresh fruit production market as well as experince in packing plant management and in postharvest treatment. Also worked in pineapple R&D for several years under different climate conditions (Costa Rico, Guatemala, Ecuador).

Ing. Alejandro Chavarría. APDO 4437-56 Pital, San Carlos. Alajuela, Costa Rica. Tel: (506) 88-20-79-55 / (506) 24-73-40-00, alechava@hotmail.com . I have worked like an International Pineapple Consulting in México, Costa Rica and Brazil. Experienced in project feasibility, plantation design, agricultural machinery, all aspects of farm crop management, post harvest management and establishment of good agricultural practices.

Dr. Mark Paul Culik. INCAPER, Rua Alfonso Sarlo 160, CEP 29052-010, Vitoria, ES, Brazil; Tel: 27-3137-9874; markculik3@yahoo.com. Experience: PhD in Entomology with more than 25 years of agricultural pest management experience in crops ranging from apples to papaya and pineapple, identification of pests and beneficial arthropods ranging from Collembola to fruit flies, and current work on scale insects with emphasis on pineapple mealybugs. Areas of specialization: Entomology, Insect and Pest Identification, Integrated Pest Management.

Dr. Francisco Gomez (E-mail: fgomez1@cablecolor.hn) and **Jose R. Vasquez**, MBA (E-mail: jrva46@excite.com). Golden Pacific Ag Services, PO.Box 15088, Lomas Miraflores, 4a. Calle, 1a Avenida # 4326, Tegucigalpa, Honduras. Phone: 504 230 1120; 504 969 5568.
Experience: Pineapple and melon production, from seed propagation-planting-field maintenance-forcing-harvesting-post-harvest management and commercialization.

Mr. L. Douglas MacClure. 360 Hoopalua Dr., Pukalani, Hawaii, U.S.A. E-mail: norfolkldm@aol.com.
Experience: More than 39 years with Maui Pineapple Company heading plantation and diversified agriculture operations and started the Royal Coast Tropical Fruit Company in Costa Rica. Collected and summarized production information in Asia and Central America. Also consulted on pineapple for companies and growers in El Salvador, Australia, Thailand and Indonesia.

Mr. Graham J. Petty 13 Somerset Place, Lambert Road, Port Alfred, 6170, Republic of South Africa. Phone: +27 (0) 46 624 4868; Tel/Fax: +27 (0) 46 625 0946; E-mail: grahamp@imaginet.co.za. Experience: M.Sc. (Agric) Pretoria : Pr. Sci. Nat. . Researcher and advisor to the South African Canning Pineapple Industry on matters of Pest Management in pineapple culture, for 34 years. Economic entomology and management of biological control agents have received particular attention.

Mr. Col Scott. E-mail: scottch45@bigpond.com. Mobile: +61 488092442; Phone: +61 7 34252417; Fax: +61 7 34252417. Over 37 years experience in all aspects of pineapple agronomy and research in Australia (32 years with Golden Circle Ltd) and South Africa (5 years with Summerpride Foods Ltd). Experience includes working with growers, researchers and fertilizer and agricultural chemical suppliers. Other production areas visited include Hawaii, Central America, Thailand, Indonesia and Malaysia.

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Book Reviews and Web Sites

Book Reviews

No reviews were provided for this issue.

Web Sites of Possible Interest

Nothing new at this time.◆

New References on Pineapple

The list below includes papers related to various aspects of pineapple culture, physiology, processing, preservation or byproducts that were published or located since the last issue of the newsletter was printed. Some papers may seem relatively unrelated to pineapple but since judgement must be exercised when including or excluding references, the decision was made to ere on the side of inclusion so as to serve as many readers as possible. Often, abstracts of the papers listed below can be found on-line and of course all abstracts of paper published in Acta Horticulturae are available from info@ishs.org.

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A pineapple references database containing over 7,600 references on pineapple is maintained by the editor. Literature searches of the database on specific topics, including abstracts where available, can be obtained by contacting Duane Bartholomew at duaneb@hawaii.edu.◆

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All contributions should be written in English. Editing assistance will be provided on request.
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