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Dear Colleagues:

Another year has gone by and we are now looking forward to hearing about plans for symposium VII. Content for the newsletter is down this year, presumably in part due to the fact that it takes time to generate research results for pineapple and because the number of researchers on the crop also continues to dwindle. Graham Petty, a well-know pineapple researcher in South Africa, retired this past year. Pineapple growers there are having a difficult time competing with low-cost producers in equatorial Africa and it seems unlikely that his position will be filled in the near future. Despite such changes, this issue includes a number of articles that I hope readers will find interesting as well as a sizable list of recent references on pineapple and related topics.

For quite a number of years I have been curious how the domination of ‘Smooth Cayenne’ in the fresh fruit pineapple market was broken. Dr. Robert E. Paull and I nominated ‘MD-2’ pineapple for the American Society for Horticulture Science 2009 Outstanding Fruit Cultivar Award. While collecting information for that nomination I also was able to obtain additional information about the history leading to the development of ‘MD-2’. Below is the story of the transformation based on the best information I have been able to collect.

**‘MD-2’ Pineapple Transforms the World’s Pineapple Fresh Fruit Export Industry**

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The world’s pineapple fresh fruit export industry went through a remarkable transformation after Del Monte Corporation introduced ‘MD-2’ pineapple to consumers in the United States and Europe, officially in 1996 (Frank, 2003). Until the mid 1990s, the world’s fresh fruit export industry was relatively small and was based mainly on cultivar Smooth Cayenne. Prior to the introduction of ‘MD-2’, the focus of the pineapple export industry was on canned ‘Smooth Cayenne’ pineapple. With the major producers focused on ‘Smooth Cayenne’ pineapple, the unanswered question is what circumstances made it possible for Del Monte Corporation to become the first multinational pineapple company in the world to break the industry fixation on ‘Smooth Cayenne’ and risk growing a new and untested hybrid pineapple?

**Some Industry History**

As the world’s economies recovered after World War II, the pineapple growers in Hawaii faced increasing competition from low-cost producers in the Philippines and Thailand. Del Monte Corporation had established a pineapple plantation and cannery in the Philippines before World War II but the Hawaii business remained mostly profitable until the late 1970s. As prices for canned pineapple dropped due to increased foreign competition in the 1950s and 1960s, the Hawaii plantations began to close. By 1980, only Del Monte, Dole and Maui Pineapple Company remained in the canning business in Hawaii. Hawaii’s glory days as the world’s leading canner of pineapples was nearing its end. Del Monte, the only large company in Hawaii that farmed on leased land, closed their Hawaii cannery in 1983 and shifted all canning operations to the Philippines and Kenya. Dole also scaled down their Hawaii operation and eventually closed their cannery in 1991. With the closure of the Del Monte cannery, the company’s options were to close the plantation or concentrate on growing and marketing fresh fruit, a major transformation because it involved developing a year-around production system that was based on a mostly full-time labor force.

Production of fruit for the cannery was concentrated in the summer months because fruit quality was better and seasonal labor, primarily students out of school, was more readily available to man the harvesting and canning operations. When Del Monte moved to an all fresh fruit production system, one of the issues the company was forced to confront was the seasonal variation in fruit quality. It had long been known that pineapple fruit quality was lower in the winter because fruit acidity was much higher during that time of year. But the major holidays that fell during the winter occurred at times when the variety and supply of other fresh fruits was reduced and thus were viewed as important market opportunities. If the quality of ‘Smooth Cayenne’ fruit had been consistent throughout the year, consumers would have been unable to compare high quality summer fruits with the lower-quality fruit produced in the winter. Thus it is likely that Del Monte management knew that something superior to ‘Smooth Cayenne’ was needed if their pineapples were going to be competitive with other fresh fruits for the consumers dollars. Where was such a superior cultivar to come from?

In 1961, the Pineapple Research Institute of Hawaii (PRI) was asked to extend its purview beyond field operations to explore uses for pineapple that might expand the market for the fruit (Gortner et al., 1963). One of the “new products” thought to have potential was field-ripe pineapple for the fresh fruit markets of the United States. Fresh fruit was first shipped from Hawaii to U.S. markets in 1849 and fruit continued to be shipped from Hawaii over the years. However, the focus of the industry in the early 20th century and onward was on processed pineapple. As a result of many years of experience with ‘Smooth Cayenne’, the mind set of
company managements was that any fruit shipped from Hawaii must look and taste like ‘Smooth Cayenne’ to be acceptable. There is more than one account of a plantation or company manager rejecting what was thought to be a promising new hybrid because it didn’t look or taste like ‘Smooth Cayenne’. In a discussion of how Hawaii might compete for the fresh fruit market with countries in the Caribbean and Central America, the Gortner et al. (1963) report stated “Hawaii has exclusive and distinct varieties available to choose from, and may be able to offer a variety better adapted to the fresh fruit market demands.” Despite that claim, the focus of the PRI research report was on ‘Smooth Cayenne’ and the focus of Hawaii’s pineapple producers remained on ‘Smooth Cayenne’ for another 20 years. No new pineapple cultivar well suited to the fresh market was being grown in more than small-scale tests.

It is understandable that a company that was mainly in the business of growing pineapple for canning would be reluctant to explore new cultivars. ‘Smooth Cayenne’ pineapple was highly productive, was reasonably resistant to the important pests and diseases, and produced a fruit that met consumer expectations when canned. Changing a large plantation over to a new cultivar is very costly and takes many years. A gradual changeover of cultivars also poses significant management problems. Care needs to be taken to prevent mixing of planting material in the field and of mixing fruits in the cannery, especially if the fruit of the new cultivar has a different appearance in the can. Growing different cultivars on the same farm also increases the chances of natural crossing, which would produce fruits with seeds. In a quality-conscious industry, seedy fruits would be rejected in the cannery, resulting in significant losses and increased costs. Further, Hawaii growers were by nature cautious because catastrophic failures were the common experience. In the early 1960s, PRI hybrid 53-116 appeared to be so superior to ‘Smooth Cayenne’ that the main Hawaii plantations had planted several hundred acres of that hybrid. Despite more than 10 years of testing, a fatal flaw, susceptibility to a fruit disease, was exposed that resulted in large losses of fruit in the cannery. The hybrid was instantly discarded and no doubt industry skepticism about the possibility of replacing ‘Smooth Cayenne’ with a more productive or higher quality hybrid was heightened.

When Williams and Fleisch (1993) presented a historical review of pineapple breeding programs in Hawaii, including that of the PRI, they gave no indication that a hybrid from the 58 year pineapple breeding program would produce a cultivar that would transform the world’s pineapple fresh fruit export industry. There is no indication from their paper that they had any knowledge that the transformation process had already begun on a new plantation established by Del Monte Corporation in Costa Rica. ‘Even as the PRI breeding and selection program ended in the mid 1980s, the focus of the program was on the breeding and selection of cultivars that would can well rather than on fresh fruit cultivars. It was up to George Yamane, Research Manager at Del Monte and, after his death, Calvin Oda, to see the transformation process through to its astoundingly successful conclusion.

The Story of ‘MD-2’

Williams and Fleisch (1993) reported that PRI hybrids had been developed that, when compared with ‘Smooth Cayenne’ had:

- better resistance to Phytophthora rots, pineapple wilt, nematodes, pink disease and internal brown spot
- higher levels of vitamins C and A
- reduced acid content in winter ripened fruit
- better harvest peaking
- higher yield
- better cannary recovery from a ton of fruit
- faster plant growth
- a range of distinctive flavors.

During intensive screening, all of these varieties exhibited flaws that prevented them from replacing ‘Smooth Cayenne’, either for canning or for fresh fruit. After 50,000+ seedlings screened from each of the most promising parental crosses made in the late 1960's - early 70's failed to produce a commercially acceptable cultivar, the decision was made to phase out the PRI breeding program. Crossing was terminated in 1972, the PRI experiment station was closed in 1975 and evaluation of all seedlings was completed at Maui Pineapple Company in 1985. Several PRI selections were released to PRI member companies for potential commercial use.

Dr. David D.F. Williams and his able assistants, Frank Bermudas and Toshio Minagawa, made many crosses in 1970 but one cross was destined to make history. That was a cross between the PRI hybrids 58-1184 and 59-443, both of which had superior characteristics but had problems that prevented them from becoming commercial successes. The seeds of that cross were planted in 1971 and among the plants that grew to produce fruit, at least two were selected as having promise in 1973. During further evaluation, those two hybrids were carried in the breeder’s book as selections 50 and 114, i.e. 73-50 and 73-114. The parentage of the parents of the two clones are relatively complex as pineapple parents go, being mixtures of ‘Smooth Cayenne’, Smooth Guatemalan, ‘Ruby’ (a ‘Spanish’ clone), ‘Queen’ and ‘Pernambuco’ (Williams and Fleisch, 1993). Both are more than 50% ‘Smooth Cayenne’, which is significant for Hawaii and the U.S. mainland because they are not considered potential fruit fly hosts (Armstrong et al., 1979; Seo et al., 1973). Between 1973 and 1980 test plantings of the two hybrids, perhaps along with others that showed some potential, were made on Maui and, between 1978-80, also on the Del Monte plantation on Oahu.

The hybrids were released by the PRI to the then member companies Del Monte Corp. and Maui Pineapple Co. for further evaluation in 1980. In 1981 Del Monte renamed 73-50 as “MD-1”, though it was never known by that name outside of Del...
Monte, and 73-114 was named “MD-2”. Both were named after Del Monte Hawaii general manager Frank Dillard’s wife Millie by Del Monte’s research manager George M. Yamane and his assistant Calvin Oda. Hybrid 73-50, which due to a patent that was obtained for what has been assumed to be that hybrid, is perhaps now most widely known as ‘CO-2’ (Chan et al., 2003). ‘CO-2’ has achieved some limited success, but no where near that enjoyed by ‘MD-2’. ‘MD-2’ is a vigorous plant with acceptable disease and pest resistance, high fruit yield and outstanding fruit quality. The outstanding fruit characteristics include golden external shell color, golden flesh color, slightly higher soluble solids content than ‘Smooth Cayenne’, considerably lower acidity, especially during the winter months, higher vitamin C content, and exceptional post-harvest shelf life. No other pineapple cultivar in the international pineapple trade holds up as well under refrigeration as does ‘MD-2’. These attributes of ‘MD-2’ apparently convinced Yamane and Oda to support expanding plantings of the hybrid. In addition, containers of planting material were shipped to Del Monte’s Philippines subsidiary in 1983-84. Test marketing of ‘MD-2’ was done in Japan, but acceptance there was slow because the cultivar was very different from ‘Smooth Cayenne’ and had not yet become popular elsewhere.

As part of Del Monte’s plan to expand their fresh fruit business, the company took over an abandoned pineapple farm of several hundred hectares in Costa Rica in the late 1970s. Armed with the knowledge that select clones of ‘Smooth Cayenne’ are superior to field-run plants, Del Monte replaced the local clone with plants of the PRI ‘Smooth Cayenne’ clone Champaka F-153, which at the time was being grown in Hawaii by both Del Monte and Maui Pineapple Company. The new clone performed poorly, which is not an unusual finding for a clone selected for the Hawaii environment. Champaka F-180, also part of the PRI ‘Smooth Cayenne’ clone collection, was inferior to Champaka F-153 in Hawaii. However, F-180 was superior the F-153 clone in the colder environment of Queenssland, Australia and became an important clone there.

In an attempt to improve production on their new plantation, company researchers Yamane and Oda had plants of ‘MD-2’ shipped from Hawaii to Costa Rica in the mid 1980s. The details of how development of ‘MD-2’ progressed in Costa Rica are not publicly available; however, Frank (2003) speculated that test marketing was done in the late 1980s or early 1990s when fruits were sold by what was then Del Monte Tropical Fruit (Fresh Del Monte Produce, 2009) in Boston and Florida under the label Del Monte Gold® Extra Sweet (for a time the boxes also carried the ‘MD-2’ label (D. Bartholomew, personal observation)). It is likely that as a result of these early test marketings, Del Monte management realized that the company had a winner on its hands. Sale of ‘MD-2’ fruits in Europe followed not long after it was introduced in the U.S. market.

The period from about 1993 until 2003 is surrounded with intrigue (Frank, 2003; Janick, 2003; Greig, 2004) that has little to do with the success of ‘MD-2’ and only impacts which companies were able to benefit from it, a not insignificant issue. What is now widely known is that fruits of ‘MD-2’ were so well accepted that they were sold at premium prices for about 10 years and, in that same time frame almost completely displaced ‘Smooth Cayenne’ as the world’s main fresh fruit cultivar grown for export (see details below).

During the period when Fresh Del Monte Produce (the name was changed in 1996) was the sole source of ‘MD-2’, pineapple sales reported by the company increased from nearly $200 million dollars in 1996 to $440 million in 2002, the last year the company broke out pineapple sales in their annual report (Frank, 2003). ‘MD-2’ is now grown by many companies and growers, both large and small, is the worlds’ main fresh fruit cultivar and the fruit is exported to temperate markets in the United States, Europe, England, Japan, Korea, Hong Kong, China, Singapore and the Middle East.

Statistics on the area planted to ‘MD-2’ and its market value are difficult to obtain because it is grown or marketed, or both, primarily by multinational corporations. The area planted to ‘MD-2’ exceeds 60,000 hectares (ha) (FAO Ag Stat 2007 and other sources). Costa Rica alone has 42,500 ha and is the country with the largest area planted to ‘MD-2’. Other countries and estimates of areas planted to ‘MD-2’ include Ecuador (5,850 ha), Honduras (2,800 ha), Guatemala (500 ha), Panama (1,600 ha), Brazil (>500 ha), Ghana (>600 ha), Cote d'Ivoire (>500 ha), Hawaii (about 820 ha) and Philippines (approximately 10,000 ha in the Davao and Bukidnon areas; the report is that one large grower plans to plant several thousand more hectares in the next five years).

Both the production and consumption of ‘MD-2’ pineapple continue to grow. Loeillet (2008) reported that in 2006 Costa Rica alone exported nearly 1.2 million metric tons (MT) of fresh pineapple, a 30% increase over 2005; increases in shipments were +25% from 2003 to 2004 and +30% from 2004 to 2005. Exports from Costa Rica have multiplied by 2.6 since 2002 and it is unlikely that such almost explosive growth in fresh sales have ever been recorded for any other fruit or fruit cultivar.

In addition to the rapid growth of ‘MD-2’ in the marketplace, its success had a devastating impact on pineapple sales from Cote d'Ivoire (Loeillet, 2008) and Ghana. In about the year 2000, Ghana experienced a dramatic drop in pineapple exports because the market favored ‘MD-2’ rather than ‘Smooth Cayenne’, Ghana’s principle export cultivar. In 2002, with $2 million of government support, tissue culture production of ‘MD-2’ was rapidly ramped up and by the end of 2007 Ghana had exported 42,000 MT of ‘MD-2’, earning $20 million in foreign currency. As a result, Ghana was ranked 3rd as an exporter to the EU (Ghana Export Promotion Council, 2008).

Based on the information obtained for this paper, it would appear that there is no simple answer to the question posed at the beginning of the article. ‘MD-2’ pineapple was introduced at a time when consumer preferences were changing, which resulted in a rapid increase in the consumption of fresh fruits and vegetables. If Del Monte had found success with ‘Smooth Cayenne’ in Costa Rica, perhaps another company would have been the first to introduce a new pineapple cultivar to the world’s consumers. But all credit must go the Del Monte researchers Yamane and Oda, or the company’s managers, or both, because they were quick
to recognize and capitalize on ‘MD-2’ once it became clear that consumers preferred it over any other pineapple cultivar then offered in the United States or Europe. Once ‘MD-2’ was accepted in the U.S. and EU markets, acceptance was rapid in Asian markets too. As a result of these remarkable successes, ‘MD-2’ is the first major pineapple cultivar to be produced by modern breeding. It has now essentially replaced the ancient ‘Smooth Cayenne’ developed by Amerindians in pre-Columbian times as the world’s principal pineapple fresh fruit cultivar grown for export. Though the total market value of ‘MD-2’ fruits is not known, it is likely that annual sales of that cultivar alone exceed $1 billion.

Dr. David D. F. Williams is now comfortably retired in the state of Colorado. In July of 2009 Williams will travel to the American Society for Horticulture Science (ASHS) annual meetings in St. Louis, Missouri to receive a medal recognizing the contributions of him and his predecessors at the Pineapple Research Institute of Hawaii in the development of PRI hybrid 73-114. And this hybrid, now known widely as cultivar ‘MD-2’, will be recognized as the ASHS 2009 Outstanding Fruit Cultivar. This article is based in part on the nomination of ‘MD-2’ for that award, which Duane P. Bartholomew and Robert E. Paull had the pleasure of preparing. With the transformation begun by ‘MD-2’, perhaps at some future ASHS or other horticultural society meeting, another new pineapple cultivar will receive an award as the outstanding cultivar and consumers will have an opportunity to choose from several pineapple cultivars produced by modern breeding.

References

7th International Pineapple Symposium
At the Pineapple Working Group meeting at the VIth Symposium it was agreed that the VIIth International Pineapple Symposium would be held in 2010. The symposium is to be organized by the Malaysian Pineapple Industry Board (MPIB) and the Malaysian Agricultural Research and Development Institute (MARDI) with the support of the Ministry of Agriculture and Agro-based Industry Malaysia (MOA). Mr. Tengku Malik (tamtm@mardi.gov.my) will lead the organization of this symposium. No formal announcement has come from MARDI to date (mid May, 2009). Malaysia is an interesting country to visit and offers many amenities as well as beautiful and unique local crafts.

ISHS Publishes Proceedings of International Pineapple Symposium VI
The proceedings of the VIth symposium were recently published and the volume can be obtained from the International Society for Horticultural Science web site at http://www.ishs.org. Plan to visit the much updated web site soon. The ISHS is one of the foremost organizations promoting cooperation and communication among horticultural researchers, growers and consumers. The ISHS continues to expand its offerings to members as well as to provide the structure under which our Pineapple Working Group (http://www.ishs.org/science/T07.php) functions. Detailed information about ISHS and the benefits of membership can be found at http://www.ishs.org or you can write to the ISHS Secretariat, P.O. Box 500, 3001 Leuven, Belgium (E-Mail: info@ishs.org).

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

News from Australia
No articles were submitted from Australia but Annual Industry Reports covering work on pineapple in the country for the years 2006-07 and 2007-08 can be obtained at http://www.horticulture.com.au/industry/annualreports.asp.
Topics covered in the 2006-07 Report include:

- Industry Tackles Challenges Through R&D
- Queensland Industry Manages Changes
- Pineapple Industry Biosecurity Plan Update
- Levies to Develop the Australian Pineapple Industry
- Across Industry Program 2006/07
- Pineapple Program 2006/07

Topics covered in the 2007-08 Report include:

- Industry supports new national levies
- Navigating change in the Queensland pineapple industry
- Development of new local fresh market pineapple cultivars
- Establishment of levies to strengthen the Australian pineapple industry
- Generation of dimethoate residue data to support ongoing use in pineapple
- Pineapple study tour of Brazil and Costa Rica
- Across Industry Program 2007/08
- Pineapple Program 2007/08
- Investing in Australian Horticulture

News From Belgium

A Novel Flowering Induction Agent for Pineapple

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Abstract

This paper is condensed from a recently published article “Determination of pineapple (Ananas comosus, MD-2 hybrid cultivar) maturity, the efficiency of flowering induction agents and the use of activated carbon” published in Scientia Horticulturae 120 (1) page 58-63. We report on a novel flowering induction agent called “zeothene”, which releases gaseous ethylene on contact with water. We evaluated the induction capacity of this agent on a commercial plantation (Ecuador) and compared its flowering induction efficiency with other common agents currently used in pineapple cultivation. The zeothene treatments resulted in homogeneous flowering, which is highly desired on commercial farms. Furthermore we evaluated the method of induction (central cup application vs. whole plant spraying) and the use of activated carbon to enhance the flowering induction treatment with ethylene gas. Our results indicated that a central cup application is more favorable to obtain a homogeneous flowering and that the beneficial effects of activated carbon are questionable.

Introduction

Pineapple (Ananas comosus) is a member of the Bromeliaceae family and is characterized by its ability to flower in response to ethylene signals (internal or external). This feature is exploited worldwide by pineapple growers to synchronize flower and fruit development, which reduces harvesting costs and optimizes market supply (Min & Bartholomew, 1996). It is very important to apply the correct flowering induction agent at the right time. This is a crucial aspect in pineapple cultivation to maximize the number of plants fruiting at the same time and, consequently, to maximize yield. In this report we focus on the correct use of different flowering induction agents.

Ethephon (2-chloroethylphosphonic acid) is a common flowering induction agent. The chemical is stable under acidic conditions (pH ≤ 3) and breaks down to release ethylene gas at a higher pH (≥ 5) (Yang, 1969; Dass et al., 1976). Usually it is applied as a broadcast spray with a boom sprayer during the day or, if temperatures are high, in the late evening or at night to prevent evaporation losses (Turnbull et al., 1999). Increased amounts or multiple applications are used if plants are expected to have low sensitivity.
Another very common flowering induction agent is ethylene gas. Ethylene has been proven more efficient than ethephon (Py et al., 1984). The gas is injected into water under pressure and applied as a broadcast spray over the plants with a boom sprayer. Activated carbon is added to the water to increase the volume of gas retained in the solution. Ethylene gas is most effective when applied late in the evening, at night, or in the early morning. Such timing reduces evaporation losses and the lower temperature at night increases the solubility of the gas in the water droplets (L’Air Liquide, 1976). It is also believed that ethylene uptake by the plant is higher at night because during this period the stomata are open (due to the Crassulacean Acid Metabolism of the plant) (Bartholomew et al., 2003). A suitable ethylene concentration used for commercial flowering induction is 2.272 kg ha\(^{-1}\) ethylene gas sprayed with 7000 L ha\(^{-1}\) water containing 20 kg activated carbon/ha (Hepton, 2003).

The aim of this research was to evaluate the flowering induction efficiency of a novel agent, called “zeothene”. This was done on a commercial plantation. Application was directly into the central cup with and without activated carbon.

Materials and methods

Flowering induction experiment

All field experiments were executed on a commercial pineapple plantation near Quevedo (Ecuador). The plantation was a monoculture of the new hybrid cultivar MD-2. The efficiency of four different flowering induction agents was tested (zeothene, ethephon, ethylene gas and ethylene dissolved in water). All treatments were applied in the early evening (5-6 p.m.) to plants of 8-9 months after planting. “Zeothene” is a special zeolite pearl retaining ethylene gas, which is released upon contact with water. This zeothene (or also called ‘ethylene pills’) was developed by M.P. De Proft (Laboratory of Plant Production, Department of 8-9 months after planting. “Zeothene” is a special zeolite pearl retaining ethylene gas, which is released upon contact with water. This zeothene (or also called ‘ethylene pills’) was developed by M.P. De Proft (Laboratory of Plant Production, Department of Biosystems, Katholieke Universiteit Leuven, Belgium). The pills (± 13.5 mg/pill containing ± 0.7 mL C\(_2\)H\(_4\)) are very effective in inducing flowering in ornamental bromeliads (Parton, 2001; Plever, 2004). To induce flowering of pineapple, 3-4 pills were dropped into the central cup of each plant. The pills release their ethylene content upon coming in contact with water standing in the central cup of the plants.

Ethephon (Ethrel, Bayer CropScience, Germany) was provided with a commercial dose of 0.5 kg ethephon per hectare (active ingredient) dissolved in 2000 L water with 5 % urea and brought to pH 3. A small amount of the prepared ethephon solution was brought into the central cup with a hand sprayer. A solution containing the equivalent of 2.272 kg ethylene gas in 7,000 L of water with 20 kg activated carbon (Hepton, 2003) was applied over the entire plants by a pressurized boom sprayer. This is the standard flower induction application of the plantation. The ethylene-water solutions were made by adding a known amount of zeothene pills to a sealed 25 L tank containing water, resulting in the following concentrations of ethylene, C\(_2\)H\(_4\) in g L\(^{-1}\): 0.389, 0.292 and 0.195, corresponding respectively to 100%, 75% and 50% of the commercial dose used for ethylene gas. Activated carbon (2.86 g L\(^{-1}\)) was added to two of the three doses tested. The prepared solutions were applied into the central cup of the plant with a hand sprayer. All treatments were applied randomly to rows of 100 plants and the experiment was repeated 3 times.

The efficiency of the flowering induction treatment can be expressed by a flowering homogeneity percentage. This percentage was obtained by counting the number of plants which were in the same floral development stage exactly 73 days after induction. We distinguished 6 main floral stages: opening of the central cup, red bud stage (= inflorescence tip is visible), 1st flower (= lowest petals are visible), 2nd flower (= middle petals are visible), 3rd flower (= upper petals are visible) and the wilting stage (= all petals are wilted) (Rohrbach & Taniguchi, 1984).

Ethylene absorption by activated carbon

Ethylene absorption of different concentrations of activated carbon-water solutions (5%, 0.5% and 0.05%; Pro-analyse, Vel NV, Belgium) was tested by means of gas chromatography. One hundred mL of each solution was added to a 300 mL air tight glass flask and a known amount of ethylene gas (1.25 ppm) was injected in the headspace of the flask. The solutions were stirred gently (150 RPM) and air samples were taken at regular time intervals. The air samples were analyzed for their ethylene content by a gas chromatograph (DI 200, Delsi Instruments, France) equipped with a flame ionization detector (FID) (De Greef & De Proft, 1978). Each concentration was tested 3 times. All measurements were conducted at normal room conditions (23°C and 1013 hPa).

Results

Flowering induction experiment

Homogeneity of induction results (Table 1) show that "zeothene" successfully induced flowering though it was only significantly better than the ethylene gas treatment (Table 1). While other treatments were not different from each other, flowering stage development of plants forced with ethephon (Table 1) was delayed compared with the other treatments. This might be explained by the fact that ethephon first has to break down into ethylene before it can exert its physiological action. The commercial ethylene application had the lowest homogeneity percentage of all treatments. This low percentage will eventually result in a more heterogeneous flower and fruit development which consequently may result in multiple harvest passes.
A water solution containing dissolved ethylene gas with and without activated carbon also induced flowering in pineapple when sprayed in the central cup. There was no significant effect of ethylene concentration and the addition of activated carbon to the ethylene solution also had no significant effect on the homogeneity percentage. These results do not provide sufficient evidence about the influence of activated carbon on the flowering induction reaction. Further research in this area is needed.

Ethylene absorption by activated carbon

To further investigate the role of activated carbon, the amount of ethylene absorbed by different activated carbon solutions was quantified. Ethylene absorption isotherms (Figure 1) for three concentrations of activated carbon showed there was very strong absorption of ethylene during the first 5 minutes and an equilibrium was reached after about 30 minutes of exposure. After 60 minutes the 5% activated carbon solution absorbed 65% more ethylene gas than pure water (0.043 µl C2H4/100 ml compared to 0.026 µl C2H4/100 ml) and the absorption was significantly greater after an exposure time of at least 20 minutes. Solutions containing 0.5 or 0.05% activated carbon did not absorb significantly more ethylene gas than water itself, during the entire exposure period.

Discussion and Conclusions

The effectiveness of flower induction mainly depends on the type of flower induction agent, the mode of application, plant genotype and environmental conditions (temperature, humidity, wind, rain…) (Bartholomew et al., 2003). The novel flowering induction agent “zeothene” proved to be very efficient in inducing flowering in pineapple. Zeothene had a higher homogeneity percentage than the ethephon and ethylene gas treatments. A solution of water with dissolved ethylene gas sprayed in the plant cup resulted in a higher homogeneity percentage than did the “commercial” broadcast spray of ethylene in water with activated carbon treatment; however, the higher percentage could be due to the method of delivery, i.e. into the central cup where as the commercial treatment was a broadcast spray.

In these experiments we only investigated flowering homogeneity. We did not take into account eventual fruit weight and quality. These aspects should also be evaluated in future research before any final conclusions can be made about the new agent. Nevertheless, it can already be stated that zeothene is a promising alternative to the currently used flowering induction agents.

Our field experiments clearly showed that central cup applications increase the homogeneity percentage and Turnbull et al. (1999) found similar results for ethephon treatments. Central cup applications have the advantage that the active ingredient is near or at the active site and is more protected from sun and wind so evaporation losses are smaller than for broadcast spraying. It is the physiologically-active apical tissue that will have to change from a vegetative into a generative status. So ethylene perception at the level of the apical meristem is a crucial aspect in pineapple flowering.

It is assumed that activated carbon enhances the flowering induction treatment by absorbing more ethylene gas, and thus increasing the total amount of ethylene gas that is delivered to the plant. Therefore this is a common practice in pineapple cultivation, although no published data on the subject could be found. Based on our field experiments no clear conclusions could be made about the influence of activated carbon on the flowering induction treatment. Recently preliminary field trials conducted by Lin (2008) have shown similar benefits of activated carbon in forcing. From our second experiment we can conclude that only very high (5%) concentrations of activated carbon absorb significantly more ethylene than does pure water under laboratory conditions without any applied pressure. During a commercial field application (0.286% activated carbon) where ethylene is sprayed with water, the contact time between the gas and the water droplets is only limited, although a lot of pressure is applied. In this short time frame it is questionable if any increased ethylene absorption is possible by the added activated carbon. Further research on the influence of activated carbon is desirable. Field trials testing different types of commercial equipment and methods of spraying can help illuminate this intriguing phenomenon.

Acknowledgments

The authors wish to thank José Ruiz and Luiz Fernando Saltarēn for their expertise and guidance. This research was partly supported by a grant from the Vlaamse Interuniversitaire Raad and the Katholieke Universiteit Leuven.

References


Table 1. The ethylene sources, with and without activated carbon, on the percentage of plants at the same flowering stage 73 days after treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethylene, g</th>
<th>Activated carbon</th>
<th>Flowering (%)</th>
<th>Flowering stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0</td>
<td>6.7 ± 2.0 (A)*</td>
<td>No flowering</td>
</tr>
<tr>
<td>Zeothene</td>
<td>-</td>
<td>0</td>
<td>92.7 ± 3.6 (A)</td>
<td>3rd flower</td>
</tr>
<tr>
<td>Ethephon</td>
<td>-</td>
<td>0</td>
<td>82.6 ± 3.3 (AB)</td>
<td>2nd flower</td>
</tr>
<tr>
<td>Ethylene spray</td>
<td>0.389</td>
<td>2.86 g/L</td>
<td>78.4 ± 7.1 (B)</td>
<td>3rd flower</td>
</tr>
<tr>
<td>Ethylene</td>
<td>0.389</td>
<td>2.86 g/L</td>
<td>90.6 ± 4.6 (AB)</td>
<td>3rd flower</td>
</tr>
<tr>
<td></td>
<td>0.389</td>
<td>0</td>
<td>83.0 ± 11.7 (AB)</td>
<td>3rd flower</td>
</tr>
<tr>
<td></td>
<td>0.292</td>
<td>0</td>
<td>89.6 ± 5.2 (AB)</td>
<td>3rd flower</td>
</tr>
<tr>
<td></td>
<td>0.195</td>
<td>2.86 g/L</td>
<td>93.2 ± 7.2 (A)</td>
<td>3rd flower</td>
</tr>
<tr>
<td></td>
<td>0.195</td>
<td>0</td>
<td>95.0 ± 7.6 (A)</td>
<td>3rd flower</td>
</tr>
</tbody>
</table>

*Treatments followed by the same letter were not significantly different (\(P < 0.05\)) from each other. (n = 100; repetitions = 3).

Figure 1. Ethylene gas absorption rates into pure water and water-activated carbon solutions (n = 3). Significant differences (\(P<0.05\)) between the 5 % solution and the other solutions are indicated by asterisks above the data points.
Flowering induction is a crucial aspect of pineapple cultivation. Growers all around the world treat their plants with ethylene, or an ethylene releasing compound, to obtain simultaneous flowering. This practice will result in a homogeneous flower and fruit development (Min & Bartholomew, 1996). It is important to apply the ethylene treatment at the right moment. Young plants will produce smaller fruits, while older plants are more subjected to natural flowering, which disturbs flowering homogeneity (Kerns, 1939). It is crucial to monitor plant development. This is done by sampling D leaves over a period of time. D-leaf fresh weight or nutrient status are commonly used parameters to monitor plant development (Malézieux et al., 2003). The optimal time for flowering induction, is the moment when plants have reached full biomass and have not yet started flowering due to natural induction. Although biomass can be monitored accurately in individual fields, the actual maturity stage of the plants is mostly unknown. In the literature this topic has been discussed but not yet fully determined. Das et al. (1965, cited by Norman, 1982) stated that pineapple plants should have at least 21 leaves before they are susceptible to flowering induction. Py et al. (1984) suggested that plants ('Smooth Cayenne') should weigh at least 1 kg before applying flower induction treatments.

We performed a field trial to find out at which exact stage during development, pineapple plants were completely susceptible to an external ethylene treatment. Our experiment was conducted on a commercial pineapple plantation in Ecuador (Quevedo) during the months of July and August 2006. The plantation is a monoculture of the new MD-2 hybrid. Plants of different physiological age, starting from 1 month after planting (MAP) up to 8 MAP were given an ethylene treatment. The different age groups were grown on different fields due to optimal management practices. All plants originated from shoots or suckers (450 - 500 g) and were treated by standard cultural practices such as irrigation, fertilization and pest control to obtain optimal plant growth and maximal fruit production. All plants used in the experiment were induced at the same moment to prevent variation in weather conditions, which can influence the flowering induction treatment. The ethylene treatment was done by dropping 3-4 zeothene pills in the central cup of each plant (n = 25). Zeothene is a new flowering induction agent (Van de Poel et al., Pineapple News, this issue; Van de Poel et al., Scientia Horticulturae, 2009). Briefly, it is a zeolite pearl containing pure ethylene gas, which is released upon contact with the water standing in the central cup of the plant. One month after flowering induction, the apical meristem was visually examined by making a longitudinal stem section. A vegetative apical meristem is characterized by a flat dome shape, while a generative plant shows a vertical elongation of the apical meristem (Kerns et al., 1936). Plant characteristics such as plant fresh weight (without roots) (g), number of leaves (including invisible young leaves), D-leaf length (cm) and D-leaf fresh weight (g) were also recorded one month after flowering induction.

At one month after treatment, plants one MAP were not susceptible to external ethylene while at 2 MAP only 27 % of the plants showed flowering (Table 1). At two MAP plants had a flowering stem 1.07 ± 0.18 cm long. At 3 MAP and older MD-2 plants were completely sensitive (100 %) to an external ethylene treatment. The average length of the flowering stem at one month after treatment was not significantly different for plants of 3 MAP up to 8 MAP (3.10 - 3.53 cm). The growth of the flowering stem was independent of plant size at one month after induction for plants at 3 MAP or later while 2 MAP plants were less sensitive to ethylene forcing and had retarded flower development. These results suggest that plants of 3 MAP are fully mature. Nevertheless, natural flowering is mostly observed much later during plant development. This might indicate that ethylene perception is different for external applied ethylene and de novo synthesized ethylene due to natural flowering conditions. Additional research on ethylene production and ethylene perception of the apical meristem, in relation to plant development, should bring forth new insights in flowering sensitivity of pineapple.

During the first three months after planting, the young plant will mainly invest in rebuilding damaged tissue and root development as they adapt to their new environment. During this period, plant growth, D-leaf growth and new leaf development are limited (Table 1, Figure 1-4). The data are presented using box plots where the smallest observation is shown by a short bar, usually below the box and easily visible in Figures 3 and 4. The lowest horizontal bar forming the base of the box illustrates the lower quartile (25%), the second, the median (50%), the third, the higher quartile (75%), and the largest observation is indicated by a short bar above the box, also easily visible in Figures 3 and 4. The mean is the filled circle in the middle of the box and the median and the mean are equal when the middle bar in the box falls on the filled circle.

After the initial lag phase, growth in terms of plant fresh weight (Figure 1) and D-leaf development (length and fresh weight) (Figure 3 & 4) becomes exponential. The total number of leaves increased linearly after the initial lag phase (Figure 2). At around 8-9 MAP when the optimal plant weight is reached, vegetative development, including new leaf development, ceases due to ethylene forcing. It is interesting that actual physiological maturity in terms of susceptibility to ethylene forcing coincides with
the end of the initial lag phase (3 MAP). These results show that young pineapple plantlets first have to outgrow an initial phase (recovery/adaptation) before they are susceptible towards an external ethylene treatment.

We can conclude that MD-2 hybrid pineapple plants reach physiological maturity by 3 MAP even though they are not yet large enough to produce a marketable fruit. We can also conclude that D-leaf length and fresh weight are reliable parameters representing general plant growth and can be used to determine the actual plant maturity border.

Table 1: Percentage of flowering and length of the flower stem one month after applying zeothane ethylene carrier and D-leaf length, D-leaf fresh weight, plant fresh weight and leaf number for MD-2 pineapple plants (n = 25) at one to eight months after planting.

<table>
<thead>
<tr>
<th>Planting time</th>
<th>Flowering %</th>
<th>Flower stem length (cm)</th>
<th>D-leaf Length (cm)</th>
<th>D-leaf Fresh weight (g)</th>
<th>Plant Fresh weight (g)</th>
<th>Plant Nr of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 MAP</td>
<td>0</td>
<td>59.7 ± 6.1 (A)</td>
<td>26.8 ± 5.8 (A)</td>
<td>354 ± 49.3 (A)</td>
<td>23.2 ±1.6 (A)</td>
<td></td>
</tr>
<tr>
<td>2 MAP</td>
<td>27</td>
<td>57.0 ± 4.2 (A)</td>
<td>24.6 ± 4.6 (A)</td>
<td>443 ± 71.12 (A)</td>
<td>24.4 ± 3.0 (A)</td>
<td></td>
</tr>
<tr>
<td>3 MAP</td>
<td>100</td>
<td>58.8 ± 5.1 (A)</td>
<td>24.6 ± 5.0 (A)</td>
<td>630 ± 90.2 (A)</td>
<td>29.2 ± 3.8 (A)</td>
<td></td>
</tr>
<tr>
<td>4 MAP</td>
<td>100</td>
<td>73.8 ± 6.3 (B)</td>
<td>46.8 ± 8.5 (B)</td>
<td>829 ± 172.8 (A)</td>
<td>32.0 ± 4.1 (A)</td>
<td></td>
</tr>
<tr>
<td>5 MAP</td>
<td>100</td>
<td>94.0 ± 7.5 (C)</td>
<td>70.4 ± 16.1 (C)</td>
<td>1338 ± 203.6 (B)</td>
<td>35.4 ± 4.5 (A)</td>
<td></td>
</tr>
<tr>
<td>6 MAP</td>
<td>100</td>
<td>105.2 ± 6.9 (D)</td>
<td>94.2 ± 15.9 (D)</td>
<td>1965 ± 406.8 (C)</td>
<td>44.2 ± 2.3 (B)</td>
<td></td>
</tr>
<tr>
<td>7 MAP</td>
<td>100</td>
<td>111.3 ± 5.7 (E)</td>
<td>113.6 ± 19.2 (E)</td>
<td>2161 ± 328.8 (C)</td>
<td>42.0 ± 4.7 (B)</td>
<td></td>
</tr>
<tr>
<td>8 MAP</td>
<td>100</td>
<td>120.5 ± 7.5 (F)</td>
<td>141.6 ± 17.9 (F)</td>
<td>2817 ± 253.7 (D)</td>
<td>48.0 ± 2.6 (B)</td>
<td></td>
</tr>
</tbody>
</table>

References
New Records of Scale Insect Pests of Pineapple and Their Natural Enemies in the State of Espírito Santo, Brazil

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Scale insects (Hemiptera: Coccoidea) are important pests of pineapple and many other agricultural crops. Integrated pest management (IPM) of such insects depends on knowledge of the pest species and natural enemies of the pests commonly present in the crops of specific areas to obtain accurate information on the best management methods available. Unfortunately, relatively little is known of the insect fauna, including scale insects and their natural enemies, in tropical areas such as the Brazilian State of Espírito Santo (Culik et al. 2007, Culik et al. 2008). Therefore, surveys of scale insects and their natural enemies were conducted in Espírito Santo to support development of integrated pest management as well as better document the biodiversity in this region.

Scale insects and their natural enemies were collected during surveys of the insect fauna of pineapple and when noticed on plants during fieldwork or other activities in Espírito Santo in 2006 to 2008. Samples of plant parts (fruits, leaves, stems) infested with scale insects were collected from locations ranging from municipalities of Serra in the north (20.13S; 40.31W) to Anchieta in the south (20.81S; 40.65W) and Vitória (20.32S; 40.35W) on the coast to municipalities in the interior of the state such as Domingos Martins (20.38S; 41.050W), from a variety of sites including experimental research plots, residences, and a greenhouse. Samples were transported to the Espírito Santo rural research and extension institute INCAPER (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural) headquarters in Vitória for preservation and identification of the scale insects and their natural enemies. Samples were also placed in plastic containers covered with cloth to allow development of natural enemies present and examined every few days for several weeks to collect adult parasites and predators that emerged. Specimens collected were sent to taxonomic specialists to confirm identifications when necessary.

Based on this research, four scale insect species that are potential pests of pineapple, Melanaspis smilacis, Unaspis citri, Dysmicoccus brevipes, and Planococcus minor, were recorded for the first time in different municipalities in the State (Table 1). In addition, a wide variety of natural enemies of the scale insect pests of pineapple were collected, including 5 new species of predators that are currently being described, and 6 species of parasitoids that are also being studied further to confirm identifications (Table 2).

Most of the scale insect species collected in this study are polyphagous and widely distributed (Ben-Dov et al. 2006). Thus, they are potential pests of many agricultural crops in many tropical areas. However, natural enemies of many of these scale insects were commonly found associated with these scale insects in Espírito Santo indicating the importance of using IPM methods, and avoiding improper and harmful management practices such as misuse of pesticides, to prevent destruction of beneficial insects and natural enemies of scale insects that may commonly help control scale insect and other pests in this area.
Acknowledgements
Research Support provided by the Fundação de Apoio à Ciência e Tecnologia do Espírito Santo - FAPES, FINEP, and CNPq.

Literature Cited

Table 1. New records of scale insect pests (Hemiptera: Coccoidea) of pineapple in the State of Espírito Santo, Brazil (2006-2008).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Municipality</th>
<th>Associated crop/plant</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DIASPIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanaspis smilacis (Comstock)*</td>
<td>Domingos Martins</td>
<td>Ananas comosus</td>
<td>New register in municipality</td>
</tr>
<tr>
<td>Unaspid ciri (Comstock)</td>
<td>Domingos Martins</td>
<td>Citrus sp.</td>
<td>New register in municipality</td>
</tr>
<tr>
<td><strong>PSEUDOCOCCIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysmicoccus brevipes (Cockerell)*</td>
<td>Serra</td>
<td>Cocos nucifera</td>
<td>New host record in State</td>
</tr>
<tr>
<td>Planococcus minor (Maskell)*</td>
<td>Vitória</td>
<td>Syzygium jambos</td>
<td>New host record in State, new register in municipality</td>
</tr>
</tbody>
</table>

*Associated natural enemies (parasitoids and predators) also collected.

Table 2. New records of predators (Diptera: Cecidomyiidae, Drosophilidae) and parasitoids (Hymenoptera: Chalcidoidea) of scale insect pests of pineapple in the State of Espírito Santo, Brazil (2006-2008).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Municipality</th>
<th>Associated crop/plant</th>
<th>Associated scale insects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CECIDOMYIIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diadiplosis sp. nov.1</td>
<td>Vitória</td>
<td>Syzygium jambos (fruits)</td>
<td>Planococcus minor, Planococcus halli</td>
</tr>
<tr>
<td>Diadiplosis sp. nov.2</td>
<td>Sooretama, Cachoeiro do Itapemirim, Domingos Martins</td>
<td>Ananas comosus (seedlings, Dysmicoccus brevipes fruit, leaves and crowns)</td>
<td></td>
</tr>
<tr>
<td>Diadiplosis sp. nov.3</td>
<td>Domingos Martins</td>
<td>Ananas comosus (fruit)</td>
<td>Pseudococcus cf. jackbeardsleyi; Saissetia cf. coffeae</td>
</tr>
<tr>
<td>Diadiplosis sp. nov.4</td>
<td>Domingos Martins</td>
<td>Coffea arabica (fruits, stems)</td>
<td>Pl. cf. citi, Ps. cf. jackbeardsleyi, S. cf. coffeae</td>
</tr>
<tr>
<td><strong>DROSOPHILIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinoleucophenga sp. nov.</td>
<td>Cachoeiro do Itapemirim</td>
<td>Ananas comosus (fruit)</td>
<td>Dysmicoccus brevipes</td>
</tr>
<tr>
<td><strong>APHELINIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encarsia cf. auranitii</td>
<td>Domingos Martins</td>
<td>Ananas comosus</td>
<td>Diaspis boisduvali</td>
</tr>
<tr>
<td>Encarsia lounsburyi</td>
<td>Domingos Martins</td>
<td>Ananas comosus</td>
<td>Melanaspis smilacis</td>
</tr>
<tr>
<td><strong>ENCYRTIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adeleencyrtus modestus</td>
<td>Sooretama</td>
<td>Ananas comosus</td>
<td>Diaspis boisduvali and Melanaspis smilacis</td>
</tr>
<tr>
<td>Anagyrus cf. cercides</td>
<td>Sooretama; Serra</td>
<td>Ananas comosus: Cocos nucifera</td>
<td>Dysmicoccus brevipes</td>
</tr>
<tr>
<td>cf. Anagyrus</td>
<td>Cachoeiro do Itapemirim; Jaguaré</td>
<td>Ananas comosus; Coffea canephora</td>
<td>Dysmicoccus brevipes; cf. Ferrisia and Pseudococcus</td>
</tr>
<tr>
<td>cf. Hambletonia</td>
<td>Sooretama; Jaguaré</td>
<td>Ananas comosus; Coffea canephora</td>
<td>Dysmicoccus brevipes; cf. Ferrisia and Pseudococcus</td>
</tr>
<tr>
<td>cf Leptomastix</td>
<td>Jaguaré; Domingos Martins</td>
<td>Coffea canephora; Coffea arabica</td>
<td>cf. Ferrisia and Pseudococcus; cf. Planococcus</td>
</tr>
<tr>
<td>Prochiloneurus sp.</td>
<td>Sooretama</td>
<td>Ananas comosus</td>
<td>Dysmicoccus brevipes</td>
</tr>
<tr>
<td><strong>EULOPHIDAE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diglyphomorpha sp.</td>
<td>Serra</td>
<td>Cocos nucifera</td>
<td>Dysmicoccus brevipes</td>
</tr>
</tbody>
</table>
Cryogenic Strategy For The Establishment Of Pineapple (Ananas Comosus L. Merrill) Germplasm Bank At Bioplantas Centre (Cuba)

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Cryopreservation of pineapple tissue has been based on protocols using the vitrification procedure. However, further research is necessary to identify the different technical factors required to obtain the appropriate cryogenic strategy for routine application to a wide number of genotypes. Moreover, the visualization of structural changes during the development of a cryopreservation procedure for pineapple has not been accomplished until now. For the above reasons in the present research different key technical issues were determined during the establishment of a cryogenic strategy to induce dehydration tolerance to a highly concentrated vitrification solution to improve the survival rates for in vitro grown shoot tips of pineapple after immersion in liquid nitrogen (LN). The best established conditions were: type of shoot tip (consisted in meristematic dome area and 3-4 primordial leaves 2.5 – 3 mm in size); 2 days of preculture in 0.3 mol L\(^{-1}\) sucrose; application of the loading solution (0.4 mol L\(^{-1}\) sucrose + 2 mol L\(^{-1}\) glycerol) for 25 min at 25°C; 7 hours of dehydration at 0°C with plant vitrification solution number three (PVS3: 50% w/v glycerol + 50% w/v sucrose). With these best conditions, histological analysis of the structural changes of cryopreserved pineapple shoot tips revealed that only cells localized in the meristematic area and in young leaf primordia had a few cellular alterations while their morpho-physiological characteristics remained almost intact. Moreover, the vitrification procedure was successfully applied to nine accessions of the in vitro collection at Bioplantas Centre. These results constitute a very important step in the validation of cryopreservation protocols for pineapple germplasm conservation and the real establishment of its cryobank.

Phenotypic Characterization of Field-grown Pineapple Transgenic Plants

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We previously introduced the bar gene, along with chitinase and AP24 genes, into the pineapple genome. The present report focuses on the phenotypic evaluation of the first vegetative generation of transgenic plants. Three plant materials were compared: macropropagated controls (non-transformed), micropropagated controls (non-transformed), and micropropagated transformed plants. From each group, 50% of the plants were sprayed with FINALE® 3 months after initiation of the experiment. The phenotypic characterization was performed after one year of field growth. FINALE® killed all non-transgenic plants. Micropropagated transformed plants sprayed with FINALE®, did not show phenotype differences from micropropagated transformed plants not sprayed with the herbicide. Between the micropropagated transformed plants sprayed with FINALE® and the micropropagated control plants not sprayed, there were two experimental differences: the genetic transformation and the herbicide application. The combined effects of these two factors caused modifications in levels of phenolics (cell wall-linked, free, total) and proteins. Moreover, they changed the fruit mass without crown. Between the micropropagated transformed plants sprayed with FINALE® and the macropropagated control plants not sprayed, there were three experimental differences: the genetic transformation, the herbicide application, and the in vitro culture. They provoked changes in levels of chlorophylls (b, total) and proteins. Furthermore, activities of phenylalanine ammonia – lyase, superoxide dismutase and glutamine synthetase -were modified. The plant height and diameter, and the crown height were also changed by these three experimental differences. Until now we have evaluated transformed pineapple plants during hardening and field growth. Although some unexpected variations were recorded, we believe that they are not relevant enough to justify rejection of transgenesis as an important tool for pineapple genetic improvement.
Proteases Expressed in Response to in Vitro Culture of Pineapple (Ananas comosus (L.) Merr.)

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Biotechnology has become an important tool to produce proteases. Bromeliaceae family plants usually contain high concentration of thiol proteases. Although pineapple plants have been found to produce proteases, most of the biotechnological investigations on this crop have been focused on propagation. Plant tissue culture techniques have provided many solutions to basics questions and practical problems in plant biology. Therefore, considerable attention has been focused on the possibility of applying efficient plant tissue culture methods to physiologically active enzymes isolation. We decided to modify pre-elongation phase during pineapple micropropagation in temporary immersion bioreactors (TIB), looking for an increase of protease excretion. Seven experiments were performed to evaluate the effects of culture duration, levels of gibberellic acid (GA), 6-benzyladenine (BA), different levels of sucrose, inorganic salts, inositol and thiamine. The following indicators were recorded: shoot fresh mass per bioreactor; and protein concentration, proteolytic activity, and specific protease activity in culture media. Specific protease activity was highest at 21 d of culture, with 90g/L of sucrose, 4.2 µmol/L of GA, 100% MS salt strength, 0.1 mg/L of thiamine. Moreover, BA and inositol produced a negative effect. Proteases expression in response to in vitro culture by 2D- electrophoresis was evaluated. We found proteolytic activity in pineapple shoots cultured in vitro. The highest specific protease activity was recorded in shoots cultured in TIB. Multiplication phase in vitro did not cause a remarkable protease production in shoots. Proteome of shoots cultured in different in vitro phase were compared. Molecular mass of some protein spots were between 21 500- 31 000 Da. This parameter was similar to those indicated for cysteine proteases from Bromeliaceae. A protease was detected in TIB culture media. Retention time in RP-HPLC and molecular mass of the major protein detected in TIB culture media showed high similarity to stem bromelain.

News From France

The Domestication of Pineapple: Context and Hypotheses

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Introduction

The present economic importance of pineapple is easily justified by its unique characteristics as a fruit, which ensured its very rapid diffusion and adoption, first at the continental level in all of tropical and subtropical America, and later at the global level in all tropical areas. However, as with other major crops of Amazonian or peri-Amazonian origin (e.g., cassava), how these unique characteristics were developed in pre-Columbian times, i.e., the process of its evolution under cultivation or domestication, has been poorly investigated. The reason for this must be mostly sought in the relatively limited interest that has long prevailed for the domestication of vegetatively propagated crops, particularly fruit crops. There also has been limited interest or limited study of the prehistory of the humid tropics, because there is poor conservation of archaeological macro-remains in the region. On the other hand, the views of archaeologists on crop domestication in relation to the birth of agriculture and the evolution of human societies have evolved considerably in the last decade. The greater acceptance within the scientific community of an Amazonian cradle of plant domestication and agriculture provides a favourable context for a new evaluation of the pineapple case. Within that context, we review here the results of recent research on pineapple genetic resources and the available archaeological data in an attempt to trace the path of development of this impressive fruit.

Crop domestication and its archaeological context

Domestication is a form of co-evolution in which humans and their crops and animals become dependent upon each other. The domestication syndrome is a set of morphological, phenological and physiological traits that are modified by conscious or
The process of crop domestication and the identification of centres of crop origin are of particular interest to plant breeders because of the importance of wild relatives and landraces as genetic resources for the improvement of modern crops and their adaptation to marginal growing conditions. With regard to domestication in the humid tropics, there was the perception of the tropical forest as a hostile habitat for the development of human societies and agriculture. With few exceptions (e.g., Carl Sauer), scientists thought that forest dwellers were relative latecomers to agriculture, first practicing agriculture between 4000 to 5000 years before the present (BP). While the explanatory model for the so-called Neolithic Revolution involving the development of cereal crops was extended to temperate and subtropical Asia, the evolution of civilization and agriculture in the two main rain forest areas, the Amazon and the Congo basins, long remained at best poorly studied. The idea that these reservoirs of cereal crops was extended to temperate and subtropical Asia, the evolution of civilization and agriculture in the two main rain forest areas, the Amazon and the Congo basins, long remained at best poorly studied. The idea that these reservoirs of megadiversity remained unexploited for agricultural development was long held by followers of Vavilov and Harlan (e.g., Hawkes, 1998), prominent among the early scientists proposing theories of crop domestication. Elucidation of crop domestication in the humid tropics was also delayed by the strong focus on major species propagated by seeds, mostly cereals and pulses, and the relative neglect of vegetatively propagated and perennial crop plants. Even in the Fertile Crescent, relatively little attention has been paid to fruits like figs, despite the antiquity and importance of their cultivation and their potential as botanical/genetic markers of human movements in the Near East and the Mediterranean. In fact, horticulture/vegeticulture may even have preceded cereal cultivation (Kislev et al., 2006).

The thinking on plant domestication and the advent of agriculture has changed considerably in recent years, with new scenarios involving the humid tropics and non-cereal plants. There is evidence of ancient major crop cultivation in the tropical lowlands, particularly New Guinea and/or Melanesia (root crops, bananas and sugar cane; see Neumann, 2003) and the Neotropics. The Amazon and its periphery have been recognized as the cradle of ancient complex civilizations (Gibbons, 1990) and domestication of cassava (Olsen and Schaal, 2001), two species of chili pepper (Capsicum baccatum and C. chinense), jack bean (Canavalia plagiostepoma), arrowroot (Maranta arundinacea), cocoyam (Xanthosoma sagittifolia), llere’n (Calathea allouia) (Piperno and Pearsall, 1998, cited in Olsen and Schaal, 2001 and Pickersgill 2007), pineapple (Leal and Coppens d'Eeckenbrugge, 1996), cocoa (Motamayor et al. 2002), and peach palm (Bactris gasipaes; Clement, 1995). Archaeological evidence, including 7000 years old pottery (Roosevelt et al., 1991), vast extensions of fertile anthropogenic soils (Amazonian dark earths; Woods et al. 2009) and topographical modifications (Heckenberger et al., 2003), indicates the very early development of agriculture in this region, leading to the establishment of significant populations (estimated between 2 and 5 million at the time of European contact; Denevan, 1992; Hornborg, 2005), and significant forest transformation through enrichment or degradation by slash and burn agriculture (Piperno, 2006; but see also Bush et al. 2007). The Amerindian occupants of Amazonia made their living by hunting, fishing and the cultivation or management of more than 138 plant species (Clement, 1999), among them a very large number of fruit trees (Miller and Nair, 2006). Combining mobile and sedentary strategies (see Rival, 2006), they managed the forest in such a way as to encourage the concentration of useful species, in processes accompanied by selection of superior genotypes of fruit tree species (Gnecco, 2003). Crop domestication appears to be quite as old as these concentration/improvement processes, and long distance exchanges ensured an early diffusion of many crops between Amazonia, the Andes and the Pacific Coast, as well as between South America and Mesoamerica (Stone, 1984). Indeed, cassava was present in northern Colombia by 7500 BP, in Panama by 7000 BP (Zeder et al., 2006), which compares well with the presence of maize, of Mesoamerican origin, in South America by 7500-7800 BP, according to data from Colombia and southwest Ecuador (Pohl et al., 2007; Dickau et al., 2007). Thus, cassava was probably domesticated roughly at the same time as maize, i.e. in an 8-10,000 BP interval (9000 BP according to genetic data of Matsuoka et al., 2002), after a long phase of wild type cultivation. This timeframe is also consistent with data for squash, which seemed to precede maize, being domesticated around 10,000 BP in Mexico (Cucurbita pepo; Smith, 2001), Ecuador and Peru (C. moschata; Piperno and Stothers, 2003; Dillehay et al., 2007).

**Pineapple domesticates and their wild relatives**

In seed-propagated crop species, the reproductive modifications induced by domestication often result in partial or complete reproductive isolation, i.e. the domesticate becomes a new species. These modifications can be so great that the identification of the crop wild relatives is problematic. Even without a sexual barrier, maize looked so distinct from its teosinte genitor, which may grow as a weed in the same fields, that the two forms of *Zea mays* were once classified in different genera.

No such difficulty appears in the case of the pineapple, and domestication has not produced any clear, qualitative, morphological or physiological differentiation, or reproductive isolation. In fact, the genus *Ananas* only includes two species, the pineapple, *A. comosus* (L.) Merrill, and the *gravata or yvira, A. macrodontes* Morren (Coppens d'Eeckenbrugge and Leal, 2003). The former is a normally diploid species (2n = 50) that includes five botanical varieties, three of which are domesticates. Its natural distribution includes all tropical South America east of the Andes. The latter is a self-fertile tetraploid (2n = 4x = 100), whose inflorescence lacks a crown and vegetative reproduction is ensured by stolons. It grows wild in forests of southern South America. Although exploited by natives for the production of fibres (Corrêa, 1952), it shows no sign of domestication. The two species exhibit limited differentiation in molecular genetic studies (Duval et al., 2001 and 2003), however their ploidy difference constitutes a clear reproductive barrier.
Cultivated pineapples

The three cultivated Ananas botanical varieties are *A. comosus* var. *comosus*, the pantropical pineapple cultivated for its spectacular and exquisite large fruit, *A. comosus* var. *erectifolius*, a small-fruited pineapple cultivated for its fibre, and *A. comosus* var. *bracteatus*, a robust pineapple with multiple uses, involving its medium-sized fruit for juice and its armed leaves for fences. The two latter varieties are now increasingly cultivated as ornamentals.

In *A. comosus* var. *comosus*, the syncarp grows very significantly after anthesis, so the fruit is generally very large and fleshy (up to several kilograms in certain cultivars; Figure 1), with many fruitlets ("eyes"); they are borne on a wide and strong, relatively short, peduncle. Seeds are rare in the fruits, because of reduced fertility, conjugated with stronger self-incompatibility and monoclonal cultivation (Coppens d’Eeckenbrugge et al., 2003). Vegetative reproduction, through shoots, in the vernacular of pineapple, slips, suckers and crowns, is often initiated after floral induction; under tropical conditions, these propagules tend to resume growth after fruit maturity. The plant has numerous wide leaves (40-80), with antrorse spines; these marginal spines are generally smaller and denser than in wild varieties and can be partially or completely suppressed by dominant mutations.

At the time of the Conquest, *A. comosus* var. *comosus* was planted throughout tropical America, and included cultivars having wide variation in fruit size, shape, colour, and flavours. Considerable morphological and genetic diversity was found in the western Amazon and the eastern Guianas (Duval et al., 1997, 2000, 2003). Variation in adaptation to different environments, including Andean hillsides was also evident. The fruit was widely consumed, and appreciated in the form of fermented drinks. Other traditional uses were based on its properties as a digestive, vermifuge, antiamoebic, abortifacient and emmenagogue, most of which are related to the presence of a proteolytic enzyme complex in pineapples as well as in many other bromeliads (Leal and Coppens d’Eeckenbrugge, 1996; Patiño, 2002).

Plants of *A. comosus* var. *erectifolius* are much less massive, with abundant and early shoots, frequent crownslets at the base of the main crown, numerous erect, fibrous leaves and a small, very fibrous, inedible fruit borne on a long and slender peduncle (Figure 2). In some clones, the fruit appears to be rare. *A. comosus* var. *erectifolius* is quite similar to the wild *A. comosus* var. *ananassoides* except for its smooth leaves, a trait which is under monogenic control (Collins, 1960). *A. comosus* var. *erectifolius* is not known to occur in the wild. It was cultivated in the West Indies at the time of Conquest, and it is still cultivated by the natives in the Guianas, including the Orinoco basin, and in the north of the Amazon basin, for the strong and long fibres associated with its typical erect habit. Indeed, the dry fibres constitute 6% of the plant weight. They are used to make hammocks and fishing nets (Leal and Amaya 1991), but now suffer competition from synthetic fibres and nylon. Vernacular names include *curagua*, *curauá*, *curána*, *kulaíwat*, and *pitte*. The typical absence of spines along the leaf margin, as well as its erect habit, is the likely result of artificial selection for high yield of easily extractable fibres among strains of *A. comosus* var. *ananassoides*. Variety *erectifolius* has recently found a new economic use in the production of cut flowers.

*Ananas comosus* var. *bracteatus* is an assemblage of two cultivated forms that show the same geographic distribution as *A. macrodontes*, and that are morphologically and genetically intermediate between *A. comosus* and *A. macrodontes* (Figure 3). The most common one corresponds to *A. bracteatus sensu* Smith & Downs, which was cultivated as a living hedge and harvested for fibre and fruit juice, or for traditional medicine, in southern Brazil and Paraguay (Bertoni 1919). Indeed, its dense, long and wide leaves are strongly armed by large antrorse spines, forming impenetrable barriers. It is very robust and still thrives in abandoned plantations, but it seems unable to colonize new habitats. The syncarp is of intermediate size (0.5 to 1.0 kg), borne by a strong peduncle, and covered by long and imbricate floral bracts, as in *A. macrodontes*. These bracts are bright pink to red at anthesis, to within-cultivar variations (Duval et al. 2001, 2003) and suggesting a very narrow origin, possibly a single genotype. The second producing a spectacular inflorescence. Morphological and genetic variations are very limited in this first form, being comparable form, corresponding to *A. fritzscheulerti* Camargo, shares an additional trait with *A. macrodontes*, as it exhibits retrorse spines on...
Figure 2A. *A. comosus* var. *erectifolius* under cultivation for fibre in the Amazon (Rio Negro basin; photograph of M.F. Duval), and Figure 2B, as an ornamental, for export, in Côte d'Ivoire (photograph of G. Coppens).

Figure 3. *A. comosus* var. *bracteatus*, a remnant from an old fence in a southern Brazil farm (machete handle provides scale; photograph of M.F. Duval), and a variegated mutant used as a garden ornamental in Martinique, FWI (photograph of G. Coppens).
the leaf base. According to Camargo (1943) and Smith and Downs (1979), it was also used in living fences. It is a very rare form, whose diversity has not been documented, only one clone being conserved in Brazil, by EMBRAPA and the botanical garden of Rio de Janeiro. The chromosome number is 2n=2x=50 (Camargo, 1943).

Wild relatives of cultivated pineapples

Wild pineapple relatives include the varieties ananassoides and paraguazensis of A. comosus and A. macrodontes.

Within A. comosus, wild botanical varieties display the highest genetic diversity, which is a common situation in crop gene pools. The most common and diverse wild variety, A. comosus var. ananassoides, is also the likely ancestor of the cultivated botanical varieties. It is generally found in savannahs or clear open forests, growing on soils with limited water-holding capacity (sand dunes or "campinas", rocks, common on and around the Guiana shield) and forming populations of variable densities. In the Guianas, it can also be found, although rarely, thriving in dense rain forest. In contrast, it is absent from the seasonally flooded lands along the Amazon and its main southern tributaries, which seem to act as a barrier dividing its distribution in two main areas; a northern one corresponding to the Guiana shield, Orinoco basin, and northern drainage of Rio Negro (i.e., from the Brazilian

Figure 4. Distribution of A. macrodontes (S) and A. comosus varieties ananassoides (A), paraguazensis (P), erectifolius (L), and bracteatus (B). Red roughly outlines the region of greatest morphological and genetic diversity within A. comosus (including var. comosus and types intermediate between var. comosus and ananassoides). Partial spininess is relatively frequent in this area. Green outlines an area with great diversity of large-fruited clones (typical of var. comosus), where the "piping" leaf trait is relatively frequent.
state of Amapá to eastern Colombia), and a southern one roughly corresponding to the Brazilian shield and north-eastern Brazil (from the Brazilian states of Acre, Mato Grosso over to Pernambuco and down to Paraguay and northern Argentina) (Figure 4).

*A. comosus* var. *ananassoides* has long and narrow leaves, up to 2 m long and less than 4 cm wide, subdensely serrate with wholly antrorse spines. The fruit peduncle is elongate (most often more than 40 cm) and slender (usually less than 15 mm wide). In the southern part of its distribution, the inflorescence is very generally small, globose to cylindrical, and it shows little growth after anthesis, so it has little flesh. The pulp is white or cream, very firm and fibrous, with high sugar content and acidity, and numerous seeds. And its habitat appears mostly restricted to areas providing an open and markedly dry habitat (grass savannahs and low open forests; Figure 5). In contrast, in the northern area, the habitats of *A. comosus* var. *ananassoides* appear more variable (Leal and Medina, 1995), and a higher morphological diversity is observed, with clones producing larger, fleshy fruits (up to 12-15 cm long) as the syncarp shows significant growth after anthesis. Their fruits were consumed in the Orinoco (Patiño, 2002) and are still occasionally consumed in the Guianas. Similar types, morphologically intermediate between the wild and cultivated forms, are sometimes found in patches in secondary forest and savannahs in French Guiana, indicating an ancient settlement, or cultivated in gardens (Figure 6). They constitute the most plausible basis for initial domestication in the Guianas. Indeed, in the study of Duval et al. (2003), these intermediate phenotypes display four haplotypes, sharing three of them with *A. comosus* var. *comosus* and all four with *A. comosus* var. *ananassoides*, which is consistent with the hypotheses of semi-domestication or introgression between the two botanical varieties.

The contribution of *A. comosus* var. *parguazensis* to the evolution of the cultivated pineapple is less likely, on geographic, morphological, and genetic grounds. Its geographical distribution appears more centred, mostly corresponding to the basins of the Orinoco and upper Rio Negro, the area of its greatest diversity, with a few observations in eastern Colombia and in north-eastern Amazon (Coppens d'Eeckenbrugge et al. 1997; Duval et al. 2001, 2003). It grows in lowland forests, under canopies of variable densities, from clearings or riverbanks to dense forest. As compared to specimens of *A. comosus* var. *ananassoides* growing in close proximity, it seems restricted to shadier environments, because of lower water use efficiency (Leal and Medina 1995). Morphologically, it differs from variety *ananassoides* by having wider leaves, slightly constricted at their base, and larger spines, some of them retrorse (Figure 7). A few Orinoco/Rio Negro phenotypes appear to be intermediate between varieties *parguazensis* and *ananassoides*, indicating some natural hybridization. However, retrorse spines and the basal leaf constriction have not been observed in the cultivated pineapples. Instead *A. comosus* var. *parguazensis* appears genetically distinct, forming a particular branch in the nuclear DNA as well as in the chloroplast DNA phylogenetic trees, with relatively few exceptions for the latter. Duval et al. (2003) explain these exceptions as the result of hybridization with var. *ananassoides* in the Orinoco/Rio Negro region and by a different genetic background for the rare specimens of eastern Guiana (which implies morphological convergence.
between wild types of distinct origins). Four of the seven *parguazensis* chloroplast genotypes, including the most common ones, are not shared with other botanical varieties (Duval et al. 2003). In conclusion, a contribution of *A. comosus* var. *parguazensis* to the genomes of the cultivated pineapples cannot be ruled out, but it would be marginal, and necessarily indirect, through occasional hybridization with *A. comosus* var. *ananassoides* as the wild ancestor of *A. comosus* var. *comosus* and *A. comosus* var. *erectifolius*.

The tetraploid *A. macrodontes* (Figure 8) only shows clear genetic affinity with *A. comosus* var. *bracteatus*, as they share rare isozymes (García 1988) as well as nuclear DNA markers, and chloroplast DNA markers in the case of the former *A. fritzmuelleri* Camargo (Duval et al., 2001, 2003). They also share most of their original geographic distribution in southern South America, and several morphological traits such as wide leaves, strong spines, the presence of retrorse spines, fruit peduncles of intermediate
length and width, and bearing medium-size fruits with floral bracts longer than the individual flowers. On the other hand, *A. macrodontes* is differentiated by the lack of a crown at the top of the syncarpic fruit and by vegetative reproduction by stolons. The fruit flesh is low in acid and it contains numerous seeds. *A. macrodontes* appears to be highly self-fertile. The natural habitat of *A. macrodontes* corresponds to humid forest areas, under semi-dense shade, from south-eastern Paraguay and north-eastern Argentina up to Mato Grosso and coastal Brazil (Coppens d'Eeckenbrugge et al. 1997).

**Domestication syndrome in the cultivated varieties of *Ananas comosus***

The clearest modifications due to domestication in *A. comosus var. comosus* (Leal and Coppens d'Eeckenbrugge, 1996) are:

- Reduced susceptibility to natural flowering induction.
- A larger number of wider, and generally shorter, leaves.
- A wider and longer stem allowing a larger/greater starch storage capacity.
- A significant increase in the number of flowers, correlated with a modification in their phyllotaxy.
- The enlargement of individual fruits (pineapple "eyes").
- A reduction in fruit fibrousness.
- Reduced seed production through the combination of lower sexual fertility and stronger self-incompatibility.

In the cultivars where the reduction of female fertility, i.e. the proportion of ovules producing a seed, is not very severe, it can be counterbalanced by the higher number of flowers. In any case, as vegetative reproduction is largely dominant in *Ananas*, this reduced sexual potential affects plant survival less than the changes in the vegetative organs and the plant vegetative cycle. Strictly speaking, the domestication syndrome in *A. comosus var. comosus* lies in its lack of adaptation to the natural conditions prevailing where the wild varieties are found. Pineapple plants from most cultivars can survive when their cultivation is abandoned, resisting competition in sufficiently open vegetation and even in dry edaphic or climatic conditions. However, they do not propagate efficiently to form subspontaneous feral populations. This may be due to the cost of an excessive harvest index (i.e., the production of a relatively large fruit), limiting the capacity for vegetative propagation, and/or the loss of dispersal capacity, as only man can transport large fruits and their crown over medium to long distances (assuming that no animal has an interest/capacity for the dispersal of other vegetative propagules). Indeed, wild pineapple populations are distributed discontinuously in the Guianese forests. They are most often found on relatively elevated areas (inselbergs, "rocky savannahs") where there is no risk of water
stagnation. Such sites are often isolated in the forest, which implies long distance vectors for seed dispersal, very probably large birds and/or monkeys. Indeed, sexual propagation might play an important role in the initial foundation of wild populations, as only one or two clones were observed at a given site (Coppens d’Eeckenbrugge, unpublished), while clones appeared distinct among sites.

The situation appears to be similar for the cultivated botanical varieties *A. comosus* var *bracteatus* and *A. comosus* var *erectifolius*, which do not show any capacity for spontaneous colonization in the wild. As in var. *comosus*, a large fruit size could be limiting in var. *bracteatus*. For the small-fruited var. *erectifolius*, the loss of leaf spines probably increases its susceptibility to herbivory, although leaf fibrousness might compensate. In addition, for those clones of var. *erectifolius* that rarely produce a fruit, there is an additional restriction on sexual recombination, and thence for the plant adaptive potential, strengthening the dependence on man. On the other hand, we must keep in mind that the main qualitative characteristic that distinguishes var. *erectifolius* from var. *ananassoides* is the presence or absence of spines. Thus, when smooth-leaved clones of var. *erectifolius* mutate back to the spiny condition, as has been observed in germplasm collections as well as under cultivation, these reverse mutants should be formally classified in var. *ananassoides*. In this case, the domesticate status and the domestication syndrome may look as fragile as the morphological difference with its wild ancestor.

**How, where and when pineapple domestication may have proceeded?**

Bertoni (1919) proposed that the pineapple was domesticated in southern South America by the Tupi-Guarani Indians who would have diffused the crop in their northward migrations. Later, most reviewers of pineapple domestication (Collins, 1960; Purseglove, 1972; Pickersgill, 1976; Sauer, 1993) accepted a southern origin. Only Brücher (1971), whose paper was written in German and subsequently ignored, underlined the presence of wild forms and primitive cultivars in the north of South America and proposed a Guianese origin. In any case, both hypotheses were based on very limited knowledge of pineapple diversity and distribution. Leal and Antoni (1981) called attention to the greater morphological diversity to be found north of the Amazon. Extensive expeditions in Venezuela, Brazil, and French Guiana (Leal et al., 1986; Ferreira et al., 1992; Duval et al., 1997), improved progressively our knowledge of the wild and cultivated forms of the plant, collected them for the morphological, biochemical, and molecular studies reviewed in the present paper, and gave substance to the hypothesis of a northern origin of varieties *comosus* and *erectifolius* of *A. comosus* (Leal and Coppens d’Eeckenbrugge, 1996; Coppens d’Eeckenbrugge et al., 1997; Coppens d’Eeckenbrugge and Leal, 2003; Duval et al., 2003).

The three pineapple domesticates have different stories of domestication, in relation to their different utilizations by man and regions of origin, so we shall consider them successively.

**Ananas comosus** var. **comosus**

The combination of morphological, biochemical and genetic data (Duval et al., 1997, 2001, 2003) clearly point to an East-Guianese origin of *A. comosus* var. *comosus*. Indeed, this area is home to its wild ancestor, *A. comosus* var. *ananassoides*. The greatest phenotypic and genetic diversity, including primitive cultivars and intermediate wild phenotypes that could be used as a basis for domestication, or that could enrich the primitive cultivated gene pool through introgression, can be found in this region. A very plausible hypothesis is that such materials were collected on "rock savannahs", sand dunes, and similar places where they thrive, and planted in home gardens and fields. Nowadays, inhabitants of the Guianese forests, and even creolized newcomers, still collect materials from the wild to incorporate them in their cultivated plots and gardens. This explains why some clones are found both under cultivation and in the wild, in patches of secondary vegetation, marking likely sites of ancient cultivation. Such practices constitute a basis for a process involving "domestication cycles". In these cycles, pineapples are sampled in the wild, put in cultivation, semi-abandoned, re-sampled for cultivation, etc., with possible selection at each step. Indeed, fields and home gardens are never completely abandoned and forgotten, as they are located near pathways and remain useful, for example for picking tree fruits that come well after first crops or hunting animals attracted by the fruits (Vélez, 1998). They also serve as stocks of useful planting materials. The most interesting genotypes are thus progressively concentrated, in a process that has been described for Amazonian fruits (Gnecco, 2003; Miller and Nair, 2006). In the long term, sexual reproduction can contribute to the exploitable diversity by the creation of new clones, some of which can be more attractive for man. On the other hand, wild types may be more highly fertile than semi-domesticated materials (Coppens d’Eeckenbrugge et al., 1993), so wild genes are probably transmitted more efficiently by sexual reproduction, reducing the effects of selection. Their robustness may also be an advantage for a safer harvest, so growers may want to maintain them among the diversity of their clones in a context of subsistence production. The result is the multiclonal production system still observed in the Guianas, which maintains an equilibrium between genotypes at very different stages of domestication. This is not a problem for a grower more interested in diversity and safety than in productivity but slows down further genetic improvement and full domestication.

The relatively slow pace of domestication in other species has also been attributed to the coexistence of genotypes at different stages of domestication (e.g., Otero-Arnaiz et al., 2005). Wild relatives of domesticates can even behave as weeds in the crop (Papa and Gepts, 2004), as is the case of teosinte in Mexican maize plots (Wilkes, 1972) or wild sorghum (Dogget and Majisu, 1968) in African fields, contaminating seed materials through pollen-mediated geneflow, and diversifying the cultivated genepool while braking the evolution to more extreme forms of the crop. Such limitations to genetic improvement obviously disappear when
the crop is grown in the absence of its wild relatives (Galinat, 1974). In the case of pineapple, this probably occurred in the western Amazon (along the upper Amazon, close to the triple frontier between Colombia, Peru and Brazil, and along the lower Rio Negro), where we can observe a high diversity of cultivars and the absence of wild types (Bello and Julca, 1993; Duval et al., 1997). There the prevalence of large seasonally-flooded areas and the rarity of elevated sites with good drainage, such as rock savannahs and sand dunes, seem to constitute a natural barrier against the extension of the Guianese types of *A. comosus* var. *ananassoides*. Once established in this area, the cultivated pineapple could evolve and diversify in completely artificial conditions, in a dynamic process combining sexual recombination restricted to domesticated germplasm, somatic mutations and clonal selection. Human societies peopling western Amazonia may have reinforced the domestication process through particular horticultural skills. Indeed, this area is also an important centre of domestication and diversification for many other fruits (Clement, 1989, 1999). Peoples like the Tikunas and the Huitotos still value and maintain a wide diversity of pineapple cultivars and other fruits. In the course of our collecting trips (Duval et al., 1997), we observed as many as twelve cultivars in a small plot maintained by a single Tikuna family. Schultes (1991) gives similar numbers for the pineapple cultivars named by the Huitotos. The species is culturally very important for peoples of the area. For example, the Yukunas celebrate nine fruit festivals yearly, five of them being pineapple festivals (Jacopin, 1988). Cristancho (2001; cited in Cristancho and Vining, 2004) ranked pineapple among the three primary Culturally Defined Keystone Species of the Letuama people (C.K.S are "species whose existence and symbolic value are essential to the stability of a cultural group over time"), along with tobacco and coca.

The existence of two centres for the diversification of *A. comosus* var. *comosus*, a primary one in the Guianas, with diversification involving clones at different stages of domestication, and a secondary one in the western Amazon/Andean foothills, with recombination between large-fruited cultivars, is also suggested by the distribution of particular leaf margin types. Thus, genotypes that present a partial suppression of spines are particularly frequent in the Guianas. The most famous such cultivar is 'Smooth Cayenne' itself, the most widely distributed pineapple cultivar, which most commonly presents a few spines at the leaf tip. This trait is under the control of the S gene, and the allele for the partial suppression of spines is dominant. In the western Amazon and in the Andes (from Peru to Colombia), leaf smoothness is determined by another gene, named P by Collins (1960). The dominant allele determines the "piping" trait, where the lower epidermis is folded over the leaf edge, and all spines, except for the terminal one, are suppressed. The existence of homozygotes for the "piping" gene (Cabral et al., 1997) indicates sexual recombination among cultivars within this western pool.

Because of very poor conservation conditions in the rain forest and the lack of archaeobotanical research in the Amazon, no ancient pineapple remains have been found in the two centres of diversification/domestication. Pineapple remains have only been conserved under arid conditions, and identified in archaeological layers dated from 3,200 to 2,800 BP on the Peruvian Coast (Pearsall 1992), while seeds and bracts were found in coprolites from the Tehuacan Valley caves dated between 2,200 and 1,300 BP (Callen, 1967). The application of historical linguistics to the names of the pineapple in Ancient Mesoamerica (glottochronology) also gives us a minimal estimation of the antiquity of pineapple domestication. Consistently, glottochronological data indicate that the crop was highly significant to Mesoamerican people more than 2,500 years BP (Brown, 2010). Thus, domesticated pineapple was traded and adopted as an important fruit crop at the continental scale more than 3,000 years BP. Such an early extension of its cultivation area implies the preliminary development of cultivars specifically adapted to the high latitudes and/or altitudes of Peru and Mexico. Indeed, highland cultivars from Andean countries show specific adaptations and tend to perform poorly in lowland conditions, presenting lower sugar, acidity and firmness, as well as frequent fruit lodging and deformation. Given the rarity of reproduction through seeds in *A. comosus* var. *comosus*, the development of environmentally specialized cultivars was necessarily a long and slow process, taking place in situ, after the arrival of the domesticated pineapple in these particular environments. In conclusion, a likely time frame for the divergence between wild and cultivated pineapple lies between 6,000 and 10,000 years BP, which is consistent with current hypotheses for other major American crops.

*A. comosus* var. *erectifolius*

*A. comosus* var *erectifolius* is morphologically very similar to the variety *ananassoides*, except for the smooth character of the leaf. Its very high genetic diversity, its scattering in the phenetic and phylogenetic trees and its proximity to various *ananassoides* genotypes, generally from the same origins, suggests that it may have been domesticated several times. According to its distribution in northern Amazonia and the Antilles, these convergent domestication events quite certainly took place in the Guiana shield. If we take the reduction in sexual reproduction capacity as an indicator of the antiquity of evolution under domestication, the highly variable fertility in var. *erectifolius* appears consistent with multiple domestication and introgression events, as some clones have partially lost their flowering capacity, whereas others show an abundant production of viable pollen and ovules (Coppenes d'Eeckenbrugge et al., 1997; Duval et al., 2003).

*A. comosus* var. *bracteatus*

The case of this botanical variety is much simpler, as it very probably corresponds to two particular clones from southern South America. The most common one (*A. bracteatus sensu* Smith & Downs) shares its chloroplast genome and most of its nuclear genome with *A. comosus* var. *ananassoides*, and the remaining part of its nuclear genome with *A. macrodontes*, which indicates a hybrid origin. The second one presents an even closer affinity with *A. macrodontes*, as, in the study of Duval et al.
Beyond the pineapple case

The most obvious interest of the authors and readers of this paper lies in the pineapple, its development as a crop and the historical and genetic processes that shaped it. However, notwithstanding this sharp interest in the crop, we cannot isolate the subject from wider contexts. As a fruit crop, pineapple occupies a very special position, because it is one of the most prominent tropical fruits and the first Amazonian fruit of local and global importance. And it has been so for millennia. Among the fruits of comparable importance, we can mention avocado (Persea americana Mill.) and papaya (Carica papaya L.), both of Mesoamerican origin.

The comparison can be extended to their history. All three species are ancient domesticates. Archaeobotanical remains attest to the antiquity of avocado domestication in Mesoamerica, beginning around 10,000 BP (Galindo-Tovar et al., 2008). For papaya, we must envisage that management by man is at least as ancient as early lowland agriculture in Mesoamerica, i.e., again somewhere around 10,000 BP (Piperno, 2006). Indeed, papaya is a very early colonizer of any open space in the forest, and it has very particular and obvious chemical properties, so early farmers or proto-farmers clearing land for cultivation must have been very familiar with the plant and the fruit. The three species also provide striking examples of the early diffusion of the most interesting domesticates across all of tropical America, similar to utilitarian crops, such as cotton and bottle gourd, and the staples, such as maize and a few important root crops. At the time of Conquest, papaya was present in all of tropical America (Patiño, 2002), where it was introduced in the form of cultivars, as can be easily deduced from the observation of feral papaya populations in South America, whose fruits differ markedly from the truly wild papayas of Central America. Archaeobotanical remains in Peru, dated 2800-3200 BP (Pearsall, 1992), confirm the antiquity of its presence in South America. Avocado cultivars may have diffused to South America as early as 10,000BP, according to seeds found in Colombian archaeological sites (Gnecco, 2003). Finally, the remarkable diffusion of the three fruits resumed immediately after the contact, as they were successfully transferred to most tropical regions of the world in less than two centuries. In particular, the pineapple had become a pantropical fruit crop before the end of the 16th century (Coppens d’Eeckenbrugge et al., 1997). This underlines the clear relation between the qualities of these plants and their universal acceptance.

Other fruit crops have had more limited prehistoric diffusion, either for ecological reasons, as the peach palm (Bactris gasipaes Kunth) that remained confined to the humid lowlands, for more limited attractiveness, or because they were domesticated much later or incipiently. However, all of them merit consideration for the study of plant domestication in Amazonia. Indeed, American agriculture is very obviously characterized by its great diversity of species, and the high proportion of fruit species, whose importance in the Amazonian diet traces back as far as the late Pleistocene, more than 10,000 BP, i.e., the same age as the many fruit remains of the Pedra Pintada archaeological site (Roosevelt et al., 1997). The review of Miller and Nair (2006) shows the link between this very particular blend of agriculture and agroforestry, based on a great variety of cultivated plants, including fruit trees, and an autochthonous cultural development, allowing the establishment of large populations with complex societies, and trade networks so highly efficient that they could reach the Pacific coast beyond the Andes, and even Mesoamerica.

As stated by Zeder (2006), the difference between domestication and other mutualisms essentially lies in the cultural component and intentionality. If we want to understand how agriculture developed and plant domestication proceeded in the Americas, and more particularly in Amazonia, we have to keep in mind the close interaction between biodiversity and traditional knowledge that are integrated and indivisible. As underlined by Vélez (1998), while the viewpoint of "western culture (and science) "is a compartmentalized vision in which a given element can be analysed and defined without considering its relationship to the whole," indigenous cultures view the same elements "as an integrated whole, considered part of a functional and compact universe in which the parts are not conceived of separately."

Accordingly, an exaggerated focus on grain and starch plant domestication cannot lead us to a correct understanding of the development of Amazonian production systems, and it is time to reconsider its major fruit component as much more than "the cherry on the cake." This means reorienting research efforts, with deeper studies of present-day agrosystems and the distribution of genetic diversity, and more attention to microbotanical remains of a wider diversity of species in archaeological sites. The potential benefits of such studies are well worth the efforts, in terms of genetic resources and agricultural/agroforestry development, as well as archaeological progress. A wider diversity of plants can tell us much more about the people that relied on them, as exemplified by the study of Brown (2010), who first extracted linguistic information on a group of Mesoamerican plant species and then reverted the process, characterizing the ecology of ancient peoples' homelands from the requirements of the array of species they exploited. In this study, less common species allowed a greater resolution in time and space, than more widespread ones, such as maize and beans.
Acknowledgements
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NEWS FROM THE PHILIPPINES

Philippines President Witnesses Signing of $500-M Contract Between the Government and Libby’s Fruits

On Saturday, June 28, 2008, NTC Marketing, a Williamsville USA-based company that imports tropical fruit sold under the Libby’s name, signed a deal to buy pineapples from the Philippines. Trade Secretary and National Development (NDC) Chair Peter Favila signed in behalf of the Philippine Government and NTC Marketing Chairman Michael DeRose signed for Libby’s Fruits. The signing was witnessed by President Gloria Macapagal Arroyo, who was visiting the United States and promoting the Philippines as a place for U.S. businesses to invest.

NTC Marketing imports millions of cans of fruit each year. It is the North American licensee of Libby’s, the brand under which the products are sold. According to DeRose, NTC’s chairman, a processing plant is being built in the Philippines and
should be finished by the end of the year. It was reported that the company will have an ownership stake in the new plant and pineapple fields to supply the cannery have already been planted. Reportedly, the deal will benefit about 5,000 farming families in the Philippines. NTC also believed the company would benefit, expecting the price would be less than fruit from Thailand, although the company expected to continue to import fruit from Thailand as well.

Ed. Note: The above information was obtained from two different web pages, one of which is no longer available.

**News from Sri Lanka**

**Antagonistic effect of Trichoderma harzianum on Thielaviopsis paradoxa - the Pineapple Black Rot Pathogen**

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**Abstract**

A local isolate of *Trichoderma harzianum* was tested against *Thielaviopsis paradoxa* (teleomorph = *Ceratocystis paradoxa*) isolated from a diseased pineapple and established as an antagonist of the pathogen. Assays for antibiosis were conducted using culture filtrates of the antagonist grown in waste residue from beer manufacture (BR) as the highest number of colony forming units (cfu) of $2.8 \times 10^6$/ml, and the highest biomass of 7.425g/250ml of BR were observed in this medium 144 hours after inoculation. While the culture filtrates had no inhibitory effect, the unfiltered broth of *T. harzianum* P3 showed total inhibition. The fungicidal effect of the antagonist was confirmed by the presence of coiling structures around the pathogen. Growth of *T. harzianum* P3 at 28 °C on solid media was slow - $10^2$ cfu 2 weeks after inoculation and $1.4 \times 10^3$ cfu at 6 weeks in white rice medium. Finger millet, red rice and rice husk media were also tested. The effect of *T. harzianum* P3 treatment to pathogen-inoculated soil was tested. Boiling tubes sterilized with 20 g soil were inoculated with the pathogen and incubated at 28 °C for 7 days. Inoculated soils were checked for presence of $10^4$, $10^5$ and $10^6$ cfu/mL of the pathogen prior to addition of the test formulation of *T. harzianum* P3. Inoculated soils were checked weekly for surviving cfu/mL of the pathogen over 10 weeks. The pathogen reached concentrations below disease causing levels ($10^6$ cfu/mL) at 6 - 10 weeks. This study indicates the possible use of *T. harzianum* P3 as a bio-control agent to control black rot disease of pineapple and BR as a potential medium for mass propagation of *T. harzianum* P3 in Sri Lanka. Further studies on the efficacy and shelf-life of the formulation are required.

**Introduction**

The black rot pathogen *Thielaviopsis paradoxa* (teleomorph = *Ceratocystis paradoxa*) is present in all pineapple growing countries. The pathogen is capable of causing the great economic loss due to rot of pineapples held in storage. In Sri Lanka the disease is problematic when fruits are harvested in rainy weather and stored prior to shipment. The disease is controlled by low temperature storage and chemical treatments (Reys et al., 2004). However alternate means of control are needed due to consumer resistance to chemicals. *Trichoderma harzianum* has been observed to be antagonistic to several plant pathogens (Perello et al., 2003; Singh et al., 2007). A local isolate of this organism *T. harzianum* P3 (TP3) was tested for antagonism against *T. paradoxa*. The fungicidal effect of the antagonist was confirmed by the presence of coiling structures around the pathogen. Selected liquid and solid media were tested for determining presence of antibiosis and developing a formulation for conducting in vitro soil assays to observe the efficacy of TP3 in controlling black rot. Soils inoculated with *T. paradoxa* were examined over 10 weeks for survival (cfu/mL) of the pathogen.

**Materials and methods**

**Isolation of *T. harzianum*** - An isolate from a pineapple plantation in Sri Lanka was purified on PDA via single spore method and given the culture number *T. harzianum* P3 (THP3). Seven day old cultures of THP3 on PDA were used to prepare the spore suspensions. Sterile water (10ml) was added to plates, the culture surface agitated, and the resulting suspension filtered through sterile cotton wool. The spore concentration of the suspension was adjusted to $1 \times 10^7$ spores mL$^{-1}$.

**T. paradoxa** - The pathogen, was isolated form the stem end of a naturally infected pineapple. Pure cultures were maintained on PDA at 28 °C, and pathogenicity confirmed via Koch's postulates (Reyes et al. 2004). Spore suspensions of the pathogen were prepared as described above.
Bio assay 1 - Four sterile PDA plates were used for the assay. Four wells of diameter 4 mm were cut equidistant from each other. Each well was filled with 75 µl of the 10^6 spore suspension of TP3. A mycelial disc of *T. paradoxa* obtained from a 7 day culture was placed in the centre of each plate prior to incubation at 28 °C for 8 days.

Bio assay 2 - The pathogen was grown in liquid medium to investigate possible antibiosis. Flasks containing 100 mL of the waste residue from beer manufacture (BR) medium, acidified with tartaric acid, were sterilized and inoculated with 1mL of 1x10^7 TP3 spore suspension for incubation at 28 °C in a 120 rpm rotary shaker for 7 days. Wells were cut on PDA plates as described previously. Wells of five plates were filled with 75 µl of culture filtrate (0.45 µm sterile membrane filter, Sartorius AG, 3400 Göttingen, SM 16510), while wells of a second set of five plates were filled with 75 µl of sterilized un-inoculated BR broth. Wells of a third set of five plates were filled with 75µl of an unfiltered 7 day culture broth. All plates were centrally inoculated with a 4 mm mycelial disc taken from the periphery of a 7 day culture of *T. paradoxa* and incubated at 28 °C for 7 days for daily observation. Slide cultures were prepared using the method described by Sivakumar et al., 2000.

TP3 growth in Liquid and Solid media - Potato Dextrose Broth, (PDB) and Czapekx Dox medium were prepared as in the Difco manual (257-258, 691-692). Brewery waste (BR) was prepared by dissolving 20 g of BR (Lion Breweries, Biyagama, Sri Lanka) and 20 g of glucose in 1000 mL of water. Autoclaved tartaric acid (1.6 mL) was added to sterilized media (250ml) to prevent bacterial growth. Each media containing flask was inoculated with 2.5 mL of 1x10^7 TP3 spore suspension, and incubated at 28 °C in a 120 rpm rotary shaker. The cultures were harvested between 48 and 360 h after inoculation by aseptic filtration (Whatmann no: 125 filter paper). The residual mycelium was dried at 80 °C to a constant weight. The culture filtrate (1 mL) was serially diluted, and plated for recording cfu/mL from each dilution for each medium after incubation at 28 °C (Singh et al., 2007). The experiment was carried out in triplicate.

Red rice, white rice, paddy husk and finger millet were used as solid media. Each medium (10g) was soaked over night in 10mL water and cooked with 0.5 mL of 10% sunflower oil, cooled, placed in polypropylene bags and sterilized. Soil dilution plate technique was used to record cfu/g from each of four replicate bags inoculated with 10^7 spore/mL of TP3 for each medium, after 2 weeks incubation at 28 ± 2°C.

Bio Assay 3 - The in vitro efficacy of TP3 was tested using boiling tubes sterilized with 20 g of soil. Four replicate tubes were inoculated with the pathogen at disease causing concentrations of 10^7, 10^6, 10^5 spores per mL and incubated at 28 °C for 7 days. The cfu/g of the pathogen in inoculated soils was checked prior to addition of 10 mL of a formulation (authors unpublished data) of 10^7 spores per mL of TP3. Inoculated soil samples were incubated at 28 °C. Efficacy of the formulation was checked by recording the number of cfu/g of pathogen each week over a period of 8 weeks.

Statistical Analysis - All results were statistically analyzed using the Statistical Analysis System (SAS) computer package - Version 6. Mean values were compared by the least significant difference test (LSD) at 5% level of confidence.

Results

The dominance of the antagonist was evident 3 days after inoculation in bio-assay 1. All plates were covered by TP3 on day 7 and it was not possible to re-isolate the pathogen from the assay plates at this stage. Growth of TP3 at 28 °C on solid media was slow with best results of 10^7 cfu /g, 2 weeks after inoculation and 1.4 x 10^6 cfu/g at 6 weeks in white rice medium. In liquid media, the highest number of 2.8x10^6 cfu/mL and the highest biomass of 7.425g/250ml of TP3 was observed in BR medium 144 hours after inoculation (Figures 1 and 2). Inhibition of the highest number of 2.8x10^6 cfu/mL and the highest biomass of 7.425g/250ml of TP3 was observed in BR medium 144 hours after inoculation (Figures 1 and 2). Inhibition of *T. paradoxa* was observed only in the unfiltered crude broth of TP3, with total inhibition observed 5 days after inoculation. Results suggest that extra-cellular metabolites do not play a role in growth inhibition of the pathogen in this case. The fungicidal effect of the antagonist was confirmed by the presence of coiling structures around the pathogen using slide culture technique. The pathogen reached concentrations below disease causing levels (10 cfu/mL) at 6-10 weeks (Figure 1). This study indicates the possible use of *T. harzianum*P3 as a bio-control agent to control black rot disease of pineapple and BR as a potential medium for mass propagation of *T. harzianum* in Sri Lanka. Studies on the efficacy and shelf-life of the formulation are in progress and are to be followed by field trials.

References


Figure 1. Growth of *T. harzianum* P3 on selected solid media at 28 °C.

Figure 2. *T. harzianum* P3 cfu/ml in liquid media.
Invited short note

**Forced Flowering of Pineapple (Ananas comosus cv. Tainon 17) With Calcium Carbide Plus Activated Charcoal and By Ice Cold Stress**

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**Increasing the efficacy of calcium carbide**

In pineapple cultivation, synchronization of flowering is an important agronomic practice that is severely disrupted by induction of natural flowering (natural induction) associated with the gradual decline in night temperature and shortened day lengths during the winter months. This problem is more serious in sub-tropical countries like Taiwan (latitude 22° to 26°N). In subtropical environments it is assumed that natural induction in pineapple is a physiological response to ethylene production in the shoot apical meristem due to low night temperature. Recent studies showing inhibition of natural induction with the ethylene biosynthesis inhibitor aviglycine (Wang et al., 2005; Wang et al., 2007), has proved this assumption. Once natural induction is prevented, flowering can be restored later at convenience through forcing with ethylene or calcium carbide (CaC₂). In Taiwan, CaC₂ remains primary choice for forcing of pineapple because of low cost and ease of application. A grower with the sole of aim of obtaining synchronized forcing would make two or more CaC₂ treatments, thus resulting in increased cost and reduced profit.

*In vitro* studies showed that the acetylene released from CaC₂ upon the addition of water was efficiently absorbed by activated charcoal and significant acetylene release could be detected even after 24 h. We then explored this property under field conditions by supplementing 0.5-1.0% CaC₂ with 0.5-2.0% activated charcoal. Van de Poel et al. (2009) reported that ethylene absorption needed at least 5% activated charcoal. This may be explained by the possible variation in the absorptive capacity of activated charcoal towards acetylene and ethylene. About 50 ml of aqueous solution containing CaC₂ with or without charcoal was applied into the leaf rosette of 11-month-old ‘Tainon17’ (‘Smooth Cayenne’ × ‘Queen’) plants during the 2007-2008 season. Supplementation of 1.0% CaC₂ with 0.5% activated charcoal improved the forcing effectiveness by up to 40%, while 1.0-2.0% activated charcoal drastically reduced the forcing percentage. Based on these results, taken together, we could assume that 1.0% CaC₂ containing 0.5% activated charcoal applied once or 0.5% CaC₂ containing 0.5% activated charcoal applied twice would

![Figure 3. Effect of T.harzianum P3 formulation on 104, 105, and 106 spore/mL of T.paradoxa in-vitro.](image-url)
force pineapple. Thus, activated charcoal provides the flexibility of reducing the number or concentration of CaC₂ or both, to force pineapple and reduce cost.

**Forcing with ice and ice water**

Though ethylene is permitted for forcing of organically grown pineapple in many countries, it still is considered unnatural by some consumers, thus making it desirable to develop a more acceptable organic method of forcing in pineapple. Organic forcing can be defined as forcing of pineapple with any agent that can elicit the endogenous production of ethylene in shoot apical tissues and initiate reproductive development without the exogenous application of ethylene or related compounds. In our laboratory, gas chromatographic analyses showed that treating shoot apical tissues of pineapple with ice for one hour stimulated a two-fold higher ethylene production than comparable apical tissues similarly treated with water at 25 °C. This prompted us to evaluate the effect of ice treatments under field conditions. ‘Tainon17’, a cultivar showing high sensitivity to natural induction was selected for the field tests. Ice treatments were applied to 11-month-old plants (3.5-4 kg mass) in the third or fourth week of October during 2006-2007 and 2007-2008 seasons. Plants were treated 1-4 times with from 500 g to 2 kg of ice crystals or 500 ml of ice water at 24 h intervals. Inflorescence emergence (budding) in the leaf whorl was counted and expressed as a percentage. Plants treated with CaC₂ (2X at a 48 h interval) ethephon (2X at a 48 h interval) and water (25°C) served as controls.

In the 2006-2007 season, budding of plants treated once or twice with ice or ice water was not observed until the third week of February. Nearly 100% budding was observed for plants treated with CaC₂ (2X-48 h interval) by the second week of December. Though ice or ice water treated plants are not induced to flower with the relative efficacy of CaC₂ bud emergence from cold-stressed plants was 1-2 weeks earlier than those treated with water (25°C), indicating that cold treatment altered the sensitivity of plants to natural induction. In the 2007-2008 season, plants treated 3 or 4 times with ice or ice water were induced to flower with an effectness almost equal to that of CaC₂ and ethephon. However, bud emergence from the cold-treated plants was delayed by 2-3 weeks. These experimental results indicate that under field conditions, 1-2 ice treatments were not sufficient to force flowering of ‘Tainon 17’, but 3-4 ice treatments readily induced flowering in ‘Tainon17’. Interestingly, four applications of 500 ml of ice water resulted in better forcing than did four applications of 500 g of ice and was equal to the results obtained on treating plants four times with 2.0 kg of ice. It is difficult to explain this scenario without data on the temporal fluctuations in the ACC synthase activity after ice-cold treatments. As expected, maturity and harvesting of fruits from ice treated plants was delayed by 2-3 weeks compared to CaC₂ and ethephon and in most treatments harvesting was completed in 3-4 harvest passes over 7-10 days. Morphology of fruits from ice treated plants showed no discernable variation from that of chemically-forced fruits, however, the qualitative parameters were not studied.

It is assumed that the high sensitivity of ‘Tainon17’ to natural induction is what accounts for the relative ease in forcing this cultivar with ice treatments in Taiwan in October, i.e. 2-3 weeks prior to natural induction. Soler et al. (2006) reported that cold water (5°C) treatment was more effective on those cultivars that are sensitive to natural induction. Forcing efficiency heavily depends on the cultivar sensitivity and prevailing weather conditions (Bartholomew et al., 2003) and, therefore, the effect of ice treatment on inflorescence emergence should be tested, (i) on cultivars differing in their sensitivity to natural flowering, and (ii) under varying climatic conditions and production seasons, before incorporating the method into the regular farming schedule.

**Acknowledgement**

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**References**


Phytophthora Heart Rot Control

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In a preliminary field test, we evaluated a vermicompost tea drench and a BTH (acibenzolar-s-methyl) dip as compared to an Aliette dip and an untreated control on the incidence of Phytophthora heart rot of pineapple. Plots were prepared by running an irrigation tube down the center of a bed and covering the bed with plastic mulch. The treatments were arranged in a RCBD with plots 1 bed (1 m) wide by 1.5 m long. Blocks consisted of one 4.5 m row. The four treatments were replicated 10 times in plots of 5 plants. Crowns of hybrid 73-50 were dipped in water, Aliette (300 g/100 l), or BTH (100 ppm) and then planted. For the vermicompost tea, water-dipped crowns were planted and then drenched with 500 ml tea/crown (1.8 mt of compost in 23,333 L ha\textsuperscript{-1} water). Crowns in the other treatments were drenched with 500 ml water. The plots were irrigated for 48 hours after planting and then again weekly for the next 12 weeks. Crown rot was recorded 3, 6, 9, and 12 weeks after planting.

More crowns (38\%) were lost in plots receiving the compost tea treatment than in any other treatment (Figures 1). Only one crown was lost in the Aliette treatment after 12 weeks. We suspect this dead plant was actually a 73-114 hybrid contaminant based upon its leaf characteristics. Sixteen percent of the crowns were lost in the untreated plots whereas only 8\% of the crowns were lost in the BTH treatment.

This vermicompost tea appeared to either stimulate or encourage Phytophthora infection. The BTH, a known inducer of systemic acquired resistance, may have induced resistance to phytophthora in these pineapples as well. Since the hybrid 73-50 is more tolerant/resistant to phytophthora than 73-114, we are repeating the evaluation using 73-114 in hopes of achieving greater infection in the untreated plots.

Evaluation of Transgenic Resistance

B. Sipes, C. Nagai, and M.-L. Wang

We have evaluated 13 lines genetically modified with a cystatine gene to control nematode reproduction in pineapple. In a group of 17 different pineapple lines (the number of individual plants per line varied), root-knot nematode (Meloidogyne javanica) infection reduced pineapple growth an average of 43\% in 11 of the lines. In the remaining two lines, plants grew more in
the presence of nematodes, however these were generally the smaller plants in the group. All of the plants supported root-knot nematode reproduction. Two lines had fewer numbers of nematode than the other lines but were not among those lines undamaged by the nematodes. We compared nematode reproduction and plant growth of one of the transgenic lines (composed of 350 plants) to that of a wild type (composed of 235 plants) in the greenhouse. Overall the transgenic plants were 37% smaller and supported 42% more nematodes per plant than the wild type plants. The cystatin levels were probably not high enough in the transgenic line, even though this line had the highest expression level of all lines based on western blot analysis. The level recorded was much lower than what has been reported in other transgenic plants like potato.

Table 1. Root-knot nematode (*Meloidogyne javanica*) reproduction on transgenic and wild type pineapple.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Nematode</th>
<th>Initial plant weight (g)</th>
<th>Final plant weight (g)</th>
<th>Shoot weight (g)</th>
<th>Root weight (g)</th>
<th>Dry root weight (g)</th>
<th>Total nematode</th>
<th>Nematode/g dry root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic</td>
<td>-</td>
<td>5.03</td>
<td>74.85</td>
<td>66.41</td>
<td>4.74</td>
<td>1.45</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>+</td>
<td>4.97</td>
<td>72.75</td>
<td>65.15</td>
<td>4.55</td>
<td>1.42</td>
<td>2366</td>
<td>1661</td>
</tr>
<tr>
<td>Wild</td>
<td>-</td>
<td>5.5</td>
<td>118.34</td>
<td>104.15</td>
<td>7.52</td>
<td>2.11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>+</td>
<td>5.34</td>
<td>119.34</td>
<td>103.87</td>
<td>7.94</td>
<td>2.31</td>
<td>1670</td>
<td>722</td>
</tr>
</tbody>
</table>

**Production of transgenic pineapple (*Ananas cosmos (L.) Merr.*) plants via adventitious bud regeneration** (accepted by In Vitro Cellular and Developmental Biology - Plant)


A new protocol for the production of transgenic pineapple plants was developed. Adventitious buds were induced directly from Agrobacterium-infected leaf bases and stem discs of in vitro plants, bypassing the establishment of callus cultures. Non-chimeric transgenic plants were obtained by multiple subculturing of primary transformants under increasing levels of selection. A total of 42 independent transgenic lines were produced from two cultivars with two different constructs; one containing a modified rice cystatin gene (Oc-IÅD86) and the other with the antisense gene to pineapple aminocyclopropane synthase (ACS). GUS histochemical staining provided the first evidence of the non-chimeric nature of the transformed plants. Their non-chimeric nature was further demonstrated by PCR analyses of the DNA extracted from individual leaves of a primary transformed plant and also from multiple plants propagated from a single transformation event. Southern hybridization confirmed random integration patterns of transgenes in the independent lines. The expression of Oc-IÅD86 gene was detected via RT-PCR at the mRNA level and translation by protein blot. Agronomic evaluation and bioassays of the transgenic plants will further validate the utility of this new tool for pineapple improvement.

**Effects of ReTain® on Natural Induction of Reproductive Development of MD-2 Pineapple**

Duane P. Bartholomew and Gail Uruu. Dept. of Tropical Plant and Soil Science, University of Hawaii, Honolulu, HI 96822. E-mail: duaneb@hawaii.edu

Natural induction (NI) of reproductive development of pineapple is a serious problem for pineapple growers and particularly for those growing cultivars highly sensitive to NI. The problem is more severe in regions with cool winter temperatures and shorter daylengths. In such environments, the incidence of NI can be high, resulting in off-schedule fruiting, a spread harvest peak and increased variability in ratoon crop fruits due to variation in sucker initiation and development. In Hawaii, NI can occur as early as the middle of November and it can continue into March.

Aviglycine, an ethylene biosynthesis inhibitor, can control NI in pineapple (Kuan et al., 2005; Lin et al., 2006; Wang et al., 2007). However, aviglycine is very costly and further studies are needed to reduce costs while maintaining efficacy in areas where NI is a serious problem. In a 2007-2008 test with aviglycine (Bartholomew and Uruu, 2008), treatments were begun on December 1 and significant NI occurred on or soon after that date, which resulted in less than ideal control of the problem. As a follow-up to that study, two tests of the efficacy of aviglycine (ReTain® and VBC-30102, a liquid formulation of aviglycine) in controlling NI of ‘MD-2’ (Dole’s MG3) were conducted during the 2008-2009 winter season. The tests were located in fields having ‘MD-2’ plants of similar age and plant size. The treatments (Table 1) were designed to test the efficacy of weekly sprays of 100 or 200 mg L⁻¹ of aviglycine and of a biweekly spray of 100 mg L⁻¹ of aviglycine in controlling NI. Increasing the concentration to 200 mg L⁻¹
and halving the volume would double the area that could be covered by one tank of spray solution. Treatments with delayed starting dates were included to help identify more precisely when NI occurs in fields of ‘MD-2’ pineapple in Hawaii. The label for aviglycine only permits a maximum of 10 applications so delaying the application of aviglycine as long as possible offers the potential to reduce costs or extend coverage later into the winter months, or both. Treatments were first applied on November 7, 2008 in Waialua field 7 (145 m elevation) and on December 1, 2008 in Brodie field 13 (275 m elevation).

Table 1. Treatments to evaluate the efficacy of ReTain® in controlling natural induction of flowering in MD-2 pineapple during 2008-09.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Product</th>
<th>A.I., mg L⁻¹</th>
<th>L acre⁻¹</th>
<th>Applications</th>
<th>Est. No.</th>
<th>Interval, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T2</td>
<td>ReTain®</td>
<td>100</td>
<td>946.3*</td>
<td>3-5</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>ReTain®</td>
<td>100</td>
<td>946.3</td>
<td>6-10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>ReTain®</td>
<td>200</td>
<td>473.1</td>
<td>6-10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>VBC-30102</td>
<td>100</td>
<td>946.3</td>
<td>6-10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>VBC-30102</td>
<td>200</td>
<td>473.1</td>
<td>6-10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>T7†</td>
<td>ReTain®</td>
<td>100</td>
<td>946.3</td>
<td>3-7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>T8†</td>
<td>ReTain®</td>
<td>100</td>
<td>946.3</td>
<td>2-4</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* Volume of 946.3 L = 250 gallons/acre and 473.1 L = 125 gallons/acre.
†Aviglycine was applied approximately one (T7) or two (T8) weeks later than the other treatments.

Plot size was 4 two-row beds by 10 m and the experimental design was a randomized complete block (RCB) with four replications. Bud emergence data were only obtained for an average of 137 plants in the two inner beds; the outer two beds served as buffers between adjacent plots. Because of time constraints, the incidence of NI was not determined in all plots. The tap water-aviglycine solution was applied as a broadcast spray in either one (473.1 L) or two (946. L) passes over the plots using a 0.98 m (60") boom with 6 TeeJet fan-spray nozzles spaced 20 inches apart and a spray pressure of 30 psi. No surfactant or other adjuvant was used.

The treatments were usually applied between 7:30 and 9:00 a.m. at the Waialua location and between 9:30 and 11:00 at the Brodie location. Air temperature data collected in the field made it possible to evaluate the effect, if any, of time of application and associated temperature changes on the efficacy of aviglycine treatments. To keep the total application time as short as possible, bulk volumes of the various sprays were mixed in the tank and then all plots for those treatments in each test were sprayed before another spray solution was mixed. The tank and hose were flushed with water between sprays having different concentrations or formulations. At the Waialua test site treatments were applied in the order 100 mg L⁻¹ ReTain®, 200 mg L⁻¹ ReTain, 100 VBC-30102 followed by 200 mg L⁻¹ VBC-30102. To minimize the time spent cleaning the spray tank and flushing the hose and boom, the last treatment at the Waialua location was the first treatment applied at the Brodie location. The last treatments at Brodie were always those containing 100 mg L⁻¹ of ReTain®.

To the extent possible, the dates of NI were estimated using bud counts collected from 10 plants forced with 10 cc of a solution containing 4% urea and 45 mg a.i. of ethephon on each treatment date at each location. In some of the plots forced during January and February, it was not possible to distinguish between forced plants and those induced naturally. Bud counts for the treated plots were converted to percentage NI and the data were arcsine transformed prior to statistical analysis using the GLM procedure. The means were retransformed for reporting purposes.

The average air temperature range for the dates and times the treatments were applied at the two sites gradually decreased over time at both locations (Table 2, Table 3). Although average air temperature at the Waialua site was about 1.0 °C (1.8 °F) warmer than at the Brodie site (data not shown), the temperatures during the time the treatments were applied was warmer at Brodie than at Waialua because of the later starting time at the Brodie field. Days to budding (approximately 1 cm open heart) of the forced plants, where they could be determined unambiguously, ranged from 52 to over 70 days at the two locations. Daily heat units (mean air temperature minus a base or threshold temperature of 12 °C (59 °F); Fleisch and Bartholomew, 1987) were calculated and summed over days to about 1.0 cm open heart for each location. Where bud emergence could be clearly associated with forcing, as opposed to NI, the accumulated heat units from forcing to 1.0 cm open heart were quite similar at both locations. These results confirm the results of Fleisch (1988) who reported that accumulated heat-units is a simple and reliable predictor of fruit development up to the time of bud emergence.

Control of natural induction (NI) at the Waialua site in early March (Table 4) was excellent when the treatments were begun before November 24, 2008. Applying aviglycine at biweekly intervals or delaying the treatment start date until November 24 still resulted in a highly significant reduction in NI as compared with the control. Treatment with 100 or 200 mg L⁻¹ aviglycine at weekly intervals provided excellent control of NI and the liquid formulation of aviglycine was as effective as the powder formulation. The excellent control of NI achieved with weekly applications of 100 mg L⁻¹ aviglycine suggests that it would be worthwhile to evaluate the efficacy of lower concentrations of aviglycine in hopes of reducing the cost of product required to...
prevent NI. Biweekly application of aviglycine also appears to offer significant improvement in control of NI and could offer significant savings in cost of product or double the period during which NI might be controlled.

Table 2. Application date, time and temperature range when aviglycine treatments were made and budding information for plants forced on the various treatment dates at Wailua field 7, block 4.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Budding‡ date, days to budding and cumulative heat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 7</td>
<td>8:00 - 9:00</td>
<td>No data</td>
<td>Dec. 29 52</td>
</tr>
<tr>
<td>Nov. 14</td>
<td>8:00 - 9:00</td>
<td>No data</td>
<td>Jan 5 52</td>
</tr>
<tr>
<td>Nov. 24</td>
<td>8:00 - 10:00</td>
<td>23.4 - 25.9</td>
<td>ND*</td>
</tr>
<tr>
<td>Dec. 8</td>
<td>7:35 - 9:15</td>
<td>20.0 - 24.8</td>
<td>Feb. 6 60</td>
</tr>
<tr>
<td>Dec. 15</td>
<td>7:50 - 9:20</td>
<td>21.4 - 24.7</td>
<td>ND</td>
</tr>
<tr>
<td>Dec. 22</td>
<td>7:40 - 9:10</td>
<td>18.9 - 21.0</td>
<td>ND</td>
</tr>
<tr>
<td>Dec. 29</td>
<td>7:45 - 9:00</td>
<td>18.6 - 23.4</td>
<td>ND</td>
</tr>
<tr>
<td>Jan. 5</td>
<td>7:45 - 9:10</td>
<td>19.5 - 23.2</td>
<td>ND</td>
</tr>
<tr>
<td>Jan. 12</td>
<td>7:45 - 9:10</td>
<td>16.4 - 20.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

‡Budding stage was 1 cm open heart.
*Not determined because natural induction confounded the observations.
†Units are Celsius or, in parentheses, in Fahrenheit.

Table 3. Application date, time and temperature range when aviglycine treatments were made and budding information for plants forced on the various treatment dates at Brodie field 13, block 13.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Budding‡ date, days to budding and cumulative heat units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec. 1</td>
<td>10:00 - 12:00</td>
<td>21.6 - 27.3</td>
<td>Feb. 2 63</td>
</tr>
<tr>
<td>Dec. 8</td>
<td>9:45 - 10:45</td>
<td>23.5 - 25.2</td>
<td>Feb 20 est. 74</td>
</tr>
<tr>
<td>Dec. 15</td>
<td>9:50 - 11:20</td>
<td>26.1 - 29.4</td>
<td>ND*</td>
</tr>
<tr>
<td>Dec. 22</td>
<td>9:50 - 11:00</td>
<td>21.4 - 23.3</td>
<td>Mar. 2 69</td>
</tr>
<tr>
<td>Dec. 29</td>
<td>9:45 - 11:05</td>
<td>24.8 - 27.1</td>
<td>ND</td>
</tr>
<tr>
<td>Jan. 5</td>
<td>9:45 - 11:00</td>
<td>22/1 - 26.3</td>
<td>ND</td>
</tr>
<tr>
<td>Jan. 12</td>
<td>9:45 - 11:00</td>
<td>24.4 - 29.4</td>
<td>ND</td>
</tr>
<tr>
<td>Jan. 19</td>
<td>7:?- 9:?</td>
<td>13.3 - 19.1</td>
<td>ND</td>
</tr>
<tr>
<td>Jan. 26</td>
<td>7:40 - 9:10</td>
<td>15.5 - 19.1</td>
<td>ND</td>
</tr>
<tr>
<td>Feb. 2</td>
<td>7:50 - 9:10</td>
<td>18.4 - 20.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

‡Budding stage was 1 cm open heart.
*Not determined because natural induction confounded the observations.
†Units are Celsius or, in parentheses, in Fahrenheit.

Table 4. Effect of aviglycine on percentage of natural induction of reproductive development of MD-2 pineapple in Wailua Field 7, Block 4. Treatments were begun on the indicated starting date in 2008 and all were ended on January 12, 2009. The means, retransformed after analysis, are based on bud counts for an average of 137 plants per plot made on March 2, 2009.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Start date</th>
<th>Interval days*</th>
<th>Replications</th>
<th>Budding, %†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nov.-7</td>
<td>NA</td>
<td>4</td>
<td>53.8 a</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Nov.-7</td>
<td>14</td>
<td>4</td>
<td>12.4 b</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Nov.-7</td>
<td>7</td>
<td>4</td>
<td>1.2 cd</td>
</tr>
<tr>
<td>ReTain®, 200</td>
<td>Nov.-7</td>
<td>7</td>
<td>2</td>
<td>1.6 bcd</td>
</tr>
<tr>
<td>VBC-30102, 100</td>
<td>Nov.-7</td>
<td>7</td>
<td>4</td>
<td>0.1 d</td>
</tr>
<tr>
<td>VBC-30102, 200</td>
<td>Nov.-7</td>
<td>7</td>
<td>4</td>
<td>1.0 cd</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Nov.-14</td>
<td>7</td>
<td>2</td>
<td>1.0 cd</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Nov.-24</td>
<td>7</td>
<td>2</td>
<td>8.4 bc</td>
</tr>
</tbody>
</table>

*ReTain spray interval in days.
†Means followed by the same letter are not significantly different from each other, P = 0.5.

Table 5. Effect of aviglycine on percentage of natural induction of reproductive development of MD-2 pineapple in Brodie Field 13, Block 13. Treatments were begun on the indicated date and all were ended on February 2, 2009. The means, retransformed after analysis, are based on bud counts made on March 9, 2009.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Start date</th>
<th>Interval days*</th>
<th>Replications</th>
<th>Budding, %&lt;ref&gt;†&lt;/ref&gt;</th>
<th>Budding, %&lt;ref&gt;‡&lt;/ref&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Dec. 1</td>
<td>NA</td>
<td>4</td>
<td>43.5 a</td>
<td>30.9 ab</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Dec. 1</td>
<td>14</td>
<td>4</td>
<td>17.1 b</td>
<td>14.8 bc</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Dec. 1</td>
<td>7</td>
<td>4</td>
<td>6.3 bc</td>
<td>4.3 cd</td>
</tr>
<tr>
<td>ReTain®, 200</td>
<td>Dec. 1</td>
<td>7</td>
<td>3</td>
<td>4.9 bc</td>
<td>2.8 cd</td>
</tr>
<tr>
<td>VBC-30102, 100</td>
<td>Dec. 1</td>
<td>7</td>
<td>4</td>
<td>0.7 c</td>
<td>0.5 d</td>
</tr>
<tr>
<td>VBC-30102, 200</td>
<td>Dec. 1</td>
<td>7</td>
<td>4</td>
<td>7.0 bc</td>
<td>5.6 cd</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Dec. 8</td>
<td>7</td>
<td>4</td>
<td>15.0 b</td>
<td>12.4 bc</td>
</tr>
<tr>
<td>ReTain®, 100</td>
<td>Dec. 15</td>
<td>7</td>
<td>4</td>
<td>51.0 a</td>
<td>37.6 a</td>
</tr>
</tbody>
</table>

*ReTain spray interval in days.
†Means followed by the same letter are not significantly different from each other, P = 0.5.
‡The column “Budding <LC” excludes data for plants where the inflorescence was at or more mature than the late cone stage from the analysis and the column “Budding <MC” similarly excludes data for plants at or older than the mid cone stage of development because these plants likely began inflorescence development prior to the start of the treatments. Detailed staging was based on the inflorescence development stages ½“ open heart, 1” open heart, early cone, mid cone, late cone, early flower, mid flower, late flower and dry petal. The respective stages occur at approximately weekly intervals in Hawaii.

Delaying application of aviglycine until December 1 can result in increased NI. Since aviglycine treatments at the Brodie location were not begun until December 1, it was important to be able to distinguish between buds that resulted from NI before the treatments began from those developing afterwards. This was done by comparing bud development of plants in an adjacent plot forced on December 1, 2008 with bud development of plants within the test plots. Bud counts in the test plots were made on March 9, 2009 and buds that were comparable in size to or more developed than those in the plot forced on December 1 were excluded from the analysis. Because it was difficult to precisely identify the stage of development in both the forced plot and the test plots, two data sets were created, one where buds more mature than the late cone stage (Rohrbach and Johnson, 2003) of development were excluded and another where plants with buds more mature than the mid cone stage were excluded. Plant numbers per plot were reduced to as few as 65 in one plot but for most plots the analysis was based on 100 plants or more. The results of the two analyses (Table 5) were similar and also were comparable to those obtained for the Waiulua block. We conclude that application of aviglycine later in the day when temperatures were warmer did not reduce the effectiveness of the treatments. As was the case for the Waiulua test, the least amount of NI occurred in plots that were treated weekly with 100 or 200 mg L⁻¹ of aviglycine. In the <LC analysis, biweekly treatment with aviglycine controlled NI significantly better than no treatment; however, in the <MC analysis, the control and biweekly treatments were not different from each other. There was a significant increase in NI when treatments were delayed until mid December. From the 2008-2009 data, for best control of NI in the Hawaii environment treatments with aviglycine need to be started by late November.

Fruit development was assessed on May 5, 2009 at both the Waiulua and Brodie locations. The range of fruit development spanned several weeks and included fruits that were at the 1.0 cm open heart stage to well beyond the dry petal stage. Some harvest data will be collected but the wide range of ages and the lack of labor for harvesting will limit the data collected to the Waiulua test.

ReTain® Applied During Early Fruit Development Causes Fruit Deformities

To date, no reports of any detrimental effects of ReTain on fruit development were found. However, in this study, ReTain sprays inhibited fruitlet development quite significantly. An unusually wide bed interspace separated beds three and four in replication 3, treatment 8 (R3T8) and replication 4, treatment 3 (R4T3). There was a high percentage of NI in the two rows bordering the wide interspace. The wide interspace had no effect on ReTain spray coverage of plants in the outside row of bed three but resulted in little or no ReTain spray coverage of the inside row of bed four in the two plots. ReTain sprays (100 ppm) were first applied to R4T3 on December 1 and, when compared to untreated fruit, development of most fruitlets of some fruit was inhibited (Figure 1). The extent of the growth inhibition/deformity was less in R3T8 and was visible only on the upper portion of fruits where ReTain sprays were first applied on December 15, 2008 (Figure 2). It appears that the growth inhibition due to application of ReTain is more pronounced when sprays are applied at about the same time that development associated with NI begins. Deformities similar to those observed in this test were also seen in plantation blocks that were treated with ReTain beginning on December 1, 2008.
The percentage of deformed fruits was relatively to very high in all treated plots in the Brodie test (Table 1). The highest percentage of deformed fruits was in the treatment where ReTain sprays were delayed until December 15. The results indicate that ReTain treatments must begin before NI occurs, not only to prevent NI but also to avoid ReTain-induced fruit deformity.

### Table 6. Effect of ReTain sprays on Percentage deformed fruit in ReTain test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Deformed fruit, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.32</td>
</tr>
<tr>
<td>ReTain®, 100 ppm, 14 day interval</td>
<td>8.42</td>
</tr>
<tr>
<td>ReTain®, 100 ppm, 7 day interval</td>
<td>11.30</td>
</tr>
<tr>
<td>ReTain, 200 ppm, 7 day interval</td>
<td>19.23</td>
</tr>
<tr>
<td>VBC-30102, 100 ppm, 7 day interval</td>
<td>9.86</td>
</tr>
<tr>
<td>VBC-30102, 200 ppm, 7 day interval</td>
<td>11.86</td>
</tr>
<tr>
<td>ReTain, 100 ppm, 7 day interval beginning Dec. 8</td>
<td>19.37</td>
</tr>
<tr>
<td>ReTain, 100 ppm, 7 day interval beginning Dec. 15</td>
<td>34.46</td>
</tr>
</tbody>
</table>

**Acknowledgements**

Help with installation of the tests provided by Jenny Antonio and her staff was gratefully appreciated. The weather data was provided by T. Matsumoto and D. Aoki of the USDA in Hilo, Hawaii.

**References**


Services

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

Commercial Services

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


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

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

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

Professional Services

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

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

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

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

Mr. Ian Greig. Greig and Associates, P.O. Box 273508, Tampa, FL 33688. Phone: (813) 908-7698; Fax: (813) 963-6229. E-mail: iang@ag-consult.com. Web site: www.ag-consult.com. Services for all phases of pineapple production but emphasis is on pineapple industry and market analysis.

Mr. L. Douglas MacClyre. 360 Hoopalu Dr., Pukalani, Hawaii, U.S.A. E-mail: norfolkldm@aol.com. Experience: More than 39 years with Maui Pineapple Company heading plantation and diversified agriculture operations and started the Royal Coast Tropical Fruit Company in Costa Rica. Collected and summarized production information in Asia and Central America. Also consulted on pineapple for companies and growers in El Salvador, Australia, Thailand and Indonesia.
Mr. Graham J. Petty  13 Somerset Place, Lambert Road,  Port Alfred,  6170, Republic of South Africa.  Phone: +27 (0) 46 624 4868;  
Tel/Fax: +27 (0) 46 625 0946;  E-mail:  grahamp@imaginet.co.za.  Experience: M.Sc. (Agric) Pretoria :  Pr. Sci. Nat.  . Researcher and advisor to the South African Canning Pineapple Industry on matters of Pest Management in pineapple culture, for 34 years. Economic entomology and management of biological control agents have received particular attention.

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

Dr. José Aires Ventura.  Incaper, Rua Afonso Sarlo 160 (bento Ferreira), 29052-010, Vitoria-ES, Brazil.  E-mail:  ventura@incaper.es.gov.br.  Tel.: 55-27-31379874.  www.incaper.es.gov.br. Area of Specialization: Plant Pathology (research in pineapple diseases management; Fusarium diagnosis, diseases resistance).

Mr. Dean Wheeler.  AgResults Inc., 609 Buchanan Street, Davis, California, U.S.A. 95616.  Phone/fax: 530-758-4620 Residence: 530-758-3354.  Email:  agresults@aol.com.  Web page at http://agresults.com/.

Book Reviews and Web Sites

Book Reviews

No reviews were provided for this issue.

Web Sites of Possible Interest

1. The CIRAD Market News Service website is http://passionfruit.cirad.fr/index.php/html)/fruitrop/fruitrop.html. The services journal FruiTrop can be found at the web site and many publications are available either for a fee and older issues are available as pdf files at no cost.


References

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

Pineapple Reference Database

A pineapple references database with over 7,000 references in it is maintained by the editor. Literature searches of the database on specific topics, including abstracts where available, can be obtained by contacting Duane Bartholomew at duaneb@hawaii.edu.


Elias Junior, J., Gomes, D.C., Matos, A.P.d., and Almeida, C.O.d., 2009. Micro and macroeconomic analyses of the pineapple industry in the state of Tocantins


Kole, C., 2007. Fruits and nuts. Genome Mapping and Molecular Breeding in Plants, (of seven volumes that provide a timely overview of the current status of genome analysis in plants including pineapple


Lorsuwan, P., Rechtanapun, C., and Chantawanarawong, S., 2008. Total phenolics, radical scavenging capacity and antimicrobial property of fruit peels, Bangkok; Thailand.


Instructions to Contributors to Pineapple News

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

Preferred contributions include:

- Timely news about research on issues related to culture, processing, storage, and marketing of pineapple.
- New, interesting, or unique problems encountered by growers.
- Country or status reports on the local pineapple industry.
- If uncertain about the suitability of material for the newsletter, contact the editor.
If possible, please send contributions by E-mail as attached files in MS Word or rich text format or on floppy disks. When sending printed copy, be sure that it is clean and sharp so it can be scanned to speed conversion to a wordprocessor format.

**Article length:** Papers usually should be no longer than 4 double-spaced pages in 12 point font or equivalent, not including tables, figures and photos. If longer than 4 pages, please contact the editor. There is no limit on the number of articles that can be submitted. However, acceptance and publication is at the discretion of the editor.

**Tables:** The preferred table format is columns separated by tabs. Authors may be asked to revise tables not in the requested format.

**Photographs:** Submit photographs that can be scanned or provide digital files in jpeg format with a minimum resolution of 300 dpi so they can be printed with acceptable resolution in grey scale with a laser printer.

Mail contributions and inquiries to: **D.P. Bartholomew, Dept. of NREM, Univ. of Hawaii, 1910 East-West Rd., Honolulu, HI 96822 U.S.A.** (Phone (808) 956-7568; Fax (808) 956-6539; E-mail: duaneb@hawaii.edu)

**Pineapple News** is available on the Web at: [http://tpss.hawaii.edu/pineapple/pineappl.htm](http://tpss.hawaii.edu/pineapple/pineappl.htm)

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