Pineapple News

Issue No. 7 Newsletter of the Pineapple Working Group, International Society for Horticultural Science June, 2000

Contents

Pineapple Working Group (PWG)

Duane Bartholomew, Editor

Dear Working Group colleagues. In Issue No. 6 of the newsletter I speculated that the PWG would benefit from greater participation of the members in our organization. While that may be true, the meager returns on the questionnaire in issue, three or four out of about 400 newsletters mailed, causes me to conclude that proposals were not adopted. The difficulty in inspiring participation and communication will result in my continuing as I have in the past. At least for the next couple of years, my primary objectives will be to do all that I can to help our colleagues in Mexico organize the Fourth International Symposium on Pineapple and to publish the newsletter.

Electronic mail is an increasingly valuable tool for communication with those interested in the PWG. Almost one-third of our membership now has access to E-mail. Most have agreed to receive Pineapple News as an attached file, to print or read it at the PWG website (see below), or to obtain the newsletter as an Adobe Acrobat PDF file. The use of electronic mail will speed distribution and reduce printing and mailing costs.

Another significant benefit of E-mail was highlighted recently when I received inquiries about the availability of 2-(3-chlorophenoxypropionic acid) for use in flowering inhibition studies, and bromelain. Special thanks are due to Dear Mr. Adriaan Dolmans, Independent Pineapple Consultant, who responded with a source of the enzyme. See that and other possible sources under Notices below. I believe no source of 2-(3-CPA) was clearly identified.

Another interesting inquiry related to flowering failure in tropical Indonesia. Material from Mr. Fauzan of P.T. Great Giant Pineapple Co. is reprinted here with permission. Mr. Fauzan stated that one of the important problems in pineapple production in Indonesia (Lat. 4°59' S, Long. 105°13' E, Alt. 40 - 50 m) is flowering failure from November to May when minimum night temperature was much higher than the average of 1989-1998 (Figure 1).

Minimum night temperature during that period ranged from 24 to 25°C. There was a tendency for flower induction to be more difficult when night temperature exceeded 22.5°C (Figure 2) and control of induction decreased as the minimum night temperature increased. Clearly minimum night temperature is not the only factor controlling induction because percentages were high in some fields but low in others. Forcing practices were about the best known, two applications of ethylene applied at 2 to 5 day intervals. To enhance the plant susceptibility to forcing, nitrogen fertilizer was withheld from 45 to 60 days prior to forcing, and ethylene

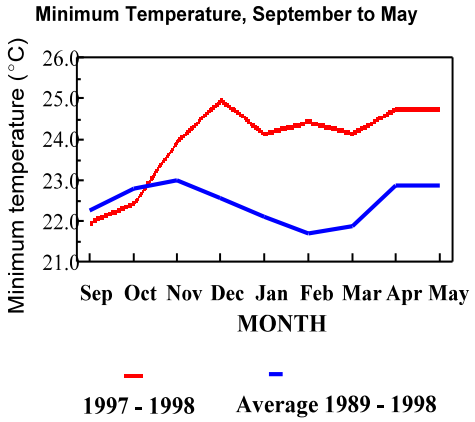

Figure 1. Average temperature for months shown.

concentration was increased 10 to 25%. I regret that I didn't inquire about the status of the different fields or forcing dates, but it would be interesting to know more about the circumstances

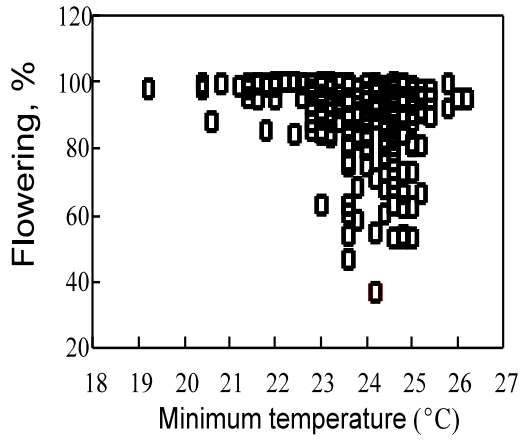

Figure 2. Percent flowering of pineapple forced with ethylene as a function of minimum temperature.

surrounding the variation among fields. Perhaps Mr. Fauzan will have additional information he can share with us at a later date.

Yet another E-mail search was undertaken to locate a source of Ginaca machines for a small cannery that wanted to begin operations in a Latin American country. Special thanks to Dean Wheeler, AgResults, Inc., who knew of the availability of used equipment. Specialized machinery needed by growers and processors of pineapple apparently isn't always easy to locate, especially for smaller operators and perhaps even for larger ones. For example, CAMECO, a U.S. manufacturer of boom sprayers and harvesters for pineapple, was purchased by John Deere Co. CAMECO no longer has a presence on the world wide web and John Deere doesn't list the machinery that was manufactured by CAMECO on their company web site, though perhaps they still manufacutre it. For future reference, Caustier FRANCE (66000 PERPIGNAN, FRANCIA (France)), a manufacturer sizers of fruits and vegetables, dumpers, grading machines, and equipment for the conditioning line sent the following message.

From : J-M Montarsolo, Export Manager
Subject : Your request for pineapple equipment
Dear sir,

Mr Max Reynes sent us on 7th April your mail concerning your request for pineapple equipment. Caustier FRANCE is able to undertake turn-key projects and ensures also the engineering and equipment for companies specialised in the packaging of fruit and vegetables. Caustier FRANCE produces a grading machine specially manufactured for mangoes, pineapples.

The value of E-mail is considerable and those that do not yet have it should consider obtaining E-mail access as soon as it becomes available and affordable.

Proceedings of the 3rd Symposium

The proceedings of the 3rd Symposium has been published by ISHS and is being distributed to those who registered for the 3rd symposium. If you wish to purchase the volume, the Acta Horticulturae volume number is 529 and the ISBN is 90 6605 902 8. The price to non-members of ISHS is 67 Euros. Those wishing to purchase a copy can order it from the ISHS (see address below).

4th International Pineapple Symposium

It was announced in Chronica Horticulturae, magazine of the ISHS, that the 4th International Pineapple Symposium will be held in April, 2002 in Veracurz, Mexico. For additional information, contact Dr. Daniel Uriza Avila, Serapio Rendom 83 Col. Sam Rafael, Del Cuauhtemoc 06470 Mexico, D.F. Tel.: (5) 51401612. Fax: (5) 55469020; E-mail: rauckv@inifap2.inifap.comacyt.mx.

I also received confirmation of plans for the 4th symposium directly from Dr. Uriza Avila. He wrote to say that the exact date is yet to be determined and that the "The headquarters would be the City and Port of Veracruz, with wide tourist infrastructure and nearness to the most important pineapple production zones of Mexico."◆

ISHS Issues and News

Pineapple Working Group members are encouraged to also become members of the International Society for Horticultural Science. For those considering joining the ISHS, it is an organization of individuals, organizations and governmental bodies interested in the field of Horticultural Research and Horticulture in general. The ISHS is registered as a society in the Netherlands. To inquire about membership in the ISHS or to order publications of the society, write to: ISHS Secretariat, K. Mercierlaan 92, 3001 Leuven, Belgium (E-Mail: info@ishs.org) or visit the ISHS web site at http://www.ishs.org/.◆

Contributions to Pineapple News

Please plan now to contribute to the next issue of Pineapple News. When submitting articles for publication in ***Pineapple News***, please follow the guidelines below.

1. All contributions should be written in English. Assistance with editing is provided.
2. Preferred contributions are timely news about the pineapple industry within a country or region and abstracts or summaries of research on issues related to culture, processing, storage, and marketing of pineapple.
3. If possible, contributions should be submitted by electronic mail or on floppy disks as DOS (ASCII) text files or as Word or WordPerfect documents prepared on computers running Microsoft DOS or Windows programs. Printed copy should be clean so it can be scanned to speed conversion to a wordprocessor format.
4. Columns in tables should be separated with tabs; do not use Tables features of word processing programs. Photographs or image files that can be printed in black and white with a good quality laser printer (600x600 dpi) are acceptable.
5. Mail contributions and inquiries to: **D.P. Bartholomew, Dept. of Agronomy and Soil Science, Univ. of Hawaii, 1910 East-West Rd., Honolulu, HI 96822 U.S.A.** (Phone (808) 956-8708; Fax (808) 956-6539; E-mail: duaneb@hawaii.edu. ***Pineapple News*** is posted on the Web after publication at: http://agrss.sherman.hawaii.edu/pineapple/pineappl.htm.
6. **Address corrections:** Please send mailing and E-mail address corrections to D.P. Bartholomew at the above address.◆

News From Australia

Tropical Fruit Symposium - Australia

The conference has received 230 abstracts from 32 countries. They are expecting 400 to 500 delegates.

1999 Pineapple Field Day

July 16, 1999

Abstracts prepared from the Field Day reports by D. Bartholomew.

Is There a Future for Fumigants for Soil Disinfestation in Australia?

Ian Porter, Agriculture Victoria, Knoxfield, P.B. 15, SEMC 3176, Victoria, Australia

With the phase out of ethylene dibromide (EDB) and methyl bromide, chloropicrin, metham sodium (methyl-isothiocyanate), and 1,3 dichloropropene and mixtures of the foregoing are being reevaluated in a variety of crops. Improved results are obtained using injection methods that ensure uniform distribution of the fumigant in the soil and sealing with plastic film. However, longer term, integrated strategies that include prediction, monitoring, biocontrol, resistant varieities, and strategic application of pesticides are thought to be the most sustainable methods of soil disinfestation.

Phytophthora Survey Results

André Drenth and Sook Han Soo, CRC for Tropical Plant Pathology, The Univ. of Queensland, St. Lucia,Australia
Ken G. Pegg, Queensland Horticulture Inst., DPI, Indooroopilly, Australia

Poor control of disease caused by *Phytophthora cinnamomi* was examined by sampling fields to evaluate development of resistance to phosphonate and metalaxyl. Isolates taken from pineapple fields and from natural areas were tested. Most farms had isolates with phosphonate resistance as indicated by the effective concentration of fungicide required to inhibit growth to 50% of the untreated fungi (EC50). However, phosphonate resistance after two and 10 years were similar and resistance to phosphonate was not associated with poor disease control. The EC50 for metalaxyl indicated no significant resistance except on one farm with a history of higher than average usage. There was no cross-resistance to the two fungicides observed; phosphonate-resistant islolates were still sensitive to metalaxyl. The metalaxyl-resistant population of *Phytophthora cinnamomi* will be monitored to see if resistant isolates spread and form a potential threat to use of the chemical for disease control.

Role of Organic Matter in Root Health

Graham Stirling, Biological Crop Protection Pty. Ltd.

In a field trial sawdust with poultry manure, fresh pineapple trash as an amendment, a mulch of fresh pineapple trash, and molasses applied after planting were compared with EDB fumigation and no treatment. Samples were collected 4 and 11 months after treatment and effects on root-knot nematodes and *Phytophthora* were evaluated. Populations of bacteria and fungi increased in amended soils, particularly in the sawdust/poultry manure treatment. When samples of treated soils were inoculated with root-knot nematodes, fewer nematodes were recovered from organically-amended soils. No amendment suppressed *Phytophthora* when inoculated into the soil. After 13 months, the crop was in good health regardless of treatment. It was concluded that plant material remaining at the end of the pineapple crop should be incorporated to take advantage of the benefits of this organic matter.

Metham fumigation–Yes or No?

Doug Christensen, Golden Circle Ltd.

Metham is well suited to deep, sandy loam soils and controls nematodes and symphylids. Trials to date show that metham at 500 L ha^{-1} supported pineapple yields to within 97% of those from fields fumigated with EDB. Metham also controls fungal diseases of pineapple even when root knot nematodes are not present. Metham does not provide the same level of nematode control as EDB; the partial kill leads to very high counts at 10 months after planting. However, metham use is expensive and a good return on investment in the application of metham has yet to be obtained from a large area of commercial pineapple fields. Other relatively effective treatments include neem seedcake with urea and large amounts of sugarcane filterpress. The additional of urea supports early plant vigor and the N content of amendments may partly explain the good plant growth responses of some treatments. EDB gave excellent root knot nematode control and while metham did not, it did support fruit yield well.

Current Nematicide Trials in Pineapple

Graham Stirling, Biological Crop Protection Pty. Ltd.

The registration label for Nemacur (fenamiphos) permits granular or liquid application prior to planting and 5 sprays during plant crop and 2 sprays during the ratoon. The total program is expensive and there is interest in knowing if reduced amounts of Nemacure are effective. Tests to evaluate modified practices were installed on three farms. At one location, root-knot nematode populations were low and good plant crop yields could be obtained without nematicides. At a second location, heavy galling due to root-knot nematodes was observed in some treatments at 14 months after planting. The data suggest that Nemacur had little effect on root galling or nematode populations, whether it was applied prior to planting or as a foliar spray. At a third location, Nemacure granules applied prior to planting controlled root-knot nematode as well as EDB. Nemacure sprays alone did not control nematodes, but spray treatments consistently increased plant size. Yield data were not yet available. Data is also being collected on the effect of metham sodium on root-knot nematode populations and on root health over a period of two years. Metham is a relatively poor nematicide as currently applied. Some nematode control was obtained with 800 to 1,000 L ha^{-1}, but even at the highest amounts was not as effective as EDB. Root health at 12 months after planting was comparable for metham and EDB treated locations. It was suggested that the fungicidal action of metham may be more important than its nematicidal effects.

Nematode Monitoring: An Aid to Managing Nematodes on Pineapples

Graham Stirling, Biological Crop Protection Pty., Ltd.

Research in Queensland pineapple fields shows nematodes vary from field to field, with some fields requiring fumigation while others do not. Monitoring involves regular collection of soil samples from a field and having nematodes in the samples counted. Nematode monitoring involves sampling about once a year. Over time, the historical data can be used to make management decisions about the need for fumigation to control nematodes. The protocol for sampling in Queensland includes:

- Collect samples prior to planting, at 12 months after planting, plant-crop harvest, and about ratoon-crop harvest.
- Sample fields of 0.5 to 1.0 hectare that are relatively uniform in soil type, aspect, and cropping history. For record keeping purposes, identify the area with a name or number.

- Use a soil sampling tube of about 2 cm diameter; collect about 50 soil cores at random from the root zone (0-25 cm depth). Mix the cores in a bucket and retain a 0.5 L sample for analysis.
- Store samples in an insulated container to prevent a large rise in temperature prior to submission to the lab for analysis. Label the sample with the block name or number, crop stage, length and kind of fallow for pre-planting samples, planting date, and nematicides applied before and after planting.

Data on root health and field history are important in interpreting nematode counts obtained in a field. Samples taken prior to planting should be low due to starvation because of lack of a food source. High counts at this time indicate to short a fallow period or the presence of a food source such as weeds or pineapple plants. Samples taken at 12 months after planting can indicate the extent of hazard for the plant crop as well as for the ratoon crop while nematode counts at ratoon-crop harvest can indicate the extent of the nematode hazard for the next crop.

Because pineapple root health is affected by disease organisms and insects as well as by nematodes, root health should be evaluated each time the soil is sampled for nematodes. A scheme has been developed for quantitatively rating root health. Growers should aim for high ratings for rooting depth, root system volume, fine feeder roots and new roots and low ratings for galling, rotting, and branching. A complete root health assessment will help to identify problems due to nematodes, pathogenic fungi such as *Phytophthora*, insects pests such as white grubs and symphylids.

Effect of Sodium Molybdate on the Juice Nitrate Concentration of Pineapples

Col Scott, Golden Circle Ltd.

Researchers in Thailand reported a decline in juice nitrate concentrations after foliar application of sodium molybdate. In two tests in Queensland, 625 g of sodium molybdate was applied in 2,500 L ha^{-1} of water in two trials. In the first trial, which was unreplicated, there was evidence of a reduction in nitrate levels in fruit. However, in a second replicated trial, neither average fruit weight or juice nitrate were significantly affected by application of molybdate. No recommendations can be made on the basis of the results and additional studies are planned.

Weather Summary for 1998/1999

Eric Sinclair, Golden Circle Ltd.

A weather summary is regularly included in the Field Day Notes. What caught this abstracters eye was an interesting comparison that was made between the long-term average hours per month between 20 and 30°C and the hours accumulated during 1998-1999. I looked at back issues of the notes and found that similar discussion goes back several years. The basis of this comparison is that the optimum temperature range for growth of pineapple is believed to be between 20 and 30°C. The hours the average temperature is not less than 20 nor more than 30°C are summed for each month. The approach is particularly interesting because it factors in the growth inhibiting effects of both low and high temperature. The process is made simple through the use of relatively inexpensive electronic data loggers capable of storing extreme temperatures as well as hourly average temperature. Once several years of such data have been accumulated, significant deviations from the average are obvious and, more importantly, may help to predict fruit development trends and explain anomalous growth responses. Looking at back issues of the Field Day Notes, in the 1997 issue it is noted by Dr. Sinclair that there was a wave of natural flowering that was associated with extreme (41.5°C!) air temperature. High incidences of fruit cripple and malformed fruit were also associated with these periods of extreme temperature. If weather records are evaluated regularly, they could indicate an enhancement or delay in crop development. Since there is no experience with this approach outside of Australia, it is not possible to speculate on its value where temperature is less extreme. Also, it would be of little value where other weather parameters, for example rainfall, were the main factors controlling growth.◆

News from Brazil

New Book on Pineapple

EMBRAPA (Brazilian Corporation for Agricultural Research) recently published the book *O abacaxizeiro: Cultivo, agroindústria e economia* ("The pineapple plant: Cultivation, agroindustry and economy") in Portugese. The book contains 480 pages and is well illustrated with more than 120 photos. The book, organized by Getúlio Augusto Pinta da Cunha, José Renato Santos Cabral and Luiz Francisco da Silva Souza, researchers from Embrapa's National Research Center for Cassava and Tropical Fruit Crops, Cruz das Almas, Bahia, Brazil, has sixteen chapters, each one written by specialists. The book covers breeding, propagation, climate and soils, all aspects of cultivation, irrigation, pests, diseases, post-harvest handling, and economics, and a reference section containing the cited references (about 700 titles from the most important research centers in the world). The book is a synthesis of the work and experience of eleven Brazilian scientists, many of them with more than 20 years dedicated to the pineapple crop. The book may be purchased at the following addresses:

EMBRAPA-SCT, SAIN Parque Rural, Av. W 3 Norte (final), Cx. P. 040315, Brasília, Brazil; Fone: 61 348 4236 or 348 4155; Fax 61 272 4168; E-mail: vendas@spi.embrapa.br; homepage: www.spi.embrapa.br

EMBRAPA-CNPMF, Rua Embrapa s/n, Cx.P. 007, Cruz das Almas, BA, Brazil; Fone: 75 721 2120, Fax: 75 721 1118; E-mail: sac@cnpmf.embrapa.br

Natural Flowering Control in Pérola Pineapple

Domingo Haroldo Reinhardt and Getúlio A. Pinto da Cunha

The occurrence of natural flowering has been the main reason for the existence of an off-season (February to May) for pineapple production in Brazil, in spite of the recent increase of the fruit offered during that period. Usually, all plantings done during the second semester of each year, are subjected to variable rates of natural flowering beginning in June of the following year, if it is not chemically forced before that.

In 1996, Embrapa Cassava and Tropical Fruit Crops started field studies on the control of the natural flowering of Pérola. Several experiments have been carried out and some interesting results have been obtained, but these have not been sufficient for getting final technical recommendations on that issue, because flower inhibition has often been accompanied by slower plant growth, at least for about a 60 day-period.

Pérola is much more susceptible to natural flowering than other cultivars, including Smooth Cayenne. The application of nitrogen fertilizers (urea) during the critical period for natural flowering did not prevent its occurrence and sometimes even accelerated flowering. Some growth regulators, such as mepiquat chlorate (Pix), gibberelic acid (ProGibb), tebuconazole (Folicur), paclobutrazol (Cultar), 2-(3-chlorophenoxy) propionic acid (Fruitone), have been tested, using several concentrations and dates of application. The latter two products have given positive results, when applied at concentrations of up to 100 mg L^{-1} (a. i.), split into three sprays of about 30 ml per plant, 2 weeks apart. The timing of the sprays has been very important. The best results, up to 85% inhibition, have been obtained for applications done from the middle of April, but not later than middle of May.

Management and Evaluation of 'Pérola' Pineapple Ratoon Crop under Semiarid Conditions

Domingo Haroldo Reinhardt, Alberto de Almeida Alves

In a commercial planting in the Itaberaba region of Bahia State, Brazil, the feasibility of the 'Pérola' pineapple ratoon crop was evaluated, as well as its improvement by cultural practices. In a randomized block design, with a 3 x 3 factorial scheme and three replications, a total of six treatments were studied, three of them related to leaf pruning (low and high pruning, without pruning) and the other three involving the application of fertilizers (without fertilization, with1/2 NPK used on the first cycle, full NPK fertilization). Fruit harvest was carried out 12 and 14 months after the first cycle harvesting. There were few significant differences among treatments for all variables studied. However, the feasibility of exploiting the ratoon crop is clear from the results obtained: good yield (17,775 fruits/ha, 10.2 slips/plant); high average fruit weight (1.79 kg); good fruit quality (13.6 ºBrix, Brix/acidity ratio equal to 1.95) and good health conditions, as well as the estimated net returns of R$ 8,700 (US$ 7,250) and R$ 2,500/ha (US$ 2,080) obtained from the selling of fruits and slips, respectively. These results were obtained in spite of a high plant dropping during the final fruit ripening phase, which caused losses of about 5,000 fruits ha^{-1}.
Source: Revista Brasileira de Fruticultura (RBF), v.20, n.3, p.323-331, dez. 1998

Evaluation of Pineapple Hybrids in Different Ecosystems of Brazil

José Renato Santos Cabral and Domingo Haroldo Reinhardt

The pineapple breeding program carried out by Embrapa Cassava and Tropical Fruit Crops, Cruz das Almas, Bahia, Brazil, has developed hybrids resistant to fusariosis, the main pineapple disease in Brazil, which may cause very high losses of plants, fruits and plantlets in most of the production areas. The program has produced 30,200 hybrids and has selected 57 genotypes that are in the final phase of clonal evaluation.

The hybrids Perolera (PE) x Smooth Cayenne (SC)-14, PE x SC-56 and Primavera (PRI) x SC-18, resistant to the fusariosis, have shown good agronomic performance during four clonal evaluation cycles, and have been micropropagated to get enough planting material for evaluations in the main ecosystems with important pineapple production in Brazil. Competition experiments between these clones and local varieties (mostly Pérola and Smooth Cayenne) are being conducted in several States and regions, such as Bahia, Pernambuco, Maranh o (Northeast), Tocantins (North), Mato Grosso, Mato Grosso do Sul, Goias, Federal District (Central West), S o Paulo, Minas Gerais, Espírito Santo (Southeast), Paraná, Rio Grande do Sul (South).

The first results are expected during the year 2000 and from 2001 the best hybrids in relation to production and consumer acceptance will be recommended as new cultivars.

Phylogenetic Relationships in Ananas and Related Genera Using Chloroplast Dna Restriction Site Variation

M. F Duval, , G. S.C Buso, J. R. S. Cabral, L. de B. Bianchetti, , F. R. Ferreira, , M. E. Ferreira. E-mail: marief@cenargen.embrapa.fr or marief@cenargen.embrapa.br

A set of universal primers for amplification of polymorphic non coding regions of chloroplast DNA (cpDNA) was used to study phylogenetic relationships involving the genus Ananas within the Bromeliads. Six cpDNA regions were studied using total DNA extracted from fresh leaves. Fragment lengths vary between 1000 and 3100 base pairs (bp), and comprise 8 to 9% of the approximate total estimated length of Bromeliads cpDNA. Polymorphism tests were conducted using a sample including clones of all seven Ananas species, and one accession whose species is unknown, one clone of the unique species of the related genus Pseudananas, one Bromelia, and one Tillandsia. Restriction tests involved 20 endonucleases of which 14 detected polymorphism at these cpDNA regions among the accessions tested. Thirty two out of 120 combinations tested displayed polymorphism at the inter-genus level, and only eight within the genus *Ananas*. The cpDNA restriction site variation was used to study phylogenetic relationship of a collection of 96 accessions of *Ananas* and its wild relatives. So far only 13 restriction site mutations and one length mutation were identified. Other polymorphic patterns were more complex, implying more than one sequence changes at these sites. Results were scored as band level presence/absence and data were analysed using Multiple Correspondence Analysis and Hierarchical Cluster Analysis based on Dice index. Preliminary results show restricted variation between the genera *Ananas* and *Pseudananas* at these cpDNA regions, in relation to variation observed between this complex and other tribes and sub-tribes represented. The cluster patterns obtained do not fit the current division of species within *Ananas*. A geographical component of the variation is clearly defined within the wild species.◆

News From Cuba

A Technology of Acclimatization of Pineapple Vitroplants

Ermis Yanes Paz[1], Justo González Olmedo and Romelio Rodríguez Sánchez
Bioplant Center, Laboratory for Plant Propagation, Carrretera a Morón, km 9 Ciego de Avila, CP. 69450, Cuba. ([1]) Corresponding author. E-mail biogenet@unica.edu.cu

There is a great demand of pineapple planting material in Cuba. Conventional methods of macropropagation do not satisfy the demand. That is why, two protocols for pineapple micropropagation have been developed, and are being applied in

commercial laboratories in Cuba. However, the bottle neck of these procedures has been the acclimatization stage. We have developed a technology for the acclimatization of pineapple that includes the management of several factors affecting plant survival and growth: quality of the *in vitro*-raised plant material, kind of substrate, biological (*Azotobacter* and mycorrhiza) and mineral nutrition, plant growth regulators, light intensity and relative humidity. This technology allow producers to reach high survival and growth rates.

Light Management During Acclimatization of Pineapple (Ananas comosus (L.) Merr.) cv. Cayena lisa 'Serrana' Vitroplants

Ermis Yanes Paz[1], Justo González Olmedo and Romelio Rodríguez Sánchez
Bioplant Center, Laboratory for Plant Propagation, Carrretera a Morón, km 9 Ciego de Avila, CP. 69450, Cuba. ([1]) Corresponding author. E-mail biogenet@unica.edu.cu

Summary

The influence of photosynthetic photon flux density (PPFD) on plant survival and the appearance of photobleaching and photoinhibition symptoms was evaluated in different stages of the acclimatization process. In the fist stage (the first month), PPFD of 222, 458, 620 and 1126 µmol m^{-2} s^{-1} were tested. In the second stage (second and third month), plants cultured at PPFD of 222 and 458 µmol m^{-2} s^{-1} were transferred to either 660 or 920 µmol m^{-2} s^{-1}. Finally, the time of permanency under shade was determined by exposing the plants to full sunlight after acclimatization for 75 or 90 days. The results permit us to establish a whole management of PPFD as follows: During the first month plants must receive PPFD varying from 222-458 µmol $m^{-2}s^{-1}$, later it must be increased to 920 µmol m^{-2} s^{-1} for a period of 45 days after which plants could be transferred outside the greenhouses.

Introduction

Micropropagation of pineapple has many advantages over conventional methods of propagation. Two protocols for *in vitro* propagation of pineapple have been recently developed in Cuba. The two are conventional micropropagation as developed by Daquinta (1998) and the temporary immersion system described by Escalona (1999). Both protocols are being applied in commercial laboratories. However, the potential use of these procedures is limited by the low survival rates and plant growth during the acclimatization stage. Plants cultured *in vitro* are exposed to high levels of organic and inorganic nutrients, high relative humidity, low light intensity, and limited exchange of CO_2 and O_2. And as a result, vitroplants have anatomical, morphological and physiological abnormalities (Ziv,1995) that make them more sensitive to environmental changes.

Among the factors affecting plantlet survival and growth during acclimatization are: light intensity, relative humidity, kind of substrate and fertilizers. In this work, we evaluate the influence of PPFD during different stages of the acclimatization process to establish a proper management of this factor.

Materials and Methods

Effect of PPFD on plant survival during the first stage of acclimatization

Plants were cultured *in vitro* as described by Daquinta (1998). Later, they were removed from the culture vessels and planted in sand in seventy-hole trays. The trays were placed in experimental chambers at PPFDs of 222, 458, 620 and 1126 µmol m^{-2} s^{-1}, measured at 12:00 M. Every treatment included one tray. An automated mist system was used to irrigate the plantlets every 30 min from 8:00 AM to 6:00 PM. The irrigation periods during the experiment were: 3 min. for the first week; 2 min. for the second week; and 30 sec. for the third and fourth weeks. Survival was recorded at the end of one month. Visual observations of leaves were also done to detect photoinhibition or photobleaching symptoms.

Effect of PPFD (µmol m^{-2} s^{-1}) on plant survival during the second stage of acclimatization

Treatments during the second phase are shown in Table . Plants were watered daily and sprayed twice a month with the fertilizer Combi II (Carisombra). Every treatment included one tray as described in the first experiment. Survival was recorded at the end of the second stage of acclimatization. Visual observations of leaves were also done to detect photoinhibition or photobleaching symptoms.

Table 1. The PPFD treatments during acclimatization were:

First stage (30 days)	Second stage (60 days)
222	660
222	920
458	660
458	920

The effect of PPFD (µmol m^{-2} s^{-1}) duration in the second acclimatization stage on vitroplants survival and growth at full sunlight was assayed by acclimating plants for 30 days at a PPFD of 222 µmol m^{-2} s^{-1} followed by acclimation for either 45 or 60 days at a PPFD of 920 µmol m^{-2} s^{-1}. After the second stage, plants were transferred to direct sunlight. Plant fresh and dry masses, "D" leaf length and width, leaf number and survival percentage were evaluated 105 days after acclimatization initiation. Experimental conditions were as described above.

Results and Discussion

Light is one of the most important factors influencing plant survival and growth during acclimatization (Agramonte et al, 1998) and survival of vitroplants of pineapple was significantly affected by PPFD (Table 1). Plants grown at low PPFD showed the highest survival rates. This was expected considering the low *in vitro* PPFD of 45-50 µmol m^{-2} s^{-1}. An abrupt change in PPFD provoked severe leaf burns (bleaching) and photoinhibition (Van Huylembroeck, 1994). In fact, the plants cultured under the highest PPFD (1126 µmol m^{-2} s^{-1}) showed bleaching and photoinhibition symptoms and eventually died. These symptoms were not frequently observed in plants growing at 620 µmol m^{-2} s^{-1} and were never seen in plants cultured at lower PPFDs.

Table 2. Effect of PPFD on survival rate of pineapple vitroplants during acclimatization.

PPFD (µmol m^{-2} s^{-1})	Survival (%)
222	97.1 a
458	100.0 a
620	95.7 b
1126	91.1 c

Means with the same letter are not statistically different (Duncan multiple range test, $p<0.05$)

On the other hand, in the second experiment all experimental groups survived and no symptoms of photoinhibition or bleaching were observed. So, a PPFD of 920 µmol m^{-2} s^{-1} would be most suitable for this stage, since it is nearer to that of field conditions.

Once the optimum PPFD for each stage of the acclimatization process had been determined, the next step was to establish how

long the plants should stay under those conditions before being transferred to full sunlight.

The time of permanency under shade did not cause total burning of plants. However, in both treatments a low percentage of plants showed light symptoms of photoinhibition and burning. It is important to point out that burning was observed over *in vitro* developed leaves that were still on plants. These leaves have an abnormal anatomy (Ziv, 1995; Desjardins, 1995), and as a result, a distortioned behavior (Pospisilova et al., 1997). Both treatments reached one-hundred percent of survival.

Table 3 shows the effect of the treatments on several vitroplants' growth variables. As seen, the time of permanency under shade did not have any effect on them (t test, $p<0.05$). This fact indicates that the exposure to full sunlight at so early stages as 75 days of acclimatization did not depress plant growth and allows to reduce cost during acclimatization.

Table 3. Effect of the duration at reduced PPFd shade on vitroplant growth at full sunlight.

Shade	Plant mass, g		Leaf number	"D" leaves (cm)	
(days)	Fresh	Dry		Width	Length
75	4.81a	0.61a	10.7a	0.75a	10.52a
90	3.96a	0.51a	11.3a	0.79a	10.33a

Plants cultured *in vitro* acclimate to the low PPFD and high relative humidity conditions prevailing there. The *ex vitro* environment must initially resemble the microenvironment of the culture vessels (Debergh et al., 1992) and then be managed during acclimatization to assure survival under field conditions. The results obtained in this study show that gradual acclimation to light, one of the principal factors to be considered because of its strong effect on plant survival and growth, assures survival in the field. Thus, a gradual increase in PPFD prior to exposure to full sunlight must be a common practice during the acclimatization of vitroplants.

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Brief Review of Some Methods to Obtain Pineapple Haploid Plants

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Key words: anther and ovule culture, haploid plants, *in situ* parthenogenesis, intergeneric crosses, pineapple

Summary: Four different methods were developed to induce pineapple (*Ananas comosus* (L.) Merr.) haploid plants: anther and ovule culture, *in situ* parthenogenesis induced by irradiated pollen and intergeneric crosses. For anther and ovule culture, we have developed a complete methodology that included: developmental stage of the microspores and embryo sac, and media and culture conditions to obtain plantlet regeneration *via* callus induction. For induced *in situ* parthenogenesis, the pollen viability and pollen tube length were dose-dependent. Pollination, using pollen-irradiation, utilized Smooth Cayenne cv. 'Serrana' and Red Spanish. Doses up to 250 Gy were not adequate to get seeds showing embryos. Some plantlets that regenerated in the different irradiation doses showed phenotypes with small and thin leaves and short internodes. The first haploid plantlets were achieved using intergeneric crosses, Smooth Cayenne cv. 'Serrana' as female parent and 'Curujey' (*Tillandsia fasciculata* Sw) as male parent. The possibilities of the use of haploid plants in the pineapple genetic breeding are discussed.

Abbreviations: BAP - 6- Benzylaminopurine, Dicamba - 3,6 Dichloro-methoxi-benzoic acid, MS - Murashige and Skoog, TDZ - Thidiazuron

Introduction

The progress reached in the genetic improvement of species such as *Solanaceae*, *Ranunlaceae*, *Cruciferae* and *Gramineae* have been possible mainly due to their response in haploid cultures. The goal of pineapple breeders is to obtain homozygous plants. For many years the improvement of these species has been carried out by methods such as selection and hybridization (Williams and Fleish, 1993). Clonal selection only serves to maintain the quality of present varieties. Hybridization has allowed the creation of new varieties, but they are mostly only of local importance (Coppens and Duval, 1995). Furthermore, hybridization is very time consuming, requiring up to 15 years for the release of a new variety. Hybridization also requires a lot of resources, with around 30,000 hybrids per cross being necessary to find a desirable hybrid (Cabot, 1997).

For pineapple breeding, using haploids is not possible because no reports of successful production are known. Therefore, we have focused the efforts in pineapple breeding on obtaining haploid plants using four methods: anther and ovule culture, *in situ* parthenogenesis induced by irradiated pollen and intergeneric crosses. This paper points out briefly the main results achieved thus far in the development of the pineapple program to obtain haploid plants.

Anther culture.

For pineapple anther culture, the studies were aiming at determining the relationships among some phenotypic characters of donor plants and the developmental stage of the microspores (tetrad, uninucleate and post-uninucleate), and media and culture conditions to get callus formation and plantlet regeneration.

Relationship among some phenotypic characters of donor plants and the developmental stage of the microspores.

The phenotypic characters such as length of inflorescence, fruitlet and anthers were analyzed on donor plants. These studies were useful as a practice method for sowing anthers containing microspores at the right stage for response to the haploid cultures. There was a displacing of the different developmental stages of the microspores from the inflorescence basal part to apical part. At 48 to 50 days after induction of flowering, the basal part showed the highest frequency of microspores containing the uninucleate stage. This stage was reached after 52 to 62 days in the middle of the inflorescence, and only after 57 to 66 days in the apical portion. Furthermore, there were correlations among such phenotypic characters of pineapple plants as length of inflorescence, floral botany and anther with the different developmental stages of the microspores.

Media and culture conditions to get callus formation and plantlet regeneration.

The combination of Dicamba and BAP was better for developing calluse from anthers than other plant growth regulators assayed for this purpose (Benega et al., 1996a). After nine weeks of culture, the anther wall breaks and calluses sprout from within anthers. They appeared mainly near the zone where anthers were connected to the filaments and the calluses had a yellowish-white or yellow color and a hard and nodular appearance. Genotype greatly influenced anther culture, and callus was only obtained from Smooth Cayenne cv. 'Oriente', Red Spanish and 'Piña blanca'. The MS (Murashige and Skoog,1962) medium supplemented by 9% sucrose was better for callus induction than 3%. The highest responses, 0.120 and 0.065, were achieved in Smooth Cayenne cv. 'Oriente' and Red Spanish (Benega et al., 1995). Out of several plant growth regulator tested to obtain plant regeneration, only thidiazuron (TDZ, 6.81 µM) gave the best results in Smooth Cayenne cv. 'Oriente', Red Spanish and 'Piña blanca' respectively.

Ovule culture

Haploid induction through *in vitro* gynogenesis by isolated ovule culture is an alternative in the haploidization techniques. Excellent results have been obtained by means of this method in those species where other haploid methods were unsuccessful (Keller, 1990). For isolated ovule culture of pineapple we have obtained a complete methodology for preparation and sterilization of inflorescence, extraction and *in vitro* ovule culture, as a first step to study the effect of the developmental stage of the embryo sac and media to get embryoid formation and plantlet regeneration *via* callus induction.

Effect of the developmental stage of the embryo sac and culture medium on callus induction

Similar to anther culture, callus formation from isolated ovules was best achieved on MS media (Murashige and Skoog, 1962) supplemented with the combination Dicamba/BAP (Benega et al., 1997a). The developmental stage of the embryo sac was one of the most important factors for callus formation. Those at the microspore developmental stage between uninucleate and first haploid mitosis stage were better than the other stages tested. Smooth Cayenne cv. 'Oriente' and Red Spanish had higher levels of callus induction (50.94 and 38.98%) than did other genotypes.

Plantlet regeneration

Of all the assayed media, only the MS (Murashige and Skoog, 1962) media containing TDZ resulted in plantlet regeneration. Embryoid formation was achieved in Red Spanish, 'Piña blanca' and Smooth Cayenne cv. 'Oriente'. However, plantlet regeneration was obtained only for Red Spanish. Values up to 20.8% of plantlet formation were achieved on calluses cultured on the MS medium supplemented by 4.54 µmM TDZ.

Dicamba is considered to be a potent auxin that has produced excellent results for growing and callus formation in crops where other plant growth regulators were ineffective (Conger et al. 1987). Moreover, TDZ has been widely used to obtain plantlet regeneration from some recalcitrant crops, as for example anther culture of apple where, 24.98 µM brought about the best results (Bouvier, 1993). Furthermore, it is being used for micropropagation of a wide array of woody species (Murthy et al., 1998). In medicinal plants (Zhou et al. 1994) and sweetpotato (Gosukonda et al. 1995) also TDZ has been used due to its tremendous ability to stimulate shoot proliferation.

***In situ* parthenogenesis induced by irradiated pollen**.

Crossing with irradiated pollen is probably the oldest technique for inducing haploid plants (Doré et al., 1995). Recently it was shown to be an efficient method of haploidization when associated with embryo culture (Raquin, 1985).

As first step in developing this technique for pineapple, we studied the effect of gamma-rays on *in vitro* pollen viability and pollen tube growth and some pollination was done on Smooth Cayenne cv. 'Serrana' and Red Spanish (Benega et al., 1996 b).

Effect of irradiated pollen on in vitro viability and pollen tube growth

Pollen of Smooth Cayenne cv. 'Serrana' and Red Spanish was gamma-ray irradiated with a ^{60}Co source at 16 Gy min^{-1}, using rate of doses from 0 to 6 kGy. There were significant difference between different doses and studied genotypes. Red Spanish ($r = - 0.8215$, $y = 57.2685 - 10.0270$ * doses) was in general more radioresistant than Smooth Cayenne cv. 'Serrana' ($r = - 0.9213$, $y = 49.7400 - 9.0060$ * doses).

Pollen tube length reductions were correlated with increasing irradiation doses as Red Spanish ($r = - 0.88$, $y = 655.0876 - 100.0338$ * doses) as Smooth Cayenne cv. 'Serrana' ($r = - 0.91$, $y = 561.8257 - 88.1924$ * doses). Moreover, the highest doses (6 kGy) promoted an absolute decrease of pollen tube length in the two studied genotypes.

Pollination using irradiated pollen

The pollination done using pollen-grains of Red Spanish irradiated at doses 0, 250, 500, 750 and 1000 Gy on flowers of Smooth Cayenne cv. 'Serrana' demonstrated that *in vitro* viability of irradiated pollen was not dose-dependent. However, there was a strong influence of irradiation dose on seed set, germinated embryo percentage and plantlet regeneration. Doses up to 250 Gy were not useful for getting either embryo formation or plantlet regeneration. Increasing irradiation dose increased the number of empty seeds ($r = - 0.69$, $y = 36.2362 + 17.3334$ * doses), and decreased the number of seeds with embryos ($r = 0.74$, $y = 63.4348 - 20.5050$ * doses) and with full contents ($r = - 0.92$, $y = 83.3998 + 22.8597$ * doses).

Some regenerated plantlets from the different irradiation doses showed phenotypes with small and thin leaves and short internodes (Benega et al., 1997b, 1998).

Intergeneric crosses

The first successful attempts to make intergeneric crosses using haploid pineapple plants were achieved with crosses between Smooth Cayenne cv. 'Serrana' as the female parent and 'Curujey'. Karyological analysis, demonstrated that 6.25% of regenerated plants from intergeneric crosses were haploid ($n = 25$). Furthermore, there were mixoploid plants with 4.66% showing cells with $n = 25$ and $2n = 33$ chromosomes, respectively. All the other regenerated plants resulted intergeneric hybrids ($2n = 33$ and $2n = 41$).

Conclusions and perspectives

- For anther and ovule culture we have developed a complete methodology that includes all the processes from inflorescence collection until plantlet regeneration *via* callus induction. These results could also be applied for mutation induction, using irradiation.
- On *in situ* parthenogenesis induced by irradiated pollen-grains there are the irradiation-doses more available to get plantlet.
- The karyological and molecular analysis demonstrated that some regenerated plants from anther and ovule culture and *in situ* parthenogenesis induced by irradiated pollen are haploids or doubled-haploids.
- The attempts achieved in intergeneric crosses upon haploid production, represent a significant step in developing a new breeding strategy for pineapple species. Haploid plants could assist greatly the hybridization programs and, thereby to obtain new varieties in a short time. After the doubled haploid plants could be incorporated to breeding traditional schemes of pineapple, a surprisingly small number of pineapple hybrids would be necessary in order to find the hybrids more available for production. This will allow pineapple breeders to concentrate efforts and resources. Haploid and dihaploid plants would also be useful in providing access to homozygous recessive genes, whenever they remain economically advantageous as in the case of deleting marginal spines (Subramanian *et al*., 1981). Moreover, embryogenic suspension cultures can be initiated now from haploid tissue, being an excellent source of material for protoplast isolation and might also be used in transformation experiments, as well as backcrossing and mapping.

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Analisys of Somaclonal Variation in Pineapple (Ananas Comosus (L) Merr) Plants Regenerated from Callus in the Field

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Abstract
The effect of *in vitro* culture on somaclonal variation of pineapple (*Ananas comosus* (L) Merr) var. Red Sapanish was determined. Callus was induced from leaf explants in Murashige and Skoog (MS) (1962) basal medium, supplemented with 0.2 mg L^{-1} 2,4-D + 0,1 mg L^{-1} kinetin. Plants were regenerated in Kartha *et al.* (1980) medium and were planted in Ferralitic red soil mixed with 50% sheep manure and adapted under cheese cloth in the greenhouse. The *in vitro* culture produced very little variation in thorny characters of leaves, while values of repeatability in plant characters were more influenced by *in vitro* culture than were fruit characteristics. Two individuals were selected, one for the low level of spines and the other for dwarfism.

Introduction

Heinz *et al.* (1977) successfully exploited *in vitro* culture of sugarcane to obtain several useful somaclons. Skirvin (1978) noted that variation associated with tissue culture included morphological and biochemical characteristics and even the number of chromosomes and chromosome structure. Larkin and Scowcroft (1981) defined somaclonal variation as variation produced when an explant (whichever part plant) was submitted to a tissue culture cycle.

Hammerschlag (1992) reported that estimated variation frequency in plants regenerated from *in vitro* culture was as high as 30-40% for some types of variation and 0.2-3% as a general rule.

Media and it's hormonal composition influenced the appearance of somaclonal variations (Ogihara, 1981). Studies that provide evidence of the effect of *in vitro* culture are Shepard *et al.* (1980), tuber color of potato; Reish and Bingham (1981), alterations in plant height; Karp *et al.* (1982), chromosome numbers; McCoy *et al.* (1982), chromosomal reorganization; and Bretell *et al.* (1986), alteration in copies gene numbers. Evola *et al.* (1984) and Novak *et al.* (1986) did not find differences between the specter of variants that appeared in *in vitro* regenerated maize plants that had been irradiated compared with unirradiated plants. Variations were found in *in vitro* cultures of pineapple (Wakasa, 1977, 1979; Dewald *et al*. 1988; Lii *et al*. 1989). Ramírez (1981) analyzed the repeatability of various characters under Cuban conditions.

The objectives of the present work were to study the effects of *in vitro* culture on regenerated plants in field conditions and select individuals with reduced spininess.

Materials and methods

A group of 400 Red Spanish plantlets was obtained from callus formed in MS basal medium supplemented with 0.2 mg L^{-1} 2,4-D and 0.1 mg L^{-1} kinetin. The plantlets were regenerated from the callus in media consisting of half MS salt, 0.2 mgL^{-1} IBA, 0.25 mgL^{-1} BAP and 0.03 mgL^{-1} AG_3, both in solid media (Kartha *et al.,* 1980). Plants were grown under shade in Ferralitic red soil with 50% sheep manure in a greenhouse and then established in the field. Clonal separation of plants generated from the same explant were realized, 20 plants of each callus proliferation were used for this purpose, 20 clones were established, replicated 20 times. These plants were selected for the spineless character.

Evaluations during growth included: Plants that remain spineless, plants that pass spiny form, plants with high wax content, plants that are spineless and have high wax content and dwarf plants. Evaluations made at harvest included: Fruit weight with crown (g), number of eyes, fruit height (cm), fruit diameter (cm), slip number, plant height (cm), and peduncle width (cm). The data collected at harvest were subjected to repeatability analysis, according to the model of single classification of randomized effects (Galvez, 1985):

$$Y_{ij} = \mu + A_i + e_{ij},$$

where Y_{ij} = is a measure of a single individual, μ = general media, A_i = variation due to individuals, and e_{ij} = error associated with those individuals. The statistical analysis was done in the following way:

Table 1. Statistical analysis of repetition.

Variation source	Degrees of freedom	Medium Square	E(MS)
Among individuals	n - 1	MS_1	$s_e^2 + s_i^2$
Among measure	n (r - 1)	MS_2	s_e^2

n = number of individuals and r =measured repetition by individuals.

The formula (1) of Galvez (1985) was used to determine repeatability.

1 $$\sigma^2_w = \frac{MS_1 - MS_2}{r}; \quad \sigma^2_R = \frac{\sigma^2_w}{\sigma^2_w + \sigma^2_e}$$

The standard error of repeatability (SeR) was calculated according to (2).

2 $$SeR = \sqrt{\frac{2(n-1)(1-R)^2[1+(k-1)R]^2(n-1)}{k^2(n-N)(n-1)}}$$

Where:

$$k = \frac{1}{N-1}\left(n - \frac{\sum_i N^2}{n}\right)$$

N- Number of individuals analyzed, n- Total number of measure, and R- Value estimated of repeatability.

Results and discussion

Of 400 plants examined, only 5 remained spineless. The remainder reverted to the spiny type. Lii *et al.* (1989) evaluated 40,000 Red Spanish plantlets produced under *in vitro* conditions and 50% of them were spineless. However, only 14.1% of these plants remained spineless. Hammerschlag (1992) noted the variation frequency of regenerated plants subjected to *in vitro* culture has been estimated to be as high as 30-40% for some characteristics and 0.2-3 % for variation as general rule. In this study, the frequency of most characteristics was low (Table 2).

Table 2. Frequency of varied characters in plant population. (400 plants).

Character	Number of varied plants	Percentage
Spineless plants	5	1.25
High wax content	6	1.50
Spineless, high wax	3	0.75
Dwarf	1	0.25

Different amounts of variation have been found when pineapple is propagated by tissue culture. Dewald *et al*. (1988) found little variation after *in vitro* propagation while Wakasa (1977) showed that variation depends on the nature of the explant. Thus he observed very little variation when axillary buds were cultivated; however, a high degree of variation was found when syncarp tissue was used. In later studies, when Wakasa (1989) analyzed a smaller quantity of regenerated plants compared with his previous investigation, he found that 77.6% of them were spineless. Wakasa did not analyze the reversion to spiny plants and did not mention the variety he used.

In the first part of this work, unlike results obtained by Lii *et al*. (1989) and Wakasa (1989), but in correspondence with the findings of Dewald et al. (1988) and Hammerschlag (1992), little variation was found and the reversion of regenerated plants from spineless to spiny was very high.

After analyzing the spininess character, Py (1968) identified three types of spiny leaves; leaves with spines only at the tip, leaves where the entire margin was spiny, as is the case for Red Spanish, and spineless leaves. Of these, the gene expression for leaves with spiny tips was highly affected by environment and there was an increase in spine number when unfavorable condition existed.

Collins (1960) proved that spiny tip (S) and spiny (s) characters were alelomorphos and, therefore, all spiny cultivars are homozygous (ss) while Smooth Cayenne is heterozygous (Ss). These criteria permit us to conclude that there is little possibility of finding spineless variants of Red Spanish, but it is possible to find individuals with fewer spines. Singh and Iyer (1974), using chemical mutagens on Queen pineapple, found spineless variants of spiny cultivars.

A second part of this work was the analysis of the repeatability of some characters, repeatability being a statistical parameter given

by the quotient of component of total phenotypic variance. According to Falconer (1970) repeatability makes it possible to relate measurements made on the same individual.

The repeatability of fruit weight and eye number were very high (Table 3) while repeatability of fruit diameter was medium and fruit height was low. Ramírez (1981) noted that permanent differences existed between individual varieties although there is a mixture between the genetic variance and the environmental variance that in our case would be the influence of *in vitro* culture.

Table 3. Values of repeatability and standard error in some fruit characters.

Character	Repeatability (R)	EsR†
Fruit weight	0.898	0.03
Fruit height	0.170	0.068
Fruit diameter	0.440	0.091
Eye number	0.990	0.003

†Standard error of repeatability .

The investigation was carried out with homogeneous plants, which were only separated by clone, planted in a homogeneous area, so we can attribute the effect of *in vitro* culture on fruit height, which presented low repeatability value. Ramírez (1981) obtained a value of 0.323 for this character in work with the Red Spanish variety in Havana province. The results for plant height were lower than values obtained by Ramírez (1981) (Table 4). Other characters analyzed also had low values, with peduncle diameter having the lowest value.

Table 4. Values of repeatability in plant characteristics.

Character	Repeatability (R)	EsR†
Plant height	0.63	0.080
Peduncle height	0.22	0.076
Peduncle diameter	0.16	0.067
No. slips	0.28	0.083

†Standard error of repeatability .

We concluded that *in vitro* culture produced very little variation in the spiny character. Also, a very high percentage of regenerated spineless plants reverted to the spiny state as adults. Values of repeatability for fruit height, peduncle height, peduncle diameter and number of slips were low. We attributed the major effects of *in vitro* culture to these characters. References related to analysis of repeatability for the three characters reported here were not found by us.

Finally, this work permitted us to select plants that were spineless or with a low level of spines; P_2R_5, P_3R_5, P_5R_1, $P_{18}R_5$ and E_1 a dwarf plant. The selections P_3R_5 and E_1 maintained character stability during four vegetative generations.

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Ed Note: Apologies are extended to the authors for changes made to improve readability that may also have altered the original meaning. Attempts to contact the authors by E-mail were unsuccessful.

News From France and Venezuela

The Application of the International Code of Nomenclature to Pineapple Cultivars

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Commercial pineapple production is mainly based on a few commercial cultivars. As these traveled extensively and have been acclimated in many different countries, they have been frequently renamed, sometimes with particular local names, and sometimes with such general (and useless) names as "Sugar Loaf". In addition, they have lost uniformity through spontaneous somatic mutation, differentiating into many regional variants, through conscious or unconscious clonal selection. These processes have generated a tremendous confusion in the nomenclature of pineapple cultivars. And the more widespread the variety, the greater the confusion maintained in the literature. Some authors have attempted to produce classifications into horticultural groups. That of Hume and Miller (1904) included three groups, "Cayenne", "Queen" and "Spanish", and a fourth "miscellaneous" category for a wild pineapple. It was completed successively by Py and Tisseau (1965), Samuels (1970), and Leal and Soule (1977) with the inclusion of two more groups, "Pernambuco" and "Maipure". The latter was renamed "Perolera" and then "Mordilona" (Py *et al*., 1987; Cabot, 1987). However, this horticultural classification is inadequate, mainly for two reasons. First, it overlooks the extent of the genetic variability in pineapples, letting aside the great majority of the pineapple cultivated germplasm. Many hundreds of recently collected genotypes could not be classified in the five groups (Duval *et al*., 1997). Second, it is confusing because the different groups correspond to different genetic concepts. While the groups "Cayenne" and "Queen" include cultivars that only differentiated by the accumulation of somatic mutations, the three other groups are defined on general appearance, or even on a single trait (conical fruit for "Pernambuco" and smooth "piping" leaves for "Perolera"), and they include cultivars of very different origins (Coppens d'Eeckenbrugge *et al*., 1997a). Thus, Coppens d'Eeckenbrugge *et al.* (1997b) proposed to abandon this classification and to come back to the international code of nomenclature for cultivated plants (IUBS, 1980).

According to the code (article 10), a cultivar is "an assemblage of plants which is clearly distinguished by any characters (morphological, physiological, cytological, chemical, or others), and which, when reproduced (sexually or asexually), retains its distinguishing characters." The term is derived from *cultivated variety*, and it is exactly equivalent to the term *variety*. However, some editors do not admit the term "variety" applied to cultivated material because of the possible confusion with the concept of botanical variety.

In the examples given by the code (article 11) is the case of a cultivar consisting of one clone or several closely similar clones, where a clone "is a genetically uniform assemblage of individuals (which may be chimeral in nature), derived originally from a single individual by asexual propagation… Individuals propagated from a distinguishable bud-mutation form a cultivar distinct from the parent plant." Also interesting for pineapple is the article 12 stating that "the practice of designating a selection of a cultivar as a strain or equivalent term is not adopted… Any such selection showing sufficient differences from the parent cultivar to render worthy a name is to be regarded as a distinct cultivar" and the code recommends naming such selections in a way that indicates the relationship. Another rule to be applied by pineapple experts should be to leave cultivar names unchanged when rendered in another language (article 32). This last rule comes too late for 'Cayenne Lisse' whose transliteration into 'Smooth Cayenne' and 'Caiena Lisa' has been widely accepted. But it should be applied to all other cultivars. Finally, cultivar names must be separated from the latin botanical name by the word "cultivar" or its abbreviation "cv.". When it is not preceded by the word cultivar, it should be written between single quotes.

Following the code, 'Smooth Cayenne' is clearly a cultivar. 'Cayenne Baronne de Rothschild' is a distinct cultivar, because it is clearly distinguished from 'Smooth Cayenne' by full spininess. Any new spiny plant derived from 'Smooth Cayenne' belongs to 'Cayenne Baronne de Rothschild' (unless it can be clearly distinguished on another character). Indeed, the code states that "the mode of origin is irrelevant when considering whether two populations belong to the same or different cultivars". Collins (1960) suggested that 'Hilo' is a distinct cultivar because it can be clearly distinguished by its numerous slips. Other names such as 'Kew' or 'Giant Kew,' still in use in some former British colonies, 'Sarawak' in Malaysia, 'Champaka', 'Esmeralda', 'Claire', 'Typhoon,' and 'Saint Michel' are not related to distinct characteristics and must be considered synonyms of 'Smooth Cayenne'.

'Queen' is also a cultivar. A detailed study might allow distinguishing clear differences between local populations or between clonal selections. However no sufficient data exist. Shoot and slip numbers are particularly variable traits. Some selections exhibit particular vigor, as 'Mc Gregor', but they cannot be distinguished from other similar selections. Thus, names as 'Mauritius,' 'Malacca,' 'Red Ceylon,' and 'Buitenzorg.' 'Ripley Queen', 'Alexandra' and 'Mc Gregor' must be considered synonyms to 'Queen'. Only the tetraploid 'Z' or 'James Queen', found in South Africa (Nyenhuis, 1974), must be considered a distinct cultivar.

The cultivar Singapore Spanish is common in Asia, where it has received many different names. Only in Malaysia, it has been known under such diverse names as 'Singapore Canning', 'Ruby', 'Red Pine', 'Nanas Merah', 'Nangka', 'Gandol', 'Betek', and 'Masmerah'. The names 'Selangor Green', 'Green Pine,' 'Nanas Hijau', 'Green Spanish,' and 'Selassie' designate a distinct cultivar, derived from a green (anthocyanless) mutant (Anonymous, 1978; Wee, 1972). According to the descriptions of traditional Taiwanese material by Sakimura (1935) and the zymotypes published by Aradhya *et al*. (1994), 'Anpi' is also a typical 'Singapore Spanish' strain while 'Oohi' and 'Uhi' belong to the green cultivar. Within 'Singapore Spanish' and 'Selangor Green', cultivars could be further distinguished on the basis of partial or full spininess.

'Española Roja' (sometimes transliterated to 'Red Spanish') has kept its name in the Caribbean basin where it is widely cultivated. However the creative people of this area gave diverse names such as 'Black Spanish', 'Key Largo', 'Havannah', 'Habana', 'Cubana', 'Cowboy', and 'Bull Head', and the Philippines have contributed with 'Native Philippine Red'. All these names should be considered synonyms. However, as in 'Singapore Spanish', cultivars could be distinguished on the basis of partial or full spininess.

In Brazil, 'Pérola' is also known as 'Pernambuco' or 'Branco de Pernambuco' but it was named 'Abacaxi', 'Abakka' or 'Eleuthera' in Florida. 'Jupi' is the name for a particular strain. However, there is no particular description in the literature, and the distinction seems insufficiently clear to raise it to the cultivar rank.

'Perolera', an important cultivar in the Andes of Colombia and Venezuela, is also known as 'Lebrija', 'Motilona', 'Capachera' or 'Tachirense'. It is heterogeneous and local farmers seem to distinguish particular types. Among these, the 'Perolera Manzana' or 'Manzana', also called 'Bumanguesa' is a distinct cultivar, which can be identified by its flatter eyes, regular shape and red shell color.

The strict application of the code of nomenclature, which gives priority to the "original" name of the cultivar, may not satisfy the scientists who need to refer to a particular local "strain" or clonal selection. Even though they are not clearly distinct, they are important for production as even a slight difference in vigor and/or fruit size translates to tons per hectare at the field level. What we have proposed is to use the name of the local population or clonal

selection, indicating the synonymy with the most common name of the cultivar (Coppens d'Eeckenbrugge *et al*., 1997b). This would give expressions such as 'McGregor' ('Queen'), 'Champaka' ('Smooth Cayenne'), or 'F-180' ('Smooth Cayenne'). We also had proposed to use 'Queen McGregor' or 'Smooth Cayenne Champaka' but it would be less correct, as it could indicate that they are derived cultivars.

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News from Malaysia

Physiological Study on the Crown and Fruit of Pineapple During and after Storage at Low Temperatures

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Being a non-climacteric fruit, the respiration and ethylene production rates of pineapple are low during ripening. In the body of this composite fruit, there is a maturity gradient from the bottom to the top of the fruit with the fruitlets at the bottom being the most mature. The crown and fruit body are different in physical characteristics and their physiological responses to environmental changes after harvest are also expected to be different. The physiological variations of the two parts were investigated in Malaysian pineapple cv. `Gandul'.

Pineapple fruits cv. `Gandul' were stored at 5, 10, 15 and 20°C for up to 5 weeks. The fruits were removed from low temperature at weekly intervals and held for 1 week at ambient (28°C) temperature. Evaluation of crown freshness (rated 1-5; 1=excellent, fresh and green, 5=dry, yellow or brown), fruit skin yellowing (rated 1-6; 1=matured green, 6=completely yellow) and total chlorophyll of both the crown and the skin during removal from low temperatures and after holding for 1 week at ambient.

The yellowing of the skin was delayed during storage under low temperatures. At 10°C, skin yellowing started after 3 weeks whereas at 15 and 20°C, skin yellowing took place a week after storage. All fruits previously stored at 10, 15 and 20°C turned yellow after they were held one week at ambient temperature. Skin yellowing did not occur in fruits stored continuously at 5°C. When these fruit were transferred from 5°C to ambient temperature, yellowing of the skin took place only for fruits stored for less than 3 weeks. Fruit stored longer at this temperature did not yellow at ambient temperature, indicating a temperature-induced chilling injury.

Crowns did not deteriorate when fruits were stored at 5°C for up to 5 weeks. The crowns of fruits stored at all other temperatures turned yellow after 5, 3 and 2 weeks storage at 10, 15 and 20°C, respectively. Yellowing of crowns of all fruits occurred when they were held for another week at ambient. The yellowing of crowns on fruits stored at temperatures higher than the optimum level suggests that crown deterioration is not related to chilling injury.

Total chlorophyll was reduced in the crown and skin of fruits stored at 10°C. The reduction in total chlorophyll in the skin is associated with the yellowing process during ripening. It is surprising to note the significant reduction in total chlorophyll of the crown a week after storage at 10°C when the fruits still appeared very fresh and green. The chlorophyll content declined further as storage period was extended. A sudden reduction in total chlorophyll of the crown was also observed doe fruits at ambient temperature whether they had or had not been previously stored at low temperature. It was concluded that the freshness of the crown was not necessarily associated with the amount of chlorophyll remaining in its tissue.

The changes in rates of respiration and ethylene production of the fruit, with and without the crown, and of the crown were studied at 2, 5, 10, 15 and 24°C. For the fruit with crown, the respiration rates increased abruptly during storage at 24°C. At 10 and 15°C, the respiration rates tended to increase as the storage period was extended, but at a much lower magnitude than the rates at 24°C. The respiration rates of the whole fruits stored at 2 and 5°C remained almost constant throughout storage. Similar respiratory patterns were also observed in the whole fruits.

A completely different respiratory pattern was observed with the crown. At 24°C, the respiration rates declined continuously during 3 weeks of storage. At 15°C, the respiration rates decreased during the first 5 days but increased and reached a peak when storage was extended. At other temperatures, i.e. 2, 5 and 10°C, the respiration rates increased at the earlier stage and declined further, followed by another increase towards the end of storage. Ethylene production of the whole fruits, detached fruits and the crowns were almost non-detectable throughout storage at 2, 5, 10, 15 and 24°C.

This study clearly demonstrated the differences in postharvest physiological behavior of the pineapple fruit body and crown. Postharvest treatments developed for the maintenance of the fruit quality during handling and distribution should also recognize the distinct difference between the two parts.◆

News from Sri Lanka

Correlation Between Calcium Levels and Variation in Storage Quality of Two Varieties of Pineapple

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Abstract

Calcium levels in the chill-sensitive Queen type Mauritius pineapple were compared with those in the chill-resistant Cayenne type Kew pineapple to investigate the possibility of a correlation between these levels and the incidence of chill-injury symptoms. Overall calcium levels in fruits together with the distribution of calcium in different locations within a fruit were recorded before and after storage at 10 °C for 17 days followed by 2 days at ambient temperature (28 °C). Total overall calcium levels were significantly higher in Kew than in Mauritius and the incidence of chilling injury was significantly lower in Kew than in Mauritius fruits. Calcium levels in Mauritius fruits decreased with maturity but were unchanged in Kew. Within-fruit total calcium levels were higher in the shell and crown regions compared with core and flesh regions where browning symptoms occurred. Following storage, calcium levels in the core and flesh regions decreased further while levels in the crown and shell regions increased. A reduction in migration of calcium ions from the core occurred when fruits were waxed with a Stafresh 7055 solution.◆

Pre-harvest Application of Calcium and the Development of Black Heart Disorder Symptoms in Mauritius Pineapples

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Abstract

The correlation between pre-harvest calcium application and the severity of black heart disorder was investigated. Pineapples of cv. Mauritius were sprayed prior to harvest with 1.2 or 2% $CaCl_2$. Soil applications of 10 or 15g per plant of CaO and $CaSO_4$ were also tested. Fruits were harvested when the shell color was between 10 and 20% yellow and were stored at 10 °C and 80 to 90% relative humidity for 17 days followed by 2 days at ambient temperature (28 ± 2°C). Fruits from each treatment were dipped in Stafresh 7055 wax solution prior to storage. Best results were obtained in the fruits sprayed with $CaCl_2$ and waxed prior to storage where 80% good quality fruits with no black heart symptoms were obtained. Unwaxed fruits sprayed with $CaCl_2$ also showed less severity and incidence of the disorder compared to untreated controls. Soil applications of CaO and $CaSO_4$ were less effective in controlling the disorder and were not significantly different from untreated controls. Endogenous calcium levels in $CaCl_2$-sprayed fruits were significantly higher than fruits from plants on plots where the soil was treated with CaO or $CaSO_4$ and from the controls.◆

News from South Africa

David Murray, Inst. for Tropical and Subtropical Crops, Bathurst, South Africa

Elmarie Rabie and myself have had quite a battle staying on top of our respective work–it just seems as if the demands mushroom the moment you turn your back for any length of time. Coupled to this, the local pineapple industry is in downturn after 3 excellent years and have had ethephon MRL problems to boot. Despite an 8 month dry spell last year, yields have increased annually with trash incorporation being the important factor I feel. The plant selections/clones are performing well and am selling all I can produce on the station. Our research function becomes more tenuous by the day–at present the stations depend on own sales (fruit, services, plants, etc) and industry financial support to cover 50% of the annual budget, the rest coming from the state. Further state funding is to some extent dependant on our involvement in agricultural socio–political projects which does'nt endear you to the commercial production sector–talk about walking a tight rope! Our brush with the EU ethephon MRL has brought about the required awareness for the South African pineapple industry to re-evaluate its production techniques in terms of worker and food safety as well as enviromental impact. Secondly, the clamor for organic pineapple is growing so we are familiarizing ourselves with earthworm secretions, microbial booster, composting, parasitic fungi, etc. This is going to be quite a challenge. There isn't a leading expert or a book on Organic pineapple in your neck of the woods? Graham Petty had a heart attack about two weeks ago whilst on the tennis court–not something I would have expected of him, but within a week he was back at work.◆

News From Taiwan

Introduction of a New Line of Pineapple in Taiwan

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A new line of Pineapple, C67-6-740, bred by pineapple breeder Mr. Chin Chyn Chang in Chia Yi Agricultural Experiment Branch Institute, Taiwan, is described. Line C67-6-740 was a cross breed between normal Cayenne and ♂1(A)1. The hybrid was selected in 1982 and tested in 6 regions over the past 16 years. The plant has average height of 71cm, has spines on leaf tips, with spineless leaf margin, which makes cultivation and management much easier. The average fruit weight is 1.3kg and turns orange-yellow when ripe. Fruits have thin skin, shallow locule, fine yellow flesh, and medium sweet taste, a Brix of 13, 0.25% titratable acidity, and a sugar/acid ratio of 52. The overall sensory evaluation of fresh fruit was rated as fine. The harvest season is between April to June. The line is expected to be designated with the official cultivator name in April of year 2000.◆

News From the United States (Hawaii)

Pineapple Research and Development Program

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Pineapple research and development for the Hawaiian pineapple industry falls into three specific areas: 1) management of mealybug wilt, 2) management of nematodes, and 3) controlling flowering and fruit quality through fruit development and post-harvest handling. World competition for Hawaii's pineapple markets have caused

Hawaii's pineapple companies to 1) identify specific market niches for high quality Hawaii products (e.g. fresh fruit varieties, fresh ready-to-eat chilled products), 2) reduce production costs by more efficient pest and disease control practices, and 3) develop practices which are more consumer and environmentally friendly (e.g. IPM, reduced pesticide use, reduced soil erosion and water pollution).

Management of Mealybug Wilt

Currently mealybug wilt of pineapple is controlled by application of Amdro bait to control ants. Several other approaches for control of mealybug wilt are currently being researched. They are:

- the use of Amdro in a bait station which will prevent release of Amdro in the environment and extend its useful life and therefore its efficiency in the field.
- the specific identification of the cause of mealybug wilt and development of genetically engineered plants resistant to the disease.
- using biological control of mealybugs to reduce the impact of ants on the population levels of both the pink and gray mealybugs
- the evaluation of newer more environmentally friendly pesticides to control ants and mealybugs.

Significant progress is being made on genetically engineering virus resistance in Hawaiian pineapple varieties and in improving the efficiency of the use of Amdro thereby reducing the amounts required.

Management of Nematodes

Pineapple nematodes are currently controlled by fallowing fields between planting, soil fumigation with Telone, and, with the post-plant applications of Telone and carbamate and organic phosphate nematicides. Studies during the last 5-10 years have dealt with increasing the efficiency of the use of these materials in order to reduce the amounts needed to maintain economically viable control. Amounts needed have been significantly reduced. However, environmental issues continue to require the search for other strategies to control nematodes. The current areas of research are:
develop resistance in the pineapple varieties using genetic engineering.
Examine the use of Systemic Acquired Resistance which is somewhat analogous to the resistance in animals induced by vaccinations.
Evaluate various cultural and biological controls such as fallow, nematode-suppressing cover crops and the use of nematode infecting nematodes.
Significant progress is being made in engineering nematode resistance and increasing the efficiency of current nematode controls.

Pineapple Flowering, Fruit Ripening and Post-Harvest Handling

The low-acid fresh fruit varieties now being developed and marketed by the Hawaiian pineapple industry frequently flower naturally more readily than does 'Smooth Cayenne'. Natural flowering increases harvesting costs and disrupts marketing plans. Additionally, knowledge of optimum cultural practices to maintain high yields and fruit quality are not as well known as those for Smooth Cayenne. Current areas of research and development are:

- Genetic engineering to control flowering and fruit ripening.
- Evaluating optimal fruit development and post-harvest handling to maintain fruit quality.
- Evaluating growth regulators to prevent out-of-cycle flowering and post-harvest fungicides to reduce post-harvest diseases.
- Determining optimum post-harvest handling conditions to maintain fruit quality.

Significant progress is being made in genetically engineering flowering control in susceptible varieties and in improving post-harvest handling and disease control.

The pineapple research and development program is publicly funded by the State of Hawaii (approximately $200,000/yr) and the federal government through the USDA-Agricultural Research Service ($250,000/yr) and the USDA Cooperative Research Education and Extension Service Special Grants through the Tropical Sub-tropical Agricultural Research Program ($43,000/yr) and the Western Integrated Pest Management Program ($50,000/yr). All funding is allocated and expended based on regular interaction with pineapple industry members. Results are reported at regular meetings with pineapple industry members and annual workshops.◆

Mealybug Wilt of Pineapple and Associated Viruses

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Etiology

We have taken two approaches to study the role of pineapple mealybug wilt associated virus (PMWaV) in mealybug wilt of pineapple (MWP). The first is to examine the distribution of PMWAV in pineapple and to associate PMWaV with MWP in the field. Field data recently collected from Maui shows a strong correlation between mealybug wilt symptomatic plants and infection with closterovirus.

The second approach is to transmit PMWAV to healthy pineapple using mealybugs to reproduce the disease. Three experiments have been conducted. First, MWP symptom induction experiment was conducted using groups of potted PMWaV-free plants and PMWaV-infected plants. The two groups received 20-100 mealybugs/plant at monthly intervals or were kept mealybug-free for the duration of the experiment. After three months only plants in the PMWaV-infected group exposed to mealybugs expressed typical symptoms of MWP; plants in the other three groups remained symptomless. Second, a randomized complete block design was used to test whether MWP symptoms could be induced under field conditions. Plots consisted of PMWaV-free plants kept mealybug free, PMWaV-free plants receiving monthly applications of mealybugs, PMWaV-infected plants kept mealybug free and PMWaV-infected plants receiving monthly applications of mealybugs. Each plot was replicated four times and contained 120 plants. Symptoms developed only on PMWaV-infected plants in the plots receiving mealybug applications. Plants in all other treatments remained healthy looking. Third, varietal susceptibility to mealybug wilt symptom development in the presence of PMWaV and mealybugs was tested. Six commercially grown *Ananus comosus* Smooth Cayenne cultivars from Hawaii were tested. All were susceptible to mealybug wilt when both PMWaV and mealybugs were present. PMWaV-free plants exposed to mealybugs showed signs of spotting caused by mealybug feeding but did not develop symptoms of mealybug wilt. PMWaV-free and infected plants kept mealybug free did not develop MWP symptoms.

In conclusion, results from the two approaches show that there is a clear association between the PMWaV presence and symptoms of MWP for some cultivars in Hawaii, and that both PMWaV and mealybugs are essential for MWP. Our working hypotheses for the etiology of MWP involves interactions between PMWaV and stress caused by mealybug feeding (i.e. insect toxins). We further

hypothesize that pineapple plants have developed tolerance to PMWaV infection and remain symptomless when infected by PMWaV. They are no longer tolerant to PMWaV when under mealybug feeding stress and develop symptoms of MWP; new leaves appear healthy when mealybug feeding stress is removed (recovery phenomenon).

Epidemiology

Transmission of PMWaV by mealybugs

Both pink and gray mealybugs can transmit PMWaV. Both PMWaV 1 & 2 can be transmitted by mealybug vectors. One mealybug can cause transmission of PMWaV; 20 mealybugs per plant cause 100% transmission. One month after transmission; virus infection can be detected by specific antibodies in tissue blotting assay.

Interactions of PMWaV with other factors on pineapple production

The impact of PMWaV-1 on pineapple growth and yield with and without drought was evaluated on the first ratoon crop. Our findings show the presence of PMWaV-1 significantly reduced mean fruit weight in the ratoon crop cycle but not in the plant crop cycle.

The impact of nematode absence or presence on pineapple plants with and without closterovirus is currently being assessed on Oahu in collaboration with Dr. B. Sipes (UH-CTAHR) and Mr. C. Oda (Del Monte). The plant crop data from the nematode/PMWaV interaction indicates plants receiving nematicide treatment produce significantly larger fruit than those receiving no nematicide. PMWaV presence in plots receiving no nematicide produced significantly smaller fruit than all other groups.

A field study of the impact of mealybugs (*D. brevipes*) absence or presence on pineapple with and without PMWAV was installed at Maui Pineapple Co., Haile Maile, Maui. The plantation has supplied the planting area, prepared the ground for planting and supplied Champaka 153 crowns for screening. Approximately 11,300 crowns were screened for closterovirus and sorted. A random block design with plots containing PMWaV-free and infected plants kept mealybug-free and plots planted with PMWaV-free and infected pineapple plants was used to test whether PMWaV, mealybugs, or both are necessary for MWP. The experimental design has for replicates with each test plot consisting of approximately 130 plants. Only plants in the PMWaV-infected plots receiving mealybugs developed symptoms of MWP.

Management

Screen for resistant germplasm

Comparisons of the negative accessions from the Philippines Breeding Institute and the Pineapple Germplasm Repository in Hilo were made to identify any additional candidates for resistance. Two of the negative samples from the Philippines were species of *A. bracteatus*. Three *A. bracteatus* from the USDA-ARS Pineapple Germplasm Repository in Hilo were also found to be negative. Various virus negative accessions (29) were obtained from the the USDA-ARS Germplasm Repository in Hilo. The accessions have been transplanted to soil and plants were inoculated. No pineapple selections obtained from the ARS-USDA Pineapple Germplasm Repository were resistant to infection with PMWaV by mealybugs.

3-2*Genetic Engineering.* We have transformed pineapple with HSP, REP, and CP genes of PMWaV in collaboration with Dr. Chifumi Nagai at HARC. Characterization of the putative transformed pineapple plants is in progress. The purpose is to develop PMWaV-resistant transgenic pineapple plants for management of MWP.◆

Fruit Sugars, Temperature and Crown Removal on the Occurrence of Pineapple Fruit Translucency

Ching-Cheng Chen and Robert E. Paull, Department of Tropical Plant and Soil Sciences, Univ. of Hawai'i at Manoa, Honolulu, HI 96822

Funded by Hawai'i Department of Agriculture Grant # 45386

Pineapple fruit translucency, of unknown cause, affects approximately 10% of fresh fruit and losses can exceed 30%, due to high translucent fruit not being harvested or damaged during shipping. A period two to three months before harvest when maximum and minimum temperatures are both low, 23° and 15°C, or high, 29° and 20°C, respectively, is crucial to the development of fruit translucency. Preliminary results suggested two hypothesis. The first is that photosynthate competition between the crown and the fruit is determined by environmental conditions during the initial period of crown growth. The second possibility is that insufficient calcium uptake during the middle of fruit growth make the fruit flesh more "leaky." Crown and fruit growth studies and defoliation and shading at different stages of growth were used to address the first possibility and calcium sprays were used for the second.

Results

Pineapple fruit translucency begins to appear 2 to 4 weeks before harvest. Flesh tissue became very susceptible to high temperature and the cell electrolyte leakage rapidly increases during these 4 weeks. Electrolyte leakage of fruit flesh is reduced by covering fruit in the field with clear-plastic or a postharvest heat treatment (48°C, 24 h). In contrast, flesh tissue electrolyte leakage is increased by the postharvest heat treatment when the fruit age was 4 weeks before harvest or older. Covering fruit with clear-plastic during the last 3 weeks of fruit development decreased titratable acidity and increased translucency severity.

The effect of temperature on fruit translucency occurrence can be divided into two stages: the period earlier than 6 weeks before harvest and the period from 6 weeks before harvest to harvest. This division was based upon high fruit temperature eight weeks before harvest or earlier reduced electrolyte leakage of the fruit flesh tissue, but increased leakage when the fruit age was 4 weeks before harvest or older.

Removing the crown either at an early or late stage of pineapple fruit development does not cause any significant effect on the fruit weight and translucency, suggesting that the crown does not play a significant role in pineapple fruit development and translucency occurrence. Defoliation conducted 4 or 3 weeks before harvest does not significantly reduce the pineapple fruit weight, but does significantly reduce the total soluble solids and fruit translucency, suggesting that the photoassimilate partitioning during the last stage of fruit development played an essential role in the occurrence of translucency.

The calcium concentration in pineapple fruit flesh declines with fruit development, possibly due to a decrease in the proportion of water imported via the xylem compared to the phloem. Mature fruit flesh tissue has a significantly reduced ability to bind divalent cations. Spraying calcium during pineapple fruit development decreased translucency occurrence at harvest.

Sugar accumulation and the activities of sugar metabolizing enzymes are related to the occurrence of pineapple fruit flesh translucency. Sucrose began to accumulate 6 weeks before harvest at a higher rate in the fruitlet than in the interfruitlet tissue. During this period the activities of three invertases and sucrose synthase were

low. Electrolyte leakage from pineapple fruit flesh increased rapidly from 6 weeks before harvest and paralleled sucrose accumulation.

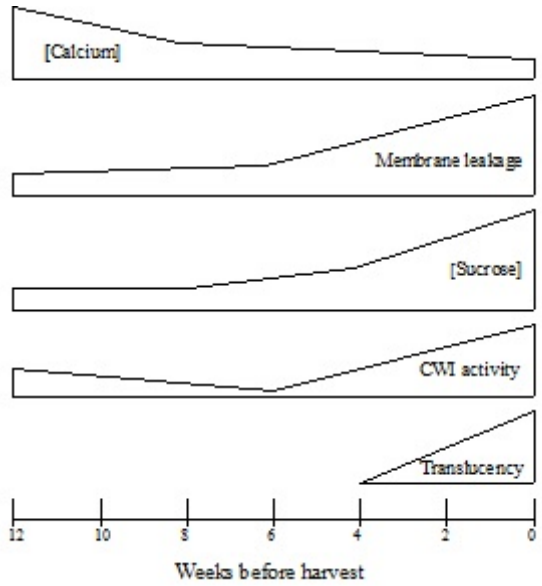
Sucrose synthase activity was high in young fruit flesh and declined with fruit development, while the activity of sucrose phosphate synthase was relatively low and constant throughout fruit development. The activities of acid invertase, neutral invertase, and cell-wall invertase (CWI) were high in the young fruit flesh and declined to very low levels 6 weeks before harvest when sucrose started to accumulate. CWI activity increased again, more in the fruitlet than in the interfruitlet tissue, 4 weeks before harvest.

Removal of 1/3 of the plant leaves 3 weeks before harvest significantly reduces fruit flesh total soluble solids, CWI activity and translucency incidence at harvest. The activity of CWI in translucent fruit flesh is significantly higher than that in opaque fruit flesh. CWI activities in the basal section of pineapple fruit flesh and in the fruitlet, where translucency first occurred, are also higher than those in the apical section and in the interfruitlet tissue, respectively.

The results support the hypothesis that high CWI activity in pineapple fruit flesh at the later stage of fruit development enhances sucrose unloading into the fruit flesh apoplast, leading to increased apoplastic solute concentration (decreased solute potential) and subsequent water movement into the apoplast. This, in turn, may reduce porosity and lead to increased fruit flesh translucency.

Conclusions

Pineapple fruit translucency occurring at the latter stages of fruit development is due to a combination of effects related to high fruit temperature and fruit maturity. Associated factors affecting the severity of translucency include a decrease in calcium concentration of fruit flesh and of divalent cation fruit flesh cell wall binding ability that lead to a loss of cell membrane integrity and cell wall fragility. These changes are followed by an increase in membrane permeability and an enhanced susceptibility of fruit flesh to high temperature. Increased sucrose accumulation and the activity of CWI favoring apoplastic phloem unloading, caused an increase in the solute concentration and liquid volume in the apoplast, that in turn lead to translucency. The results are summarized below.◆



High Pressure Treatment of Pineapple Slices

Nancy Jung Chen[1], Robert E. Paull[1] & Aurora Hodgson[2]
[1]Dept. of Tropical Plant and Soil Science; [2]Dept. of Food Science and Human Nutrtion, University of Hawai'i at Manoa, Honolulu, HI 96822 U.S.A.

Introduction:

Hawaii's ability to expand export acreage and compete on selected crops will depends on the success at production and marketing a premium high quality product. Minimal processing offers some potential to solve the fruit fly problem, and at the same time provide consumer a high quality convenience food. Recent development in high pressure food processing of pineapple suggested the possibility of processing a very high quality fresh chilled "ready to eat product". The advantage of this high pressure processing over the conventional minimal processing are its product's enhanced flavor and extended shelf life.

High pressure treatment is a process in which foods are sealed in flexible packages and subjected to high pressure. Pressure, unlike heat, acts immediately and is transferred uniformly throughout the food and is not a function of container size. The high pressure sterilizes the food without the changes in flavor, color, texture, aroma, or nutritional value. Pressures of 600 to 900 MPa (5,921 to 8,882 atmos.; 5,000 to 9,000 bars; 10,000 to 135,000 PSI) are used. Fruit spoilage organisms such as yeast and lactic acid bacteria are killed by high pressures of 600 to 700 MPa for 10 minutes. The low pH of the fruit also suppresses bacterial spore growth. Fruit fly larvae and egg show no survivors at greater than 125 MPa for 20 min. Additives and mild heat are also used in conjunction with pressure.

Results:

The high pressure treatments tested did not consistently produce sterile products, however it did significantly reduce the bacterial count. In most case, the counts were not detectable. Spoilage was noted within 5 days when the product was stored at ambient temperature. Fruit slices treated with pressures higher than 400 mPa for 10 min can be stored at 2°C for more than one month without microbial spoilage.

Laminated PE bags, though more difficult to handle, are readily sterilized. The rigidity of these laminated bags makes it difficult to remove all the air from the package. It was noted that there was a relationship between the amount of free air in the sample package and the success of sample sterilization: the less free air in the package, the easier it was to create a sterile product. If liquid is not added to the bag, sterilization was not consistently obtained.

All three color values; 'L', 'a' and 'b' decrease in response to pressure with the yellow color intensifying after pressure treatment. 'L' value showed a 30 to 40% decrease while the change in 'a' value is less pronounced. The reduction in 'b' value varied with the pressure, duration of the treatment and the maturity of the pineapple fruit.

The yellow flesh color begins to fade in treated pineapple slices within 2 week, when stored at ambient temperature. Fading is noticed in untreated slices at 2°C after 4 weeks and turn brown within 7 weeks, at ambient temperatures browning occurred within 1 week. The addition of low concentrations of ascorbic acid to treated slices held at ambient temperature does not prevent browning. Ascorbic acid concentration higher than 0.25% effectively delay browning of treated pineapple at 2°C. When stored at ambient temperature, 0.25% and 0.5% ascorbic acid are not effective in delaying browning, concentration higher than 1% delayed browning for 2 to 6 weeks. It was noted that after 6 weeks, pineapple slices turned orange red instead of brown following treatment with ascorbic acid. Cysteine is similarly effective in delaying browning at similar concentrations to ascorbic acid. Calcium lactate at 2% does not delay browning during storage at 2°C.

Holding intact pineapple fruit at 10°C before preparing the fruit for pressure treatment alleviated the reduction in yellow color. There is no difference between fruit held at 10°C and fruit treated within 48 hours of harvest in texture, TSS, pH and TA in response to pressures.

There are no significant differences in slice firmness, TSS, pH and titratable acidity between control samples and those treated with high pressure at varying temperatures. However, at elevated temperature, the threshold time to sterilize pineapple is reduced. A minimum of 10 min is required to sterilize pineapple slices when treated at ambient temperature and 600 mPa. At room temperature, more than 20 minutes at 500 mPa is required to create a sterile product. At higher temperatures a lower pressure is required to achieve sterility. Our machine's limits are 60°C and 670mPa.

Conclusions:

1. Fruit slices treated with pressures higher than 400 mPa for 10 min can be stored at 2°C for more than one month without microbial spoilage.
2. Holding intact pineapple fruit at 10°C before preparing the fruit for pressure treatment alleviated the reduction in yellow color.
3. When stored at ambient temperature, 0.25% and 0.5 % ascorbic acid are not effective in delaying browning, while concentration higher than 1% delayed browning for 2 to 6 weeks.
4. There are no significant differences in slice firmness, TSS, pH and titratable acidity between control samples and those treat with high pressure at varying temperatures.◆

Alternative Chemicals for Control of Nematodes

B. S. Sipes, Dept. of Plant and Environmental Protection Sciences, Univ. of Hawai'i at Manoa, Honolulu, HI 96822.

An emulsifiable formulation of 1,3-dichloropropene (Telone II EC) is effective for preplant control of rootknot and reniform nematodes. Since this product can be applied through the drip irrigation system, we are evaluating postplant applications of the product. We are comparing multiple postplant applications of 80 L ha^{-1} of product to single postharvest applications at 120, 160, or 238 L ha^{-1}. Average first ratoon fruit weight was similar among the treatments multiple applications at 80 L ha^{-1}, a single application at 120 L ha^{-1}, and multiple Nemacur treatments. The postharvest quantities of 160 and 238 L ha^{-1} reduced reniform nematode soil population densities by an average of 52%. First ratoon average fruit weights were 1.0, 1.0, and 0.9 kg, respectively for the Telone II EC trteatments 0, 160, and 238 L ha^{-1}. The higher amounts of Telone II EC might be phytotoxic.

DiTera, the dried fermentation solids and solubles of *Myrothecium verrucaria*, a product from Abbott Laboratories, is being applied through the drip irrigation system. Plant and first ratoon crop average fruit weights were similar, being 1.8 kg for plantation practice and 1.7 kg for DiTera treatments.

We are also evaluating Sincocin, Maxicrop, and Agri 50, biologically based products for nematode control. Treatments of Sincocin, a multi-component product, produced similar average fruit weights compared to standard plantation practices in one field evaluation, although reniform nematode populations were not affected in greenhouse experiments. Maxicrop, an algal based product marketed as a fertilizer reportly toxic to nematodes, is in greenhouse tests. Agri 50, a colloidal solution from Organic Solutions, L.L.C., is being widely tested for nematode and insect control and we have greenhouse tests in progress. These biologically-based products generally do not seem to have the nematicidal activity associated with products like Nemacur, Mocap, or Rugby but merit evaluation because of their lower toxicity. Application timing and amounts may need to be tailoring and tweaking to maximize their efficacy.◆

Cover Crop for Nematode Management

Koon-Hui Wang and B.S. Sipes. Dept. of Plant and Environment Protection Sciences, University of Hawai'i at Manoa, Honolulu, HI 96822.

Current nematode management tactics in Hawaii rely on fallowing with pineapple residue for 6 to 12 months, preplant fumigation with 1,3-dichloropropane (1,3-D) or methyl bromide, and application of postplant non-fumigant nematicides. While the industry remains dependent on nematicides, the United States Food Quality Protection Act of 1996 may drastically affect the availability and registration of currently used nematicides. During the fallow period, plant-parasitic nematode populations remain at damaging thresholds. Reniform nematodes also survive by dormancy achieved via anhydrobiosis during the fallow period. Thus, alternatives to current nematode management practices are necessary.

One alternative is to employ non-hosts or allelopathic hosts of nematodes as a cover crop prior to pineapple planting, followed by incorporation of the crop into the soil as green manure. Current cover crops under investigation are sunn hemp, rapeseed and marigold. Rapeseed is difficult to establish where soil pH is low, and is a good host for root-knot nematodes, also a damaging nematode for pineapple. The marigold tested, *Tagetes erecta* 'Cracker Jack', established well in Hawaii, but is short lived, susceptible to thrips damage, and is a good host for reniform nematode. To date, sunn hemp, *Crotalaria juncea* 'Tropic Sun', is the most promising cover crop. It is a poor host to reniform nematode, produces a leachate toxic to reniform nematode, and reduces nematode egg hatching. Sunn hemp also enhances nematode-trapping fungal population density in the soil (Fig. 1); densities increased as the sunn hemp growth period increased (Fig. 2). Research is under way to observe how long can this nematode-trapping fungal enhancement effect carries over after pineapple planting.

Sunn hemp tolerates drought conditions and low soil fertility. It is a legume and contributes up to 200 kg N ha^{-1} after 2 months of growth. Fast establishment of sunn hemp also provides erosion control as compared to bare fallow. Although extra labor is needed for cover crop management, sunn hemp's lower price compared to fumigant nematicides make it a cost-effective management option.

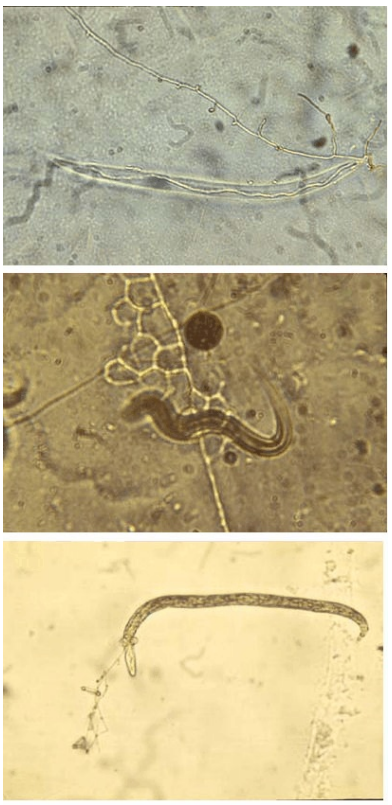


Figure 1. Nematode trapping fungi that form a constricting ring, an adhesive knob, and an adhesive net.

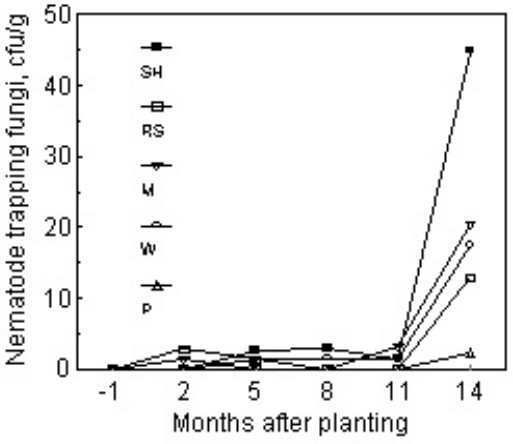


Figure 2. Nematode-trapping fungi colony forming units per gram of soil in *Crotalaria juncea* (SH), *Brassica napus* (RS), *Tagetes erecta* (M), and pineapple (P) plots.

Control of the big-headed ant, *Pheidole megacephala*, in Hawaii with ant bait stations

Glenn Taniguchi Department of Plant Pathology, University of Hawai'i at Manoa, Honolulu, HI 96822.

Ants have been a problem of the agricultural profession from the beginning of such practices. In Hawaii, the big-headed ant (*Pheidole megacephala*) is a common pest of many agricultural crops, but particularly for the pineapple industry. The association between *Pheidole megacephala* and the industry is indirect. *Pheidole megacephala*, as with most ants, it is attracted to two species of pineapple mealybugs, *Dysmicoccus brevipes* and *D. neobrevipes*, for its honey dew. The problem for pineapple is that the big-headed ant protects the mealybugs, which are known to carry and transmit the devastating disease known as mealybug wilt. *Pheidole megacephala* is very protective of mealybugs, as are all ants that feed on honey dew.

Control of mealybugs is achieved by controlling *Pheidole megacephala*, which will then allow prasites and predators to enter the picture, a classic case of biological control. In the past, *Pheidole megacephala* was best controlled by myrex and heptachlor, both of which are banned from use. In the years following their ban, numerous pesticide-ant bait combinations were tested as potential replacements for myrex. One such pesticide-bait combination that showed great potential was Amdro (hydramethylnon). However it had two draw backs; it is unstable in light and in water. In light, activity is lost within 24 hours while in water, activity is lost within 92 minutes. These two factors restricted the field-application of Amdro to periods of favorable weather and at night, or with protection from sunlight.

The idea of an ant bait station for agriculture use was tested in the study reported here. While most bait stations gave control, the Perimeter Patrol system of B & G Equipment Co. was selected for further study. Preliminary results were astounding. Amdro in the bait station was exposed to normal environmental conditions for periods of 1, 2, 3, and 10 weeks. Laboratory efficacy testing revealed no difference in efficacy of ant control between exposure at week 0 and week 10.

In the field, results were equally satisfactory. Control of *Pheidole megacephala* in block-size trials was impressive. Complete ant control was achieved within a month in test plots with bait stations containing Amdro and spaced at 7.62 by 7.62 meters (Figure 1). The duration of ant control was further enhanced by the addition of the ant growth regulator Distance® (pyriproxyfen) prior to installing the Amdro bait stations (Figure 2). Spacing of 7.62 by 7.62 or 15.25 by 15.25 mters resulted in comparable levels of control and it is not possible to draw conclusions about the superiority of the two spacings, because plots were not replicated. However, there was no reduction in ant numbers at a bait-station spacing of 30.5 meters.

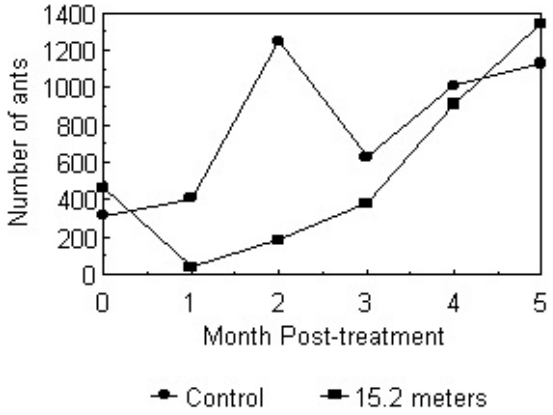


Figure 1. Ant control with Amdro alone in bait stations placed as indicated.

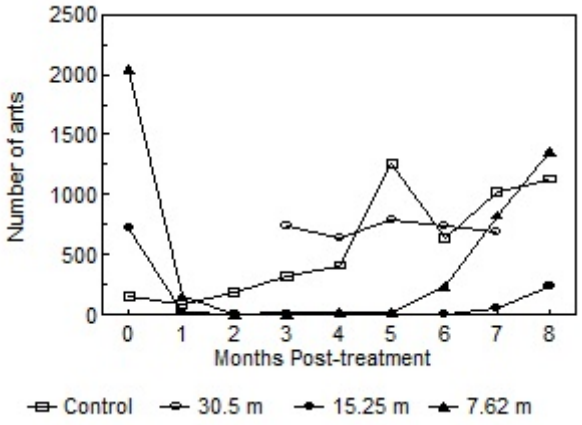


Figure 2. Ant control with Amdro plus Distance in bait stations spaced as indicated.

Bromelain, Health Food for Dairy Cows?

Abstracted from *Bromelain–Health Food for Bossy, Too* by Judy McBride in the Nov., 1999 issue of Agricultural Research (USDA Agricultural Research Magazine, 5601 Sunnyside Ave, Beltsville, MD 20705-5130)

Dairy cows can have chronic udder infections that increase the white cell count in milk. Milk quality is based on that count and U.S. dairy producers get paid extra for milk having a white cell count

under a certain level, which varies from state to state. Producers in the U.S. can't sell milk with white cell counts that exceed 750,000 per milliliter and the level may be lowered. Levels allowed in Canada are 500,000 per mL and are lower still in Europe. Max Paape, Dairy Scientist, Immunology and Disease Resistance Laboratory, Beltsville, Maryland (mpaape@lpsi.barc.usda.gov), agreed to test bromelain on cows with chronic mastitis. The bromelain is sold by Ajinomoto Co., Inc. of Tokyo, Japan. Bromelain, a mix of enzymes extracted from pineapple stems, is sold in health food stores under claims it combats heart disease, arthritis, and other maladies. According to Paape, it appears to reduce inflammation in dairy animals by interfering with the synthesis of prostaglandins and other inflammatory substances. Paape divided 10 cows into two groups having average cell counts a little over 300,000. For 4 weeks, one group received pellets containing 75 g bromelain in their feed while the control group got no bromelain. One week after the first trial ended, Paape switched groups, with the former control group getting bromelain while the former treated group did not. In both trials, bromelain reduced white cell counts in milk by 100,000 during each trial and cell counts did not exceed U.S. or Canadian limits as they did when cows did not receive bromelain. Milk with low white cell counts also had more milk protein or casein, which is preferred by cheese makers.◆

Notices

Availability of Chlorflurenol for Commercial Production of Pineapple Planting Material

As a convenience to readers, we again want to inform you that Chlorflurenol (as Maintain CF-125) is available for the production of pineapple planting material. For further information, contact N. Bushan Mandava, Repar Corporation, P.O. Box 4321, Silver Spring, MD 20914, U.S.A.; Phone (202)-223-1424; Fax: (202)-223-0141

Availability of Bromelain and 3-Chlorophenoxypropionic Acid

As a result of requests for sources of bromelain and 3-chlorophenoxypropionic acid, Mr. Adriaan Dolmans, Independent Pineapple Consultant, van Wassenaerlaan 31,7218 AT Almen, The Netherlands, provided the following.

Bromelain: Hong-Mao Biochemical Limited, 111 Moo 2 Tambol Nicompatana, Ampur Nicompatana, Rayong Thailand 21180 Tel. 038-636-088; Fax 038-636-087.

Bromelain also was to be available sometime in the year 2000 from: Upapaina S.A. de C.V., P.O. Box 151, Colima, Col. 28000, Phone: +52 3315-0218; +52 3315-0501; Fax: +52 3315-0009; E-mail: ultrapap@prodigy.net.mx; papaina@prodigy.net.mx; Web Page: http://colima.podernet.com.mx/ultrapapaina/homepage.html

Giovanni Marangoni (E-mail: gmarangoni@euronet.be) states that Great Food Biochem, no address given, also in in Thailand, produces bromelain. Giovanni lives in Belgium and his first language is French.

3-chlorophenoxypropionic acid: Mr. Pieter Erasmus, formerly at Applied Chemicals in South Africa, is now Managing Director, Querkus Co., P.O.Box 12245, Aston Manor 1630, Republic of South Africa; Tel. Office +27-11-972-7724, Fax: +27-11-972-7724, Mobile Tel. +27-82-453-1564. Perhaps Mr. Erasmus is still able to provide 3-CPA to those interested in evaluating it.◆

Meetings

International Meeting on Plant Biotechnology
"Plants Genetic Improvement: Trends and Prospects". Centro de Bioplantas. Universidad de Ciego de Avila.Ciego de Avila, Cuba. April 16 to 20, 2001. For more information, contact: Dr. C. Oscar Fernández García, Manager MERCADU S. A., Universidad de Ciego de Avila., Carretera de Morón km. 9, Ciego de Avila C.P. 69450, Cuba. Fax: 53(33) 266340; Email: ofdez@unica.edu.cuo r mercadu@unica.edu.cu

8th International Controlled Atmosphere Research Conference
Rotterdam, Netherlands, July 8-13, 2001. For more information contact: Conference Secretariat, Eurocongres Conference Management, Jan van Goyenkade 11, NL-1075 HP Amsterdam, The Netherlands. Phone 31-20-679-3411; Fax: 31-20-673-7306; Email: CA2001@eurocongres.com

International Symposium on Foliar Nutrition of Perennial Fruit Plants
Meran/Merano, Italy, Sept. 11-14, 2001. For more information, contact: Prof Dr. Massimo Tagliavini, Dipartim. Di Colture Arboree, Universita di Bologna, Via Filippo Re 6, 40126 Bologna, Italy or W. Drahorad, Sudtiroler Beratungsring fur Obst- und Weinbau, Kirchgasse 4, 39018 Terlan (BZ), Italy. Phone: 39-047-125-7198; Fax: 39-047-125-7800; Email: beratungsring.terlan@rolmail.net.

CAM-2001: The III International CAM Congress
Coconut Beach Resort, Queensland, Australia, August 24-28, 2001. For more information contact Dr. Joe Holtum, Dept. of Tropical Plant Sciences, James Cook Univ., Townsville 4811, North Queensland, Australia. Phone: 47-81-4391; Fax: 47-25-1570; Email: joseph.holtum@jcu.edu.au.◆

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This list includes papers published or located since the last issue of the newsletter was printed. **Please help** keep this section current by sending citations or copies of recent publications to D.P. Bartholomew .

Reprints of most of the publications listed below should be obtainable through any university library or by writing to: Library External Services, Hamilton Library Room 112, University of Hawaii, 2550 The Mall, Honolulu, HI 96822 U.S.A. Charges are approximately $14.00 per article plus postage for the first 20 pages and $0.25 per page over 20 pages.

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Directory of Professionals

This listing is maintained as a convenience for those seeking the assistance of professionals with experience in pineapple production and processing. If you have such expertise and wish to have your name listed here in a future issue, please send your name, address, Email address, and a brief resume to D.P. Bartholomew at the address on page 1.

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