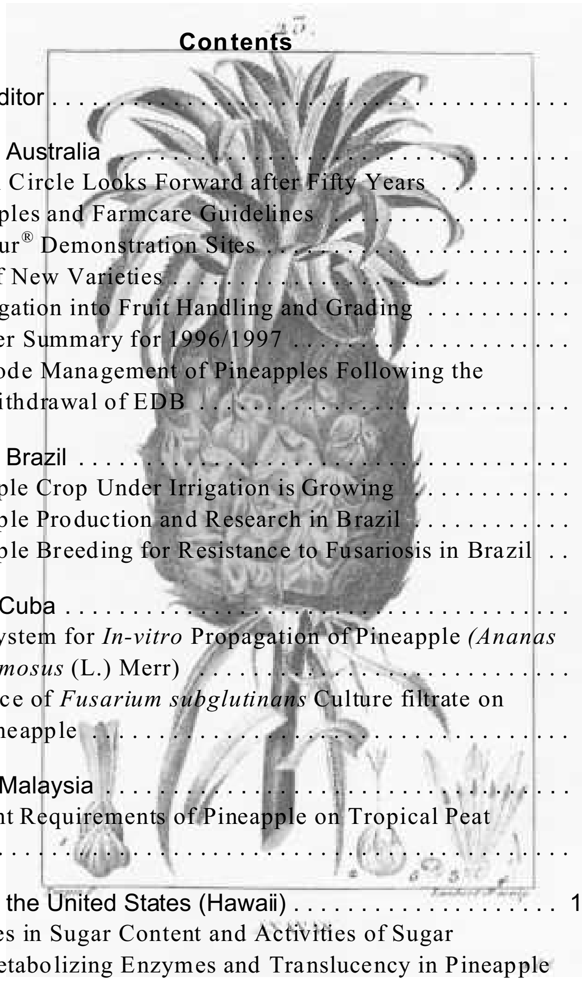
# Pineapple News

Newsletter of the Pineapple Working Group, International Society for Horticultural Science

No. 5 April, 1998



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# From the Editor

## About the Newsletter

**Printing delayed**: Printing of this issue of Pineapple News was delayed primarily due to delays in getting material for the newsletter. It is difficult to maintain a feature-packed newsletter when support for agricultural research has been reduced in many areas of the world, including Hawaii. A weak local economy has reduced state funding for all agricultural research, including that for pineapple. The Hawaiian pineapple industry provides some direct support to university faculty and graduate students for pineapple research, but that research is primarily limited to nematodes and mealybug wilt so the number of research projects dealing with pineapple has declined.

**Change in numbering**: The numbering system for Pineapple News is being changed to make it consistent with standard library cataloging practices. Because only one issue is published each year, Pineapple News falls in the category of randomly issued publications and so should be numbered sequentially. Since four issues have been published (Vol. I, No. 1, Vol. I, No. 2, Vol. II, No. 1 and Vol. III, No. 1) this issue becomes No. 5. Lastly, the cover was eliminated to reduce printing and mailing costs and production time. The cover used on previous issues was more costly to purchase and print while offering little in the way of additional value to readers.

**Address corrections**: To be sure of receiving the next issue of Pineapple News, please send corrections or changes in your mailing and Email addresses to the editor.◆

## 3rd International Pineapple Symposium

The announcement for the 3rd International Pineapple Symposium in Pattaya, Thailand was sent out several months ago and and all who responded to the first mailing should have received it. The dates of the symposium were changed to November 17-20, 1998. Important deadlines for those planning to attend are: June 30, 1998, paper titles and abstracts due; July 31, 1998, deadline to notify National Organizing Committee of requirements for presenting a poster or exhibit; August 31, authors and exhibitors receive notification of acceptance of papers, posters, and exhibits; August 31, 1998, deadline for payment of registration fee; September 30, 1998, last date for withdrawal of papers, posters, and exhibits; October 1, 1998, hotel room reservations due; deadline for cancellation of registration; November 24, 1998, deadline for submission of full papers. For further information, contact Mr. Somchai Watanayothin, Secretary, Third International Pineapple Symposium, Horticulture Research Institute, Department of Agriculture, Chatuchak, Bangkok 10900, Thailand; Tel (66) (2) 5798553, 9405485-5; Fax. (66) (2) 5614667; email: hortdoa@mozart.inet.co.th.◆

## Proceedings of 2nd Symposium Published

The proceedings of the 2nd symposium has been published by the International Society for Horticulture Science (ISHS) and copies were mailed to those attending the 2nd symposium in early February, 1998. The proceedings, Acta Horticulturae Vol. 425, can be obtained for 215 Dfl. from the ISHS secretariat at the address below.◆

## Impact of the Computer Revolution

I recently received a catalog from a U.S company that sells instruments to measure and monitor soil, plants, and the atmosphere. The array, relatively low cost (US$200-500), and portability of instruments available surprised me. It probably shouldn't have because it reflects what has happened to the power (significantly up) and cost (significantly down) of personal computers over the last few years. Sensitive and inexpensive handheld instruments are available to measure soil, water, and spray solution pH values, electrical conductivity of dissolved salts in soils and solutions, nitrate and potassium in plant sap, chlorophyll content as a measure of plant greenness and nitrogen status (nitrogen status in the Hawaii pineapple crop log was based on leaf color and chlorophyll is a more accurate measure of greenness), and instantaneous and long-term weather. Some of the instruments will download data directly into a personal computer, making it easy to further analyze the data. Eric Sinclair of Golden Circle Ltd, in Queensland, Australia wrote in the Pineapple Industry Field Day Notes (see News From Australia) of how weather data led to a better understanding of the effects of short-term weather on fruit development and quality. Understanding a problem and its causes is the first step in developing solutions to the problem. The dramatic impact of two very hot days on fruit size and appearance makes one speculate about the possibility of alleviating heat stress with a mid-day spray of water.

Growers need to be aware of changes in availability and cost of instrumentation and technology that have the potential to improve their understanding of or control over their crop. But they also need to know how they are going to use new technology and how their farm operation will benefit from it before responding to an enthusiastic sales pitch.◆

## Membership in ISHS

The International Society for Horticultural Science 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 (Email: ISHS@agr.kuleuven.ac.be). The ISHS maintains a web site at: http://www.agr.kuleuven.ac.be/ishs/ishshome.htm.◆

## Contributions to Pineapple News

I am always looking for contributions to Pineapple News so please plan now to contribute to the next issue of the newsletter. The greatest weakness of the newsletter to date has been current information about the state of the pineapple industry in the various countries where the crop is grown. If any reader has knowledge about the industry in their country that can be shared with other readers, please write an overview for the newsletter.

When submitting articles for publication in ***Pineapple News***, please follow the guidelines below. All contributions should be written in English. I will assist writers whose native language is not English to prepare contributions for the newsletter. If possible, please submit contributions 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. Use tabs to separate columns in tables. Since the objective of the newsletter is to promote communication among pineapple researchers, specialists, and growers, the preferred contributions are abstracts or summaries, including only essential tables and figures, of research in progress or completed. Also of interest are overviews of pineapple production and processing within a country or region, and brief review papers in the writers area of expertise. At this time, cost and time prevents the publication of photographs. News articles are arranged alphabetically by country, and within a country section are arranged alphabetically by author. Please submit all 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.◆

# News From Australia

## Pineapple Industry Farm Field Day

The 1997 Pineapple Industry Field Day was held on Friday, July 18. The summaries below were abstracted by D. Bartholomew.

## Golden Circle Looks Forward after Fifty Years

Mr. Greig Nissen, Chairman, Golden Circle Cannery Board, briefly reviewed the past fifty years of operation, but spoke mostly about the future. He reviewed some of the issues faced by the cannery and by grower-owners. Important issues include Field technical problems, Environmental issues, Urbanisation, Marketing, Production scheduling and raw material quality, and Housekeeping and labour relations. The most pressing field technical problem facing the industry is the loss of the fumigant ethylene dibromide (EDB) in 1999 for nematode control. Other technical issues include natural flowering of pineapple and lack of control of some pests. Important environmental issues include soil erosion, fertilizer use and waste, and pesticide use and storage. Dealing with these issues will take time and increase costs to growers and the cannery. Urbanisation brings people into closer proximity to farms resulting in complaints about noise, dust, and use of chemicals. Ultimately, it will also increase land costs to the point where farms may need to be moved to less populated areas. Marketing problems are related to changes in consumer preferences; consumers want unsweetened products that are fresher and in packages that are easily opened and disposed of. Production scheduling is being implemented to assure that the highest prices are paid for high quality fruit and a true payment-for-quality system is envisaged in the near future. A computer-based system is being developed to predict harvest date on a field by field basis. Fruit handling systems are being studied to improve the quality of fruit delivered to the cannery. Labour relations are affected by rules for dealing with staff that are becoming more complex. To remain viable, the industry needs to reduce its dependence on unskilled labor.◆

## Pineapples and Farmcare Guidelines for the Environment

Chris Lindsay, Environment Policy Officer, Queensland Fruit and Vegetable Growers

The issues that relate to environmental management include 1) the realization that long-term farm productivity is tied to farm resource sustainability, 2) new environmental protection legislation in Queensland, 3) urban residential settlement near production areas, 4) more stringent domestic and export market requirements, 5) increasing consumer expectations about food safety, and 6) greater on-farm awareness of health and safety issues facing farm families and workers. Among the issues discussed at meetings held with growers are:

1) the need to manage soil to maintain fertility levels and prevent erosion. Current practice of using soil tests to assess nutrient levels prior to planting and careful control of nitrogen applications to prevent nitrate accumulation in fruit going to the cannery ensure

that fertilizer inputs are neither inadequate or excessive. Phosphorus inputs in the industry are relatively low compared to other crops, with 30 kg/ha P applied over 4 years.

Soil erosion is a concern where pineapple is grown on steep slopes to take advantage of their superior drainage and frost-free growing conditions. Trials show that 10-20% of applied nutrients, primarily calcium and potassium, can be lost with eroded soil. Losses in runoff water may reach 30-50%of individual nutrient applications. Pineapple plant residue as a surface mulch can reduce soil losses to 1/100th that of conventional practice.
2) controlling pest problems with an integrated approach. Current practice is to program pest controls based on pest type, pressure, and time of year. With the loss of EDB in 1999, chemical control of nematodes will become more expensive and hazardous. The nonvolatile nematicides are highly toxic and water soluble so they have the potential to leach through the soil. Detailed sampling shows that nematode problems differ across farms and paddocks, so the challenge will be to develop an integrated approach to nematode management that takes this variability into account. Research is being done to improve the accuracy of nematode populations and to develop alternatives to chemical control such as fallowing, green manure crops (forage sorghum and velvet bean), and organic amendments.
3) managing dust, spray drift, odors, and other air quality impacts, and managing noise. These problems have become some of the biggest headaches for growers as inappropriate local government planning and a shift away from rural priorities results in residential development in the midst of agricultural businesses. The challenge for the industry is to manage to minimize adverse impacts on the urban neighbor despite the lack of any economic benefit.◆

## Nemacur® Demonstration Sites

Robert Vitelli - Research & Development Officer - Bayer Australia

Demonstration sites are being established on six farms located throughout the pineapple growing areas to help smooth the transition from the use of EDB to nonvolatile nematicides. The aims are to 1) demonstrate correct application techniques for granular Nemacur 100G pre-plant; 2) demonstrate application techniques for Nemacure 400 as a foliar spray; 3) discuss application technology; 4) discuss nematode monitoring techniques; and 5) discuss safety precautions and procedures when using Nemacur.◆

## Role of New Varieties in Developing the Australian Pineapple Industry

G.M Sanewski (sanewsg@dpi.qld.gov.au) and R.J. Nissen, Marrochy Horticultural Research Station, Nambour, Queensland, Australia

The authors conclude that better fresh fruit varieties could help offset foreign competition in the canned pineapple market. Smooth Cayenne, the mainstay of the industry in southern Queensland, has a number of well-known problems. Fruit produced in summer are prone to translucency and may have a green skin when ripe while winter fruit have low sugar, high acid, and are prone to blackheart. New pineapple varieties produced in summer could offer consumers greater variety, a more yellow, crisp and firm flesh, a smaller core, more attractive external appearance, and a better relationship between external color and internal ripeness. Benefits to growers could include increased disease resistance, less translucency and the attendant fruit fragility, spineless leaves, longer storage life, faster cycles, and more abundant planting material. The greatest benefits are thought to come from fruit produced in summer but fruit of new varieties produced in winter could have higher sugars, lower acid, and blackheart resistance. No mention is made of whether new varieties are close to being released to the industry. New cultivars with unique qualities also may open export opportunities.◆

## Investigation into Fruit Handling and Grading

Jason Kruger - Golden Circle Horticulture

**Fruit Handling**: An extensive evaluation of the handling system for cannery fruit was undertaken. The fruit handling system consists of harvest, transport, and receipt. Under normal conditions, damage due to static loading (squashing due to stacking) in 0.81 m deep bins had not effect on fruit recovery in the cannery. However, if fruit were previously damaged, were over-ripe, or translucent, the reduced fruit strength could result in higher losses. It was estimated that 10 to14% of first grade fruit could be bruised by drops (dynamic loading due to impact) of up to 1.0 m in height. Increased bruising is reflected in reduced slice recover and with increased recover of pieces and crush. It is recommended that drop heights during harvesting and unloading be 0.5 m or less. Up to 3% of the fruit mass could be lost by dehydration during a 48 h transport from farm to factory and, as time from harvest increased, slice recovery decreased and pieces and crush increased. There was no difference in recovery of fruit transported by road or rail.

**Grading*:*** Tapered fruit yields fewer first-grade slices than does fruit with a constant diameter along its length. Studies are being conducted to identify a method of grading that will increase recovery of first-grade slices from tapered fruit.◆

## Controlling Blackheart by Genetic Engineering

Lien Ko and Mike Smith, Maroochy Horticultural Research Station, Nambour, Queensland, Australia

'Blackheart' causes losses estimated to be up to $3 million a year in the Queensland pineapple industry due to reduced production and loss of consumer confidence. Efforts are underway through a collaborative project between Queensland Dept. of Primary Industries, CSIRO and counterparts in Malaysia to overcome the problem by genetic engineering. Transformation involves the insertion of so-called antisense gene(s) that block the activity of an enzyme or enzymes responsible for blackheart development. Selection of transformed cells can take place at the callus level. An efficient system exists to produce new plants from the transformed callus.◆

## Weather Summary for 1996/1997

Eric Sinclair (esinclair@gcl.com.au), Golden Circle Ltd.

Weather summaries for the Beerwah farm were reported and contrasted with the longer term averages and the implications of weather fluctuations were discussed. Of note were temperatures that exceeded 40 °C on November 15$^{th}$ and 30$^{th}$, 1996. The check in growth caused by these temperatures brought on a wave of natural flower induction; these fruit were harvested in late June and July. The high temperatures also injured young fruit with fruit harvested in April being damaged the most. Fruit were deformed and development of the top 2 or 3 fruitlets on each spiral were arrested resulting in an elongated neck below the crown. Ratoon fruit harvested in May and June were short but wide. This effect was attributed to high temperature interference with fruitlet initiation. Subsequent to fruitlet initiation, growing conditions were good so fruitlets were well filled out. (Ed. Note: Southern Queensland is a climate of extremes, both hot and cold, compared with some of the more tropical (Thailand, Philippines, Malaysia) or oceanic (Hawaii) areas where pineapple is grown. Dr. Sinclair makes some interesting observations on the effects of extreme temperatures that deserve further comment. Since it became common practice to force flower induction with growth regulators, natural flower induction is reported to be a problem in the late fall-early winter period when photoperiods are short and days are cool. This is the first instance I have heard about where flowering appears to have been induced by high temperature. Dr. Sinclair's statement that the short, wide fruit "seemed to be caused by few eyes having been laid down at the time of induction, but subsequent good growing conditions allowed these eyes to fill out well" is consistent with Dr. X.J. Min's research (Acta Horticulturae 425:329-338), which showed that leaf titratable acidity, a measure of $CO_2$ fixation, and fruitlets per fruit were reduced when plants were induced at a 30 °C night temperature even if plants were subsequently moved to a 30 °C day-20 °C night regime. The plants were harvested before the fruit matured, but at harvest mean fruitlet weight was greater for the plants induced at the 30 °C night temperature.◆

## Nematode Management of Pineapples Following the Withdrawal of EDB

Graham Stirling, Biological Crop Protection Pty. Ltd., and Col Scott, Eric Sinclair, and Doug Christensen, Golden Circle Ltd.

An appendix in the Field Day Notes provided a comprehensive overview of nematodes and their management in pineapple. Major topics included: 1) Introduction; 2) Background technical information; 3) Monitoring as an aid to nematode management; 4) Assessing other components of the root disease complex; 5) Chemical control of nematodes; 6) Non-chemical control of nematodes; and 7) Planning for a future without EDB. Since it isn't possible to abstract such a comprehensive review here, an abbreviated version of the checklist for growers provided at the end of the article is paraphrased here. Growers are advised to:

- be familiar with the various nematodes and management options
- learn to correctly diagnose root health and disease problems
- begin monitoring nematodes on the farm and use the results to better understand the problem and improve nematode management practices
- base nematode control programs on nematicides that have previously been tested on the farm
- ensure that the soil management program recognizes all soil-borne problems (white grubs, symphylids, *Phytophthora*)
- if non-volatile nematicides are applied, use appropriate and accurately calibrated application equipment
- when working with nematicides, know and follow the all safety precautions
- enhance the effectiveness of the nematode control program by utilizing non-chemical control measures (extended fallow, rotate with a non-host crop, increase soil organic matter content)
- when possible, cooperate with scientists who are developing alternative nematode control measures.◆

# News From Brazil

## Pineapple Crop Under Irrigation is Growing

Luiz Francisco da Silva Souza (soil fertility) (luiz@cnpmf.embrapa.br) and Domingo Haroldo Reinhardt (plant physiology) (dharoldo@cnpmf.embrapa.br) - EMBRAPA Cassava and Tropical Fruit Crops, Caixa Postal 7, 44.380-000, Cruz das Almas, Bahia, Brazil.

During the 90s the area used for cropping pineapple under irrigation has grown strongly in Brazil. This expansion has occurred in regions where pineapple was traditionally grown without irrigation, as well as in new areas, especially under semi-arid conditions. Even though there aren't official statistics on the subject, it is estimated that about 10 % of the 55.000 ha of pineapple harvested in 1997 were irrigated. The main reasons for the expansion are: 1) to get a better and more stable fruit quality, which is very important, as the majority of the pineapples are consumed fresh in the inland market, and 2) to move a large portion of the yields to the off-season period, from February to May, when the fruit prices paid to the growers are about twice as high as in the main harvest season (November to January). The expansion of the pineapple crop into the semi-arid region, where irrigation is needed to obtain good yields of high quality fruit, has also been motivated by the unfavorable conditions for the development of fusariosis caused by *Fusarium subglutinans*. The semi-arid conditions help to control this disease, which is the most important one for the pineapple crop in Brazil. Further, the expansion of the area of irrigated pineapple has led Embrapa's National Research Center for Cassava and Tropical Fruit Crops, located in Cruz das Almas, Bahia State, to dedicate part of its R & D activities to developing or adjusting technologies for irrigated pineapple cropping systems for the different environmental conditions of the country.◆

## Pineapple Production and Research in Brazil

Domingo Haroldo Reinhardt (plant physiology) (dharoldo@cnpmf.embrapa.br) and José da Silva Souza (economy), EMBRAPA Cassava and Tropical Fruit Crops, Caixa Postal 7, 44.380-000, Cruz das Almas, Bahia, Brazil.

This is a synthesis of a paper presented by the first author in the Pineapple Club Convention, Interlaken, Switzerland, May 1996, with some new data from 1997.

Pineapple is one of the major tropical fruits grown in Brazil, the largest fruit producer in the world (about 10 % of the world production). Brazil harvested about 1.60 million tons of pineapple in a harvested area of about 55.000 ha in 1997. The Northeast (from Bahia State northward to and including Maranhão State) and the Southeast (São Paulo, Rio de Janeiro, Minas Gerais and Espirito Santo States) are the major producing regions, being responsible for about 78 % of the total volume of pineapple produced in the country. However, the North (South of Pará and North of Tocantins States) has been the region of fastest growth, going from an insignificant volume harvested in the late 1980s to a production of about 300 million fruits, representing around 20 % of the country's production in 1997.

Most of this fruit is consumed fresh in the inland markets, giving an annual consumption of about 10 kg of pineapple per capita. About 1.0 % of the production is exported as fresh fruit, mostly to neighbor countries economically integrated by the MERCOSUL (Market of the South Cone). The income due to pineapple juice exports has been rather low, declining from US$ 10.8 million in 1988 to about US$ 1.5 million in 1995.

The inland fresh fruit market prefers the 'Pérola' pineapple, a cultivar whose origin is in Brazil and which has some characteristics differing from the 'Smooth Cayenne' variety, such as: Whitish and less fibrous flesh, making even the fruit central core edible; lower acidity and a higher TSS/Acids ratio; a cone fruit shape with lower canning output for slice production; a slightly smaller fruit and lower yield potential; and leaves with spines. 'Smooth Cayenne' is mostly grown for canning purposes and a few special fresh fruit markets, mostly in the Southern part of the country.

Average pineapple yields in Brazil are still low (around 23.400 fruits or 30.4 t/ha in 1997), but they have increased by more than 150 % from 1975 to 1997. This increase occurred even though most of the country's production has been from small growers with limited availability of capital. Hence they experience difficulties in acquiring and using all the inputs indispensable for getting good yields, such as superior planting material, fertilizers and pesticides.

Strong research and technology transfer efforts, mainly carried out by EMBRAPA (Brazilian Corporation for Agricultural Research) via the National Research Center for Cassava and Tropical Fruit Crops (CNPMF) at Cruz das Almas, Bahia, over the past 20 years, have contributed to that good result. In this context, the following technologies should be mentioned: - Planting densities increased from about 20.000 to around 35 - 50.000 plants/ha, using both single and double rows planting systems; - integrated fusariosis control methods (rouging, selection and production of healthier planting material, fungicide sprays just before and during flowering, displacement of flowering and fruit production to periods with climatic conditions less favorable for the fungus). Other improvement include higher efficiency of flowering induction treatments, improved mineral fertilization (doses, forms and periods of application), development and use of sprinkle irrigation systems, better pest control methods (fruit borer, mealybugs, mites and other pests), and general improvement of all other cultural practices (weed control, plantation management etc.).

New average yield increases and better fruit quality are being pursued by concentrating research activities on the development of new cultivars resistant to *Fusarium subglutinans*, 'Pérola' ratoon crop growing techniques, the control of precocious flowering during the short day- period of the year, and the further improvement of the quality (vigor and health) of planting material and the irrigated pineapple crop management, with emphasis to plant nutrition aspects.◆

## Pineapple Breeding for Resistance to Fusariosis in Brazil

José Renato Santos Cabral (plant breeding) (renato@cnpmf.embrapa.br) and Aristoteles Pires de Matos (plant pathology) (apmatos@cnpmf.embrapa.br)
- EMBRAPA Cassava and Tropical Fruit Crops, Caixa Postal 7, 44.380-000, Cruz das Almas, Bahia, Brazil.

EMBRAPA-CNPMF has been carrying out a pineapple breeding program aiming to obtain cultivars showing resistance to fusariosis, a disease caused by the fungus *Fusarium subglutinans*, which is a serious problem for most pineapple producing areas in Brazil. 'Perolera' and 'Primavera', resistant to fusariosis, have been crossed with 'Smooth Cayenne' and 'Pérola', susceptible to the pathogen, in order to obtain segregating populations. The evaluations for resistance have been carried out with seedlings by wounding them at the base and dipping them in an inoculum solution containing $10^5$ conidia/ml, for three minutes. Genotypes showing resistance to fusariosis have been transferred to field where other horticultural characteristics were evaluated. The evaluation of 28.800 hybrids, during the sexual cycle, allowed the selection of 26 genotypes, that had total soluble solids equal or higher than 14ØBrix, titrable acidity from 5.5 to 10 meq/ml, among other desirable characteristics. The genotypes which keep those characteristics during the clonal evaluation will be recommended as cultivars resistant to the pineapple fusariosis.◆

# News from Cuba

## New System for *In-vitro* Propagation of Pineapple *(Ananas comosus* (L.) Merr)

M. Escalona ; J.C Lorenzo ; B. González ; M. Daquinta ; Z. Fundora ; C.G. Borroto; P. Espinosa; D. Espinosa; E. Arias; M. E. Aspiolea. Centro de Bioplantas, Universidad de Ciego de Avila CP 69450, Cuba (celulas@bioca.edu.cu).

Micropropagation of pineapple plants has many advantages over conventional methods of vegetative propagation. This technique allows an efficient and rapid increase of selected varieties. The most important Cuban pineapple producer enterprise is located in Ciego de Avila province. A commercial laboratory (Biofactory) for pineapple-plant micropropagation has been established that integrates all stages from in *in-vitro* culture to field establishment. Since 1995, standard tissue culture methods have been introduced that involve sequential culturing on liquid medium for meristem and axillary shoot bud multiplication (Daquinta et al., 1991). Using this approach, annual pineapple production is currently around one million plants. However, the commercial utility of conventional pineapple micropropagation is limited as a result of the large number of pineapple plants needed annually to start up new farms. The commercial use of micropropagation is currently low because of high production costs. They result primarily from high labour cost, low multiplication rate, and poor survival rates during acclimatization.

Much research has been recently conducted on automation to reduce manual operation in micropropagation. Improvements include automation of liquid medium preparation and feeding, plant image recognition and processing, microcutting and transplanting (Aitken Christie et al., 1995; Kozai et al., 1995, Smith, 1995). Automation of some or all stages of organogenesis is necessary to reduce tissue handling,and thereby reduce plant costs (Chu, 1995).

A temporary-immersion system has been described by Teisson & Alvard (1995) for plant propagation. It has been successfully used with *Coffea arabica, Hevea brasiliensis, Elaeis guineensis, Citrus deliciosa,* and *Musa sp*. This system, named RITA, uses small vessels and is very expensive. For this reason its use for commercial production is limited.

An automated system for large scale pineapple propagation was designed using the temporary immersion technique. Higher efficiency, lower costs, and better survival rates during acclimatization were achieved in comparison with conventional micropropagation practices. The system utilizes two containers, one for culture medium and another for plants. Culture medium is transferred by air pressure to immerse the plants and then withdrawn after a fixed period of time. This cycle is carried out several times a day. Nutrients are supplied and plants are aerated without the use of sophisticated technology. The system combines the advantages of low cost and ease of use and morphogenic responses can be controlled.

The protocol used with this technique involves the following steps: 1) *In-vitro* buds are initiated cultures established using conventional containers (Daquinta et al; 1991); 2) Shoot multiplication is performed in a 10 L bioreactor vessel where shoots are only immersed periodically in liquid medium; and 3) Shoots elongate in a bioreactor vessel for 30 days. The use of paclobutrazol in this system is an innovation and the elongation procedure makes it possible to achieve higher levels of plant uniformity and recovery. There are three stages in the bioreactor vessel including shooting for four weeks, cluster bud differentiation for seven days, and elongation for three weeks.

**Comparison of Conventional and Temporary Immersion Systems**

*In vitro*-cultured pineapple plants with two small shoots were used as explants. Murashige & Skoog (1962) culture medium with 2.1 mg.$L^{-1}$ benziladenine and 0.3 mg. $L^{-1}$ naphthaleneacetic acid was used as recommended by Daquinta et al. (1991). The effects of the traditional vessel was compared with the temporary immersion system were compared at paclobutrazol levels of 0.0, 0.5, and 1.0 mg.$L^{-1}$. Multiplication rate was evaluated after six weeks of culture (Table 1).

Table 1: Effect of paclobutrazol and culture system methods on multiplication rate.

| Culture method | Pacolbutrazol (mg.$L^{-1}$) | Multiplication rate |
|---|---|---|
| Traditional vessel[z] | 0.0 | 8.8 |
| | 0.5 | 9.0 |
| | 1.0 | 11.1 |
| Temporary immersions system[y] | 0.0 | 22.2 |
| | 0.5 | 37.7 |
| | 1.0 | 68.8 |

[Z]6.5 cm base diameter, 10 cm height, 25 mL culture medium volume, 5 explants/container.
[Y]Volumes were culture container, 1.0 L, culture solution 1.0 L, plant container 1.0 L; there were 5 explants/container with a 2-minute immersion period once very 3 hours.

The combination of a temporary immersion system with 1.0 mg $L^{-1}$ paclobutrazol greatly increased the pineapple multiplication rate. The positive effect of paclobutrazol on pineapple multiplication rate was previously described by Escalona et al. (1995). The temporary immersion system combines the advantages of solid and liquid medium culture. Solid culture medium allows explant aeration but limits contact with nutrients. Liquid culture medium permits an efficient nutrient uptake but related troubles are often present. The use of paclobutrazol also promoted the formation of compact bud clusters with limited leaf development while avoiding unnecessary leaf growth during shoot formation stages. Dynamic of shoot formation in temporary immersion with paclobutrazol was established in experiment 2.

***Effect of duration of shoot formation period in temporary immersion systems on multiplication rate***: This experiment was carried out to determine the optimum duration of the shoot formation stage. Durations of shoot formation in weeks and their respective shoot multiplication rates were 4, 4.4; 6, 3.3; 7, 1.1 and 8, 6.6. The highest multiplication rate was found when explants formed shoot during seven weeks. Longer periods (eight weeks) promoted shoot deformation. The results of our experiment demonstrate that the temporary immersion system is a better way to increase pineapple multiplication rate.

***Shoot Elongation in Temporary Immersion Systems***: Shoot formation during seven weeks was the optimum (1666.5 shoots/L) but the plant container was not large enough to allow plant elongation (Table 2). The number of plants suitable for rooting *ex-vitro* and acclimatization was affected by the vessel size and design. A large number of the plants were etiolated. Two strategies were followed: reduction of shoot formation period or decrease of initial number of explants. The percentage of longer plants increased as the shoot formation period and number of initial explants decreased (Table 2).

Table 2. Effect of weeks of shoot formation and initial explant number on plant elongation in a temporary immersion system (TIS).

| Weeks | Explants/L | Length classes (cm) | Percentage |
|---|---|---|---|
| 7 | 5.0 | <4 | 61.0 |
| | | 4-6 | 21.8 |
| | | 6-8 | 12.2 |
| | | 8-10 | 3.5 |
| | | >10 | 0.4 |
| | 2.5 | < 4 | 42.5 |
| | | 4-6 | 20.8 |
| | | 6-8 | 11.4 |
| | | 8-10 | 13.9 |
| | | >10 | 11.1 |
| 4 | 5.0 | < 4 | 42.5 |
| | | 4-6 | 14.3 |
| | | 6-8 | 14.7 |
| | | 8-10 | 15.9 |
| | | >10 | 12.3 |
| | 2.5 | < 4 | 17.7 |
| | | 4-6 | 14.1 |
| | | 6-8 | 24.7 |
| | | 8-10 | 16.2 |
| | | >10 | 29.5 |

MS medium and MS medium with 1 mg/L of gibberellic acid (GA) were first used to elongate bud clusters in the TIS. This showed the need to differentiate bud clusters prior to treating shoots with gibberellic acid. Treating cultures with benziladenine and GA 7 days prior to use of 1.0 mg $L^{-1}$ GA (unpublished data) achieved the best results on shoot elongation and plant uniformity.

**Genetic Variability**

A key requirement prior to introducing a new propagation method using tissue culture is the evaluation of genetic variability of regenerated plants. Using four enzymatic systems (peroxidase, diaforase, alcohol-dehydrogenase and malate dehydrogenase) to evaluate genetic variability, no differences were detected between plants from conventional micropropagation and plants the from temporary immersion bioreactor. Till now, the amount of phenotypic variation is low. Evaluation of the frequency of spiny leaves and physiological development of plants propagated by field techniques, and by conventional and temporary immersion micropropagation has been encouraging. Field tests for clonal fidelity are in progress.

**Production Cost**

Pineapple production costs achieved by temporary immersion in this system are lower than those of conventional micropropagation. The following considerations are key targets for reducing cost:
1. Reduce container number, which in conventional micropropagation increases exponentially during the shoot development stage.
2. Elimination of shelves in culture room.

3. Reduction of material that must be moved.
4. Elimination of cutting and planting, the most costly operation in the production process (Chu, 1995)
5. Biological optimization.
6. Elimination of *in-vitro* rooting.
7. Reduction of contamination levels.

This system has begun to be used for large-scale production of pineapple plants. Most of the obstacles currently found in conventional micropropagation have been overcome. It is estimated that this protocol will reduce costs per plant by 80% comparison with the conventional method. New methods for pineapple micropropagation have been developed to reduce the cost for commercial applications (Firoozabady et al., 1995).

**Summary**

The Bioplant Centre (Cuba) has developed a temporary-immersion bioreactor for various economically important crops, including pineapple. Significant changes to the pineapple propagation scheme established by Firoozabady et al. (1995) are reported in this paper including:
1. The use of paclobutrazol to increase an axillary multiplication rate during the shooting stage in the bioreactor, resulting in enhanced multiplication.
2. Shoot elongation is carried out in the bioreactor in two steps, the first one to achieve bud cluster differentiation, and the second one to develop shoots.
3. Shoots were separated from clusters and placed directly on substrate for *ex-vitro* rooting and acclimatization after four months.

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## Influence of *Fusarium subglutinans* Culture filtrate on Pineapple (*Ananas comosus* (L.) Merr.) Callus growth and Plant Regeneration


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**Summary**


Two pineapple varieties differing in resistance to fusariose were examined for the phytotoxic effect of *Fusarium subglutinans* culture filtrate. The cultivars were Perolera, which is resistant to *Fusarium subglutinans*, and Smooth Cayenne, which is susceptible. The phytotoxic effect of culture filtrate was assessed on callus growth, plant regeneration, and leaf chlorosis. Smooth Cayenne was most sensitive to the culture filtrate whereas Perolera showed resistance to it. Perolera calli grew and regenerated plants in the presence of high concentrations of culture filtrate where Smooth Cayenne callus could not. Besides, chlorotic leaves were frequently observed in Smooth Cayenne plants.


**Introduction**

Fusariose, produced by *Fusarium subglutinans*, is the most serious pineapple disease in Brazil where it was first reported in the State of Sao Paulo (Kimati & Tokeshi, 1964). Besides causing losses of up to 80% of the marketable fruit, the pathogen infects approximately 40% of the asexual propagative materials and kills about 20% of the pineapple plants prior to harvest (Matos, 1995).

Fungi of the genus *Fusarium* are known to produce a variety of biologically active substances in their culture filtrate reported to be toxic to callus, cotyledons, germinating seeds, and plants of many species (Wood et. al., 1972; Kern, 1972; Durbin, 1981; Hartman et. al., 1983; Jin et. al., 1996a). Culture filtrates from isolates of *Fusarium solani* causing Sudden Death Syndrome (SDS) of soybean were toxic to callus; however, variability in the response of the calli of different soybean cultivars to culture filtrate from different fungal isolates was also observed (Lim et al., 1990). Plant tissue response *in vitro* to culture filtrates from a pathogen correlates with disease reaction of the host variety so it may be possible to use culture filtrates of pathogens to select varieties that have disease resistance (Daud, 1986). The phytotoxic effect of *Fusarium subglutinans* culture filtrates on Perolera and Smooth Cayenne callus growth and plant regeneration is examined in this paper.

**Materials and Methods**

***Culture filtrates***. An isolate of *Fusarium subglutinans* (provided by National Research Centre for Cassava and Tropical Fruit Crops) was grown on potato-dextrose-agar (PDA) dishes for 7 days at 26 ±2.0 °C in 12-h alternating light (4,000 lux warm white fluorescent light) and darkness. Fungus mycelium from the dishes was inoculated into 100 milliliters (mL) of Czapek-Dox broth in a 250-ml flask and grown at 26 ±2.0 °C with the same light conditions described above. After three weeks of stationary cultivation, the culture filtrate was obtained by filtering the mycelium and conidia through Whatman No.1 filter paper (Whatman, Clifton, NJ) and a 0.2 m Millipore membrane

(Sartorius, NG). The culture filtrate was concentrated 80% (v/v) prior to use.

***Callus growth bioassay***. Calli of both varieties were established aseptically according to Daquinta et al. (1996) on Murashige and Skoog (1962) (MS) culture medium with 0.5 milligrams per liter ($mgL^{-1}$) of benzyladenine (BA) and 2.5 $mgL^{-1}$ of Dicamba and grown in the dark at 26 ±2.0 °C. After four subcultures, 10 mg of callus was transferred to the above culture medium supplemented with 0, 4, 8, 12, 16, or 20% (v/v) *Fusarium subglutinans* culture filtrate and cultured as described above. Fifty calli of each variety were used in each treatment. Callus fresh mass was recorded after 30 days of culture as recommended by Arai & Takeuchi (1993).

***Plant regeneration bioassay***. Establishment of callus cultures was as described above. Calli were placed on MS culture medium with 0.5 $mgL^{-1}$ BA supplemented with 0, 4, 8, 12, 16 or 20% (v/v) of *Fusarium subglutinans* culture filtrate. The cultures were maintained in the light at 26 ±2 °C. Plant formation and chlorotic leaves per plant were recorded after three months of culture.

***Data analysis***. Treatment differences were evaluated by analysis of variance and Duncan's new multiple range test using SPSS/PC (Statistics Package for Social Science) (1992). Means were compared by least significant differences at $P < 0.05$ unless otherwise stated.

**Results and Discussion**

Perolera callus fresh weight was not greatly affected by the culture filtrate and regenerated plants had a relatively low percentage of chlorotic leaves (Table 1). Smooth Cayenne callus growth and plant regeneration were reduced in the presence of the higher concentrations of culture filtrate and chlorotic leaves increased to 100% at a culture filtrate concentration of 12%.

We found previously (Borrás, et. al., 1998) that the phytotoxicity of *Fusarium subglutinans* culture filtrate was greater on calli of pineapple cultivars that were more susceptible to fusariose. Similar results were obtained by Jin et al. (1996b) for the highest levels of *Fusarium solani* culture filtrate. Arcioni et. al. (1987) reported that calli derived from resistant material of *Medicago sativa* grew on higher concentrations of toxins than calli from sensitive material.

The results reported here indicate a positive relationship between the degree of virulence of *Fusarium subglutinans* isolates and the degree of toxicity of its filtrate to both pineapple varieties *in vivo* and in callus culture (Table 1). Nidelnik (1988) found a similar phenomenon in tests on plants and callus, where high virulence was always accompanied by a high level of filtrate toxicity.

The correlation between pineapple cultivar susceptibility and the toxicity of culture filtrates suggests that filtrates could be used to screen *in vitro* for disease resistance. Tissue culture systems can provide a means of rapid screening for disease resistance when a toxin is involved in disease development. However, it would be still premature to conclude whether any of these characteristics can be used for *in vitro* screening of germplasm for fusariose disease as has already been attempted in several host parasite systems (Kaur et al., 1987). There is a need to extend the investigation on more cultivars/germplasms with varying degree of fusariose resistance.

Table 1. Effect of *Fusarium subglutinans* culture filtrate on pineapple callus fresh weight, plant regeneration, and leaf chlorosis.

| Variety | Filtrate[Z] | CFW[Y] | R | CL |
|---|---|---|---|---|
| Perolera | 0 | 16.8 a | 75.5 | 0 |
| | 4 | 16.3 a | 70.3 | 0 |
| | 8 | 15.9 a | 64.2 | 2.4 |
| | 12 | 15.4 a | 60.3 | 5.7 |
| | 16 | 15.2 a | 57.4 | 11.4 |
| | 20 | 14.9 a | 51.3 | 13.5 |
| Smooth | 0 | 16.1 a | 80.2 | 0 |
| Cayenne | 4 | 13.2 b | 33.5 | 22.4 |
| | 8 | 10.1 c | 22.0 | 70.4 |
| | 12 | 7.8 d | 7.1 | 100 |
| | 16 | 4.8 e | 0 | - |
| | 20 | 2.3 f | 0 | - |
| C.V.[X] | | 23.4 | | |

[Z]The *Fusarium subglutinans* culture filtrate was concentrated 80% (v/v) and the culture medium was supplemented with the indicated percentage of filtrate (v/v). Means for treatments followed by the same letter were not significantly different at P=0.05.
[Y]CFW, calli fresh weight measured as recommended Arai & Takeuchi (1993) after one month in culture; R, regeneration percentage after 3 months in culture; CL, percentage chlorotic leaves.
[X] Coefficient of Variation (%).

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# News from Malaysia

## Nutrient Requirements of Pineapple on Tropical Peat in Malaysia

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Pineapples are grown mainly on peat in Malaysia and the fruits are used solely by the canning industry. Gandul, a Spanish group cultivar is grown principally for canning and is well adapted to peat soil. Despite its cultivation in large areas, information regarding the fertilizer requirements of this cultivar are limited. So, field experiments were conducted to study the effect of nutrients on growth, yield and quality of pineapple cv. Gandul on tropical peat at two locations of Peninsula Plantations Estate, Simpang Rengam, Johore from June 1996 to September 1997. At location 1, pineapple has been cultivated for about 20 years and location 2 has been under cultivation for about 30 years. Yields at the two locations were different. Six levels each of nitrogen, phosphorus, potassium, calcium, magnesium and copper were studied separately. The levels in kg ha-1 were as follows: N (0, 200, 400, 600, 800 and 1000), P2O5 (0, 24, 48, 72, 96 and 120), $K_2O$ (0, 267, 534, 800, 1066 and 1334), CaO (0, 7.5, 15, 30, 45 and 60), MgO (0, 6, 12, 24, 36 and 48), CuO (0, 0.8, 1.6, 3.2, 4.8 and 6.4). The sources of nutrients were: N, urea; P, China rock phosphate; K, muriate of potash; Ca, gypsum; Mg, magnesium sulphate; and Cu, copper sulphate. A basal application in kg/ha of 600 N, 72 $P_2O_5$,800 $K_2O$, 30 CaO, 24 MgO, and 3.2 CuO was applied. The experiments were conducted in a randomized complete block design with three replications. All fertilizers except copper were applied in four splits at 2, 4, 6 and 8 months after planting. For copper, a single application was made as a foliar spray for all doses at two months after planting. Data on plant height and number of leaves were recorded ten months after planting. Fruit weight, length, diameter, sugar (estimated as Brix, %), titratable acidity (% citric acid equivalent) and total dry matter were determined at harvest. Total nutrient uptake was calculated from leaf nutrient concentrations and total biomass at harvest.

Application of nitrogen significantly increased plant height, which generally increased with increasing nitrogen up to 800 kg and then tended to decrease but the increase was significantly different only with the control. In the case of the number of leaves per plant, the change did not follow any regular trend. However, the largest number of leaves was recorded with 400 kg N which was identical to all other doses except for the control at location 2. At both the locations, fruit length and diameter increased with increasing levels of nitrogen up to 800 kg and then declined. Although fruit sugar did not change significantly with nitrogen application, sugar content decreased consistently with increased nitrogen application at both the locations, the highest being recorded with the control; there was no change in fruit acidity. Bigger fruits (1.18 kg) were produced with 1000 kg N at location 1, but the size was statistically similar to fruits produced with all nitrogen levels except for 200 kg and the control. Although not significant, the biggest fruits of 1.07 kg were produced with the application of 1000 kg nitrogen at location 2. At location 1, total dry matter production increased gradually with increasing nitrogen up to 800 kg, but the effect was not significant. At location 2, the highest dry matter was produced with 600 kg of nitrogen, but plant dry mass was identical to all other doses except for 200 kg and the control. At both locations, nitrogen uptake increased with increased nitrogen applied. The highest N uptake at location 1 was 276 kg ha-1 while for location 2 it was 266 kg. Pineapple yield response to N fertilizer at both locations can be described by the equation Y = a + bN - cN2. The maximum level of nitrogen calculated from the response function equations were 919 and 782 kg ha-1 for location 1 and 2, respectively. But the economic optimum level for location 1 was 857 kg ha-1 and that for location 2 was 720 kg ha-1.

Phosphorus significantly increased plant height, fruit diameter and fruit sugar at location 1and fruit diameter and fruit length at location 2. The greatest plant height was recorded at location 1 with 24 kg phosphorus applied and this was similar to all other doses except for the control. Leaf number was not affected by phosphorus application. The largest fruit length and diameter were achieved with 24 kg phosphorus application but there was no significant effect of other doses except for the control at either location. Fruit sugar increased with increased phosphorus application, the highest being recorded with 120 kg at location 1 and with 96 kg at location 2. No regular trend was found with P application for fruit acidity. Although insignificant, the largest fruits of 1.22 kg and 1.01 kg were produced with 24 kg phosphorus application at location 1 and location 2, respectively. Phosphorus had no significant effect on total dry matter production. Phosphorus uptake was variable but tended to increase with increased fertilizer applications.

A significant effect of potassium was observed for number of leaves and fruit diameter at location 1 and that for fruit length and fruit weight at location 2. Though not significant, the largest fruit (1.26 kg) were produced with 534 kg potassium application at location 1. For location 2, significantly larger fruits (1.09 kg) were produced with 267 kg potassium, but fruit weights were identical for all doses except for 1066 and 1334 kg ha-1. Fruit weight decreased with high application of potassium at both locations. From the response function equation, the maximum level of potassium was found to be 432 kg and the economic level calculated from the equation was 274 kg ha-1 at location 1. However, at location 2, 267 kg ha-1produced the highest yield. Total dry matter increased with moderate application of potassium but decreased with high doses at every location. Total K uptake increased up to 800 kg fertilizer applications at location 1, but at location 2, K uptake increased up to 534 kg application and then decreased gradually with increased applications.

None of the crop characters was significantly affected by the application of calcium except for the fruit length at location 2. At this location, significantly longer fruits were produced with 15 kg of calcium, though this length was similar to all other doses except for the control. Although not significant, the largest fruits (1.27 kg) were produced with 30 kg calcium at location 1, at location 2, the greatest fruit mass (1.12 kg) was recorded 7.5 kg of calcium. The maximum level and economic optimum level of calcium calculated from the response equation was 31 kg ha-1 at location 1. Total dry matter production per plant and calcium uptake increased gradually up to 30 kg and then fell with further applications at both locations except for calcium uptake at location 2, where the trend was highly irregular.

Number of leaves per plant and fruit acidity were the only parameters affected by magnesium at location 2. The greatest number of leaves were produced with 36 kg Mg followed by other doses except for 6 kg ha-1. Fruit acidity increased significantly with 24 kg magnesium but the increase was similar to all other doses except for the control at location 2. There was no effect of magnesium on fruit weight. However, fruit weight was greatest (1.31 kg) with 24 kg magnesium at location 1 but was greatest with 6 kg magnesium at location 2. The maximum level and economic optimum level determined from the response curve was 25 kg ha-1. Magnesium uptake increased with the increasing doses of Mg, however, at the highest doses this relationship broke down.

Individual fruit weight and total dry matter production increased significantly with the application of copper at location 2. At that location, the largest fruit (1.15 kg) were with 3.2 kg copper and this was followed by other doses except for the control. Although not significant, the largest fruit (1.23 kg) were obtained at location 1 with 4.8 kg copper. The response function equation indicated that 3 kg ha-1 of copper would produce the maximum and the economic optimum yield for 'Gandul' at location 2. Significantly higher amount of dry matter was produced with 1.6 kg of copper at location 2 and this was similar to other doses except for the 4.8 kg and control levels. There was no change in Cu uptake as a result of the application of variable doses of copper fertilizers in the field.◆

# News From the United States (Hawaii)

## Changes in Sugar Content and Activities of Sugar Metabolizing Enzymes and Translucency in Pineapple Fruit Flesh

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Changes in sugar content, activities of sugar metabolizing enzymes and translucency in pineapple fruit flesh during development were studied. Fruit were picked biweekly from 12 weeks before harvest. In the early stages of fruit development, glucose and fructose were the predominant sugars. Sucrose started to accumulate rapidly 6 weeks before harvest, with more in the fruitlet than in the interfruitlet tissue, and ultimately exceeded glucose and fructose concentration. The activities of sucrose synthase, soluble acid invertase and netural invertase were high in the young fruit and declined to very low activity with development. The activity of sucrose phosphate synthase was relatively low and constant throughout fruit development. The translucency symptoms started to occur, mainly in the fruitlet tissue, 2 weeks before harvest, and followed a rapid increase in the activity of cell wall-bound acid invertase 4 weeks before harvest. There was a positive correlation between the activity of cell wall-bound acid invertase and the severity of translucency. The results of this study indicate that activities of the sucrose cleavage enzymes including sucrose stnthase, soluble acid invertase and neutral invertase are important in determining the components of stored soluble sugars in pineapple fruit flesh. The high activity of cell wall-bound acid invertase in translucent fruit suggests that this enzyme might play a significant role in the occurrence of translucency.

## Transmission, Epidemiology, and Management of Viruses in Mealybug Wilt of Pineapple

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**Study the role of PCV in MWP**.

We have taken two approaches to study the role of PCV in MWP. The first is to examine the distribution of PCV in pineapple and to associate PCV with MWP in the field. The second approach is to transmit PCV to healthy pineapple using mealybugs to reproduce the disease. Field data recently collected from Maui shows a strong correlation between mealybug wilt symptomatic plants and infection with closterovirus. Plants showing typical wilt symptoms in a plant crop field were sampled and tested for closterovirus with a tissue blotting using a specific monoclonal antibody. All symptomatic plants (104/104) sampled were positive for closterovirus. Closterovirus infection rate was only 40-60% (20/50 and 30/50) for symptomless plants sampled in areas of the same field, but away from the area developing wilt. The 40-60% infection rate is referred to as a background infection rate and is generally repeatable for a given selection or hybrid unless mealybugs have alterated (increased) the proportion of infected plants. This data was combined with previous field data collected from Oahu (Hu et al., 1997) and subjected to analysis of variance. There is a strong correlation between the closterovirus presence and symptoms of wilt ($.01 < P < .05$).

Induction of wilt symptoms in pineapple grown in pots was achieved by infesting PCV infected plants with either *Dysmicoccus brevipes* or *D. neobrevipes*. PCV-free plants infested with either species did not show classic wilt symptoms although spotting from grey mealybug feeding was evident. Wilt symptoms were not generally present on the new growth.

***Determine efficiency of PCV transmission by different mealybug species*** PCV were acquired and transmitted by the pink pineapple mealybug, *D. brevipes* and the grey mealybug, *D. neobrevipes*. Mealybugs acquired PCV from infected pineapple plants or detached leaves. The virus was detected with tissue blotting immunoassay and confirmed with immuno-labelled electron microscopy. Plants exposed to mealybugs reared on PCV-free pineapple tissue remained uninfected. The presence of ants increased the rate of virus spread when caged with *D. brevipes*. All stages of *D. neobrevipes* acquired PCV although vector efficiency dropped significantly in older adult females. Both species of mealybugs acquired and transmitted PCV from infected pineapple material that had been clonally propagated for decades and both acquired PCV from sources previously infected with the virus by the other mealybug species.

**Genetic Engineering**. High-molecular-weight dsRNAs have been isolated from PCV-infected pineapple plants in Hawaii and used in collaboration with researchers at the University of Florida to generate several PCV-specific cDNA clones using the degenerate primer approach aimed at the HSP70 gene. Based on results from preliminary sequence analysis and Northern blot hybridization, PCV has been shown to be a mixture of at least two genetically unrelated closteroviruses. Two gene constructs have been developed using the HSP70 gene and polymerase gene and transformed into pineapple. Pineapple transformation and regeneration systems have been developed by Drs. Nagai and Nan at the Hawaii Agriculture Research Center. Putative transgenic pineapple plants are being characterized.◆

## Intercycle Cover Crops for Nematode Management in Pineapple

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Several methods of non-chemical management of plant-parasitic nematodes in tropical crops are being investigated, including the use of cover crops during the inter-cycle. Sunn hemp (*Crotalaria juncea* L.), yellow mustard (*Sinapis alba* L.), and marigold (*Tagetes erecta* L.) were grown as intercycle crops between pineapple crops for 3 months at Whitmore. Two additional treatments were weed fallow and 1,3-dichloropropene (1,3-D). Reniform nematode (*Rotylenchulus reniformis*) population densities were determined before cover crop planting, 3 months after planting, 1 month after cover crop incorporation into the soil, and bimonthly after pineapple planting. The cover crops were poor hosts to *R. reniformis* and suppressed nematode population densities during the intercycle period. Nematode reproduction rate at 6 months after planting pineapple was lowest in plots previously planted with *C. juncea* ($P=0.07$). *S. alba* reduced *R. reniformis* number during the intercycle period ($P<0.05$). Cover crop incorporation increased bacterial and fungal feeding nematodes with the highest number on *C. juncea* (100 per 250 $cm^3$ of soil). The number of fungal colony forming units (CFU) on the Wahiawa soil was greatest for *T. erecta*. In a separate experiment, *Brassica napus* suppressed weeds and simulated growth of tomato in a bioassay. However, the nematode population under *B. napus* did not decrease after cover crop incorporation.

The biology the *R. reniformis* was not affected by the Pineapple Clostero Virus (PCV) in the greenhouse. PCV (+) pineapples growing in nonfumigated plots were the smallest plants in the field. PCV (-) plants growing in fumigated plots were the largests plants at 6 months.

Preliminary analysis of damage threshold data of *R. reniformis* in pineapple shows that moderate preplant population densities (40-70 nematodes/250 cc soil) caused significant yield loss.◆

## Hawaii Integrated Pest Management (IPM) Goals

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The U.S. Department of Agriculture has targeted funds to help state extension programs work with growers to develop IPM programs on 75% of U.S. farm acreage by the year 2000. Hawaii's Performance Plan goal for the 1998-2001 time frame is to promote adoption of IPM on 75% of crop acres. The acreage targeted includes about 10,000 of the 20,800 acres planted to pineapple in Hawaii. Hawaii's pilot project was begun as a partnership between the College of Tropical Agriculture and Human Resources (CTAHR) and Maui Pineapple Company. IPM guidelines were prepared jointly by CTAHR scientists and extension specialists and the grower. A point system and certification process was developed that is similar to programs already developed in the Eastern United States. To receive certification, growers must acquire 80% of the total points established in the program. The primary motivation is environmental stewardship, but the potential exists for premium price incentives for growers meeting IPM standards.

The elements of the Pineapple IPM program are made up of the best IPM practices developed for the management of each type of pest. The pests considered are the major ones affecting pineapple production in Hawaii and include ants, mealybugs, reniform and rootknot nematodes, the fungal diseases caused by *Phytophthora*, *Chalara*, and *Pythium* fungi, and grass, broad-leaved and viney weeds. In addition to committing to a regular soil testing program, relatively new or modified practices designed to reduce pesticide usage include: monitor ants on a regular basis and apply ant bait as needed; determine plant-parasitic nematode control strategies using field history and nematode population densities; monitor nematodes on a regular basis during the plant crop vegetative growth cycle; and use raised bed/ridge to reduce root rot in wet areas. Monitoring identifies areas where pest populations are potentially injurious and makes it possible to selectively target those areas with an approved pesticide. On an experimental basis on 100% of their acreage, Maui Pineapple Company plans to monitor and establish weed maps during fallow periods to determine which weeds are dominant in specific sites, and cover crops will be used on 0.10% of the cultivated area to explore their potential to depress nematode populations or increase soil nitrogen levels. While most of the above-mentioned practices generally are not new to the industry, they represent a more holistic approach to nutrient and pest management than was common in the past. Readers interested in obtaining more information on the program should contact Dr. Ron Mau.◆

## Efficacy Studies With New Formulations of Amdro For Control of the Big-headed Ant, *Pheidole megacephala*

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The big-headed ant, *Pheidole megacephala*, has long been a major problem for the pineapple industry of Hawaii. The relationship between ant, pineapple mealybug and the mealybug wilt virus is a major concern of the pineapple industry of Hawaii. Since the loss of Mirex ant bait 20 years ago, no new ant bait has been registered for ant control in the pineapple fields.

Recently, Amdro was given a section 18 clearance for use on pineapple, while a permanent label was being developed based on residue studies. In the mean time, American Cyanamid Company has developed 3 new formulations of Amdro. The new formulations differ from the original bait by the reduced percent active ingredient of Hydramethylnon. The new formulations are 0.1%, 0.01%, and 0.001%. The original formulation is 0.73% active ingredient.

Based on the studies conducted, the new formulations of Amdro is not as effective as the original formulation. Mortality was observed on all 3 new formulations, however after 3 weeks, the queen and a few workers remained alive and active. Treatments using the original formulation resulted in 100% mortality in 7 days with only one application of the bait.

Table 1: Percent mortality of big-headed ants, *Pheidole megacephphala*, to new formulations of Amdro[1] ant bait.

| Trial | Amdro[2] | Amdro 1 | Amdro 2 | Amdro 3 | Control |
|---|---|---|---|---|---|
| 1 | 100 | 13.5 | 76.0 | 49.5 | 4.5 |
| 2 | 100 | 66.5 | 90.5 | 97.5 | 28.5 |
| 3 | 100 | 9.0 | 70.0 | 70.0 | 12.5 |
| Mean | 100 | 29.7 | 78.8 | 72.3 | 15.2 |

[1] Amdro (Hydramethylnon) product of American Cyanamid Company.
[2] Amdro Regular (0.73% a.i.), Amdro 1 (0.001% a.i.), Amdro 2 (0.01% a.i.), Amdro 3 (0.1% a.i.).◆

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This list includes papers published since the last issue or references that were unavailable or unknown when the previous issue was printed. Please help keep this section current by sending citations or copies of recent publications to the editor at the address on page 1 of the newsletter. Since the last issue of Pineapple News, the proceedings of the 2nd Symposium have been published. The references in Acta Horticulturae 425 are listed here to provide complete citations for the papers in that volume.

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