



# Pineapple News

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## Pineapple Working Group News

Dear Colleagues:

I am sorry for the delay in getting issue No. 18 of Pineapple News out to readers. I spent most of March-May doing a final review/edit of the papers submitted for inclusion in the proceedings of the 7<sup>th</sup> Symposium. Ninety papers were submitted and 80 papers will be included in the proceedings, 16 more than any of the six previous pineapple symposium proceedings.

During the editing process I gained experience with MS Word and plan to use that software to prepare this and future issues of Pineapple News. That necessitated developing a new logo for the newsletter. I also have modified the newsletter format to more closely follow that used by ISHS for *Acta Horticulturae*. That change will make it possible for contributors to Pineapple News to use the ISHS author guide when preparing contributions to newsletter (see instructions at the end of the newsletter). I haven't had time to conjure up an article from the editor so I hope you find the content below interesting.

### 7<sup>th</sup> International Pineapple Symposium

D.P. Bartholomew<sup>1</sup>, T.M. Tengku Ab Malik<sup>2</sup>, H. Abdullah<sup>2</sup>, Y.K. Chan<sup>3</sup> and M.N. Latifah<sup>2</sup>

<sup>1</sup>Dept. of Tropical Plant & Soil Sci., Univ. of Hawaii, Honolulu, HI USA

<sup>2</sup>Horticulture Research Centre, Malaysian Agric. Research & Devel. Inst., 43400 Serdang, Selangor, Malaysia

<sup>3</sup>Malaysian AgriFood Corp., 47160 Puchong, Selangor, Malaysia

The 7<sup>th</sup> International Pineapple Symposium was held almost one year ago. For those unable to attend, the abstracts of the symposium were provided as Pineapple News No. 17 Addendum, which is available from the Pineapple Working Group pages at the ISHS web site.

The symposium was held from July 13 to 15, 2010 at the beautiful Persada Johor International Convention Center, Johor, Malaysia, near the heart of Malaysia's pineapple growing area. The Symposium was supported by Malaysian Agricultural Research and Development Institute (MARDI), Malaysian Pineapple Industrial Board, Department of Agriculture Malaysia, Federal Agricultural Marketing Authority, The International Tropical Fruits Network and Tourism Malaysia and held under the auspices of the International Society for Horticultural Science. The theme of the symposium was "Global Pineapple Industry – The Way Forward." There were 421 registered participants from 22 countries, certainly making it one of the largest pineapple symposiums held to date.



Figure 1. Participants attending the 7th International Pineapple Symposium in the Johor convention center (MARDI photos).

There were 32 oral presentations and 115 posters and the papers were organized into six topics, namely (abstract numbers in parentheses) Industry and Trade (4), Biotechnology and Breeding (15), Plant Physiology and Cultural Practices (32), Pest and Disease Management (18), Postharvest Handling and Product Development (40) and Consumer and Marketing (6). The last session of the symposium was a panel discussion led by Ying Kwok (YK) Chan on the subject "Global pineapple industry: The way forward".

After the opening ceremonies, which included a welcome from the Director General of MARDI and an official opening by the Minister of Agriculture, speakers at the first session provided overviews of the pineapple industries of Malaysia, Philippines, Taiwan, Thailand and North East India. The oral presentations were supplemented by poster presentations on the pineapple industry in China and in the Commonwealth of the Northern Mariana Islands. The presenters highlighted interesting contrasts between the smaller-scale operations in the foregoing countries and the very large and highly integrated operations in Mindanao, Philippines and Sumatra, Indonesia. Growers in the Philippines produce for the fresh and processed market while production in Indonesia is destined primarily for processing. In Thailand, most of the fruit is produced by literally thousands of smallholders who supply about 30 canneries and 95% of country's production is processed. Presenters from Indonesia and Malaysia indicated their governments were trying to help small-scale farmers obtain an increased share of the market, an issue that was explored in more detail at a panel at the end of the symposium (see below). There was general optimism about the potential for growing the industry in most countries but the prospects and problems varied with the country. Small holder production in North East India is unique because the fruit is produced entirely by small holders and production techniques are entirely organic, which it was believed offered good opportunities in the high-value organic niche.

With 15 papers included in the Biotechnology and Breeding section, the breadth of coverage in the oral and poster sessions ranged from the molecular (screening pineapple bacterial endophytes for cytokinin-like compounds; differential expression of microRNAs during fruit ripening) to the whole plant (pineapple with landscaping potential; a new pineapple cultivar) and everything in between. Brazilian researchers continue to explore the genetic diversity of their large germplasm collection while work in Thailand produced a new hybrid with herbicide resistance based on a genetically transformed parent. If the amount of work being done to engineer cultivars resistant to important diseases was disappointing, so is the public acceptance of such cultivars. Permanent solutions to the major pest and disease problems of pineapple and their respective reductions in costs to human health, the environment and to producers remain relatively unexplored because the public has been deceived about the risks involved.

Topic coverage by the 32 papers in the Plant Physiology and Cultural Practices section was broad but generally quite site specific. Among those that bring a new approach to old problems was new research from Reunion that indicates it is possible to use a heat unit model to help manage pineapple crops in diverse environments. While the model appears to be more relevant to cooler subtropical environments, it could speed the development of cultural practices tailored to new cultivars and add needed sophistication to the management of small holder pineapple farms in the cooler regions of the tropics. The wide-spread adoption of the 'MD-2' cultivar brought with it the problem of managing natural flowering. Until a genetically engineered solution becomes acceptable, three papers reported on the continued development and success of aviglycine in controlling this important problem. While a costly solution, cost-benefit analyses show that when used successfully, the benefits in both mother plant crop and ratoon exceed the costs.

The keynote presentation in the Pest and Disease Management section highlighted integrated pest management as the best and most economical solution to the myriad of problems confronting pineapple growers. However, the session was dominated, nine of 18, by papers dealing with various aspects of mealybug wilt. The etiology of this complex disease, typically ranked first or second in importance in areas where pineapple is grown, is still being worked out. With more scientists bringing new tools to bear on the detection of the viruses involved in disease development, it seems likely that all of the characteristics of the disease will soon be known. The next problem will be to develop cost-effective solutions for its control as the array of pesticides available to control it continue to dwindle. Perhaps the "way forward" for this problem will be via a biotechnology approach.

A highlight of the meeting for those interested in what happens to pineapple once it is harvested was the rich coverage of such issues in the Postharvest Handling and Product Development section. The papers highlighted the wide ranging expertise of Malaysian scientists in this area, evident from the fact that 37 of 40 papers were by Malaysian authors. Of particular note was the broad focus on product development with products ranging from vacuum fried pineapple chips to utilization of waste products recovered during fruit processing in high fiber products such as juice and cookies and in the production of organic dyes.

The country reports mentioned above indicated plans were to continue the expansion of pineapple plantings for fresh fruit production in tropical Asian countries. In contrast to such optimism, the lead paper in the Consumer and Marketing section raised concerns about the decreasing quality and decreasing prices of 'MD-2' fruits in the European market. But perhaps the Asian market is not yet saturated with high quality pineapple fruits.



Related to fruit quality, one of the four papers in the section dealt with assessing consumer preferences for fresh pineapple. It was reported that consumers prefer 'MD-2' fruits over other cultivars currently being grown in Malaysia and the authors recommended that the 'MD-2' cultivar be used as the benchmark when selecting hybrids in a pineapple fresh fruit breeding program.

The last session of the meeting was a panel discussion that focused on the theme of the symposium, The Global Pineapple Industry- The Way Forward. Panel members were: Y.K. Chan (Facilitator), D.P. Bartholomew, D.H. Reinhardt, A.P. de Matos, G. Sanewski, A. Hassan and Y. Ahmad (Fig. 2). Dr. Chan provided the following summary of the discussion.



Figure 2. Malaysian hosts M.M. Selamat (1<sup>st</sup> row, 2<sup>nd</sup> from left), Convenor T.M. Tengku Ab Malik (1<sup>st</sup> row, 6<sup>th</sup> from left), M.N. Latifah (1<sup>st</sup> row, 2<sup>nd</sup> from right), and H. Abdullah (2<sup>nd</sup> row, 5<sup>th</sup> from left). Discussion panel members include D.P. Bartholomew (1<sup>st</sup> row, 5<sup>th</sup> from left), D. H. Reinhardt, chair, Pineapple Working Group, (1<sup>st</sup> row, 6<sup>th</sup> from right), A.P. de Matos (1<sup>st</sup> row, 4<sup>th</sup> from left) and G. Sanewski, co-host, 8<sup>th</sup> International Pineapple Symposium (1<sup>st</sup> row, 3<sup>rd</sup> from right) with some of the local and international participants at the 7<sup>th</sup> International Pineapple Symposium, Johor, Malaysia (DPB photo).

The Global Pineapple Industry provides equal opportunities for all countries/ players to grow, market and trade in fresh pineapple on an international level playing field. Is this realistic or plain idealism based on naivety?

This certainly is not happening now as the global pineapple industry is dominated by multinational giants like Dole, Del Monte and Chiquita. Likened to a poker game with the big players having a mountain of chips, where and how would a new player with a couple of chips break in?

The situation is made worse by some of the pressing issues faced by the fresh produce industry today:

1. GAP (Good Agricultural Practices) certification for traceability on food safety, environmental protection and sustainability and workers well-being. This is all good, but it marginalizes the small producers who can ill-afford such expensive certification.
2. Sanitary and Phytosanitary (SPS) restrictions imposed by many importing countries are sometimes viewed as trade barriers that have the effect of precluding the small growers from exporting their fruits because SPS treatments are done with expensive equipments that require specialized technical expertise to operate
3. Food Miles/ Carbon Footprints are yet another constraint for growers to export their fresh produce. Big players have invested huge sums to develop technology for export by sea reefers that have reduced carbon footprint, but the average producer does not have the technology, equipment or volume to go by this means.
4. In the past, germplasm accessions were freely exchanged, but today they are severely restricted by Intellectual Property (IP), Variety Protection, Breeders' Rights, patents and Access and Benefits Sharing (ABS) agreements. In the days to come, small growers will have no opportunity to benefit from new varieties developed by research organizations without paying hefty royalties.

5. The advent of 'MD-2' dramatically changed the fresh fruit pineapple market, a market currently dominated by multinationals who grow the hybrid in low-cost countries like Costa Rica and the Philippines. To obtain market share, the smaller players are rapidly changing over to this cultivar. The result is a concern not only in the glut of 'MD2', but more importantly, continuous monoclonal culture will lead to loss of diversity resulting in genetic vulnerability.

In 'Going Forward', it is unrealistic to expect the global pineapple industry to change very much in the coming years. It will continue to be dominated by the multinational giants. The role of small growers can be increased but they need the help of the bigger "boys" in fulfilling their corporate social responsibility. There is some evidence of this already happening when contract farmers are roped in to produce for the multinationals such as Dole in the Philippines. There is also evidence that the government can help in integration of the small farms into economically viable and competitive enterprises. The Farm Consortiums reported in Indonesia and the Technology Management initiatives in NE India are cases in point.

On the last day of the meeting, the organizers recognized and presented small gifts to the speakers. Prof. Reinhardt, Chair, ISHS Pineapple Group announced that Australia would host the next, the 8<sup>th</sup>, international pineapple symposium in 2014. At the gala dinner held in the evening, Prof. Duane Bartholomew was recognized for his contributions to the world pineapple community and was presented with a special gift by Convener Tengku Ab. Malik Bin Tengku Maamun (Fig. 3).



**Figure 3. T.M. Tengku Ab Malik, Convener of the IPS2010, gives a special gift to Prof. Duane Bartholomew during the Gala dinner.**

The meeting closed with a one-day field trip to Johor Tropical Products (JCorp) packaging center and to the Malaysian Pineapple Industry Board Pineapple Technology Development Center. Featured at JCorp was the packing plant operation and right-sized equipment for use on mineral soils on the smaller farms typical of areas of mineral soils in Malaysia where pineapple is grown (Fig. 4).

Next was a visit to the Malaysian Pineapple Industry Board (MPIB) experimental farm, which is located in Alor Bukit, Pontian, Johor. Malaysia is the only country in the world with large-scale pineapple cultivation on peat soil. The farm covers approximately 40 hectares and has samples of most of the pineapple cultivars found in the country. Visitors had a chance see and taste the pineapple accessions 'Josapine', 'Maspine', 'Moris', and 'N36' and were given a brief synopsis of each commercial cultivar developed by MARDI. For many foreign visitors it was the first time they had a chance to see well-grown pineapple on peat soil.

The last stop of the day was at MARDI's Technology Development Center. This government run and operated facility was established to create and develop new products and uses for pineapple. This facility has the capabilities to produce juice concentrate, dehydrated slices, candied pine pieces, pineapple chips, sauces and salsas. Newly developed technologies are later turned over to private companies for large scale productions.



Figure 4. At the Johor Tropical Products Sdn. Bhd. farm, displays included a poster and a live demonstration of equipment suitable for use on smaller farms where pineapple is grown on mineral soil (DPB photos; left photo is of MARDI poster).

### Proceedings of the 7th International Pineapple Symposium

The 80 papers to be published in the *Acta Horticulturae* volume have been with ISHS editors for about a month now and I was recently informed that the volume is almost in press and publication is projected for August, 2011. The volume of the *Acta* will be announced at [www.actahort.org](http://www.actahort.org) and [www.ishs.org/news/](http://www.ishs.org/news/) and will be available as soon as it is published.



## News from Australia

### 8<sup>th</sup> International Pineapple Symposium

This is to confirm the 8<sup>th</sup> International Pineapple Symposium will be held in Brisbane, Australia from Monday 18<sup>th</sup> August- Friday 22<sup>nd</sup> August, 2014 in conjunction with the 29th International Horticultural Congress (IHC). Some general information about the IHC can be found at the website (<http://www.ihc2014.org/>) but a dedicated linked page will eventually be developed for the pineapple symposium. More information about the venue, the Brisbane Convention centre, can be found at it's website ([www.bcec.com.au/](http://www.bcec.com.au/)). While the venue is close to the central business district of the major capital city of Brisbane, it is only a one hour drive to major pineapple growing districts. As in previous symposia, the event will include tours of growing districts to see the latest developments in the Australian pineapple industry. Enquiries can be directed to G. Sanewski ([garth.sanewski@DEEDI.qld.gov.au](mailto:garth.sanewski@DEEDI.qld.gov.au)).



Figure 1. Organising committee members (left to right) Chris Doyle (inset), Garth Sanewski, Simon Newett and Mike Smith.

## The Effect of Temperature on Pineapple Pollen Tube Growth Rate

G. Sanewski. Agr-Science Queensland. Department of Employment, Economic Development and Innovation, Nambour. Qld. Australia. E-mail: garth.sanewski@deedi.qld.gov.au

### INTRODUCTION

Most pineapple varieties exhibit gametophytic self-incompatibility but some will occasionally set selfed seed and are considered pseudo self-incompatible (Cabral et al., 2000; Cabral et al., 2003; Coppens d'Eeckenbrugge et al., 1997). The mechanism of pseudo self-incompatibility is not understood but environment is thought to play a role. S-RNase compounds are considered central to the pollen tube–pistil incompatibility response (Cruz-Garcia et al, 2003; Chen et al, 2010). Temperature is known to affect pollination in many crops and self-incompatibility can be circumvented in some through high or low temperature regimes (Dane and Melton, 1972; Ronald and Ascher, 1975; Larsen, 1984; Gertz and Wrickle, 1991; Keiichi and Murakami, 1992; Boyle et al, 1994). High temperature for instance is thought to denature the S-RNase in the pistil thus allowing self pollen tubes to grow down the style. Temperature could also affect endogenous aspects of pollen tube growth. Warm temperatures are thought to improve pollination by selfing in pineapple (Kerns et al., 1968; Williams, 1970).

While there are several papers dealing with aspects of pollination in Bromeliaceae including pineapple, none have investigated the optimum temperature for pineapple pollen tube growth. Wee and Rao (1979) used 27°C in studies of pollen germination in *Ananas comosus* var. *comosus*. Bhomik (1980) used 20°C for *Ananas comosus* var. *comosus*. Parton et al (2002) used 21°C for the Bromeliaceae species *Aechmea*, *Vriesea*, *Guzmania*, *Tillandsia* and *Pitcairnia*. The effect of temperature on pollen tube growth rate in pineapple was examined in this short study.

### METHOD

Anthers were collected from newly opened flowers before 9am and placed in 9 mL sterile pollen germinating solution in a sterile 10 mL tube. Pollen germinating solution contained 15 % sucrose, 100 ppm boric acid and 200 ppm calcium nitrate. These are the same concentrations optimised for pineapple pollen germinating media by Rao and Wee (1979). The solution containing the anthers was gently shaken and 1 mL pipetted into each of 10 tubes. Each tube was placed in a beaker of water in a different compartment along a temperature gradient bar with light from fluorescent Sylvania *Growlux* tubes. The temperature gradient bar covered the temperature range from 16-33°C over 10 compartments. Pollen of 6 varieties including *A. comosus* var. *ananassoides*, *A. comosus* var. *bracteatus* and *A. comosus* var. *comosus* were examined in this way. The pollen solution was incubated overnight. Wee and Rao (1979) used a relatively short incubation time of only 6 hr and stated that pollen tube length had reached it's maximum by that time. Parton et al (2002) in their study of Bromeliad sp indicted pollen tube growth rate at 21°C was usually fastest in the first 10 hrs, slowed substantially from 10-24 hrs but maximum length was only reached in several species after 24 hrs. In the present study, an incubation time of approximately 24 hrs was used for convenience and to ensure the majority of pollen germination and tube growth had been achieved. After incubation a drop of the solution from each tube was pipetted onto a microscope slide and the length of 50 pollen tubes measured with a graduated eyepiece. Only pollen tubes longer than the diameter of the pollen were measured. The mean pollen tube length per hour of incubation ( $\mu\text{m/hr}$ ) for each temperature was calculated.

### RESULTS AND DISCUSSION

Pollen tube growth was most rapid at 21 °C (Figure 1). At temperatures around 28 °C or more, few pollen germinated, pollen tubes were very short and many abnormalities such as extrusion of pollen contents were apparent.

Wee and Rao (1979) reported a pollen tube growth rate in a 'Spanish' variety of up to 163  $\mu\text{m/hr}$  at 27°C. This is less than the maximum single data sample of 209  $\mu\text{m/hr}$  achieved in one variety in this study but much greater than any varietal mean here. Observations in the current study suggest there are large varietal differences in pollen tube growth rate. There was also a large variability between pollen tubes within varieties. In most cases the standard deviation of the mean was as large as the mean. It is suspected that some pollen had germinated in-



vivo before treatment and were responsible for the small number of unusually high measurements in each treatment which contributed to the high standard deviations.

While some varieties may have exhibited shorter pollen tubes than others, the response to temperature was generally similar. Good pollen tube growth appeared to be obtained in the 19-23°C range and 21°C is suggested for studies involving measurements of pollen tube growth.

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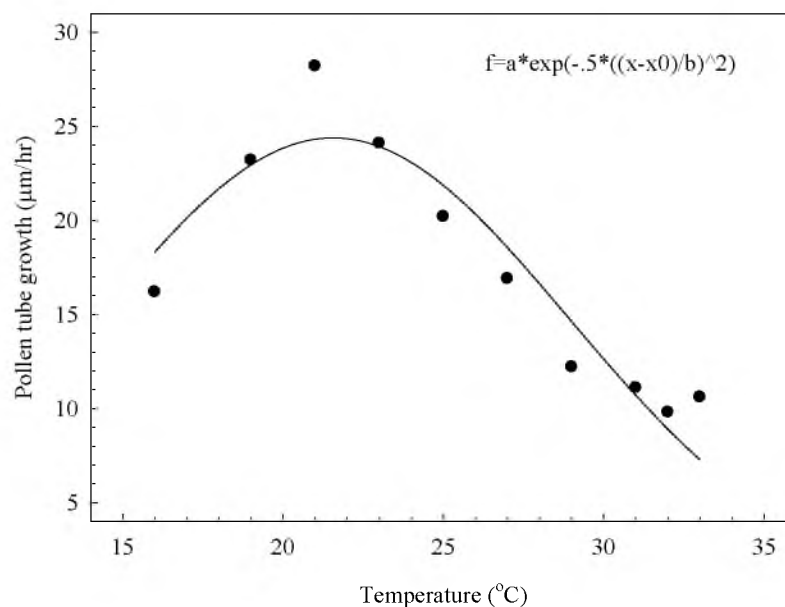


Figure 1. Mean pollen tube growth rate for pineapple. Each data point is the mean of 50 pollen tubes for each of 6 varieties (300 measurements per data point). The equation fitted is a Peak Gaussian, 3 parameter with an  $R^2$  of 0.89.

## News from Brazil

### Production of ‘Pérola’ Pineapple Plantlets by Stem Sectioning Technique

Carlos Vinícius de Jesus Lima<sup>1</sup>; Susana Cristine Siebeneichler<sup>2</sup>; Ronice Alves Veloso<sup>1</sup>; Ívio Alves Milhomem<sup>1</sup>; Cleidivone Soares da Silva<sup>1</sup>; Aristoteles Pires de Matos<sup>3</sup>

<sup>1</sup>Undergraduate Student, Federal University of Tocantins, Gurupi, TO; <sup>2</sup>Professor, DSc, Soil Sciences, Federal University of Tocantins, Gurupi, TO, [susana@uft.edu.br](mailto:susana@uft.edu.br); <sup>3</sup>Plant Pathologist, PhD, Senior Researcher, Embrapa Cassava & Fruits, Cruz das Almas, Bahia, [apmatos@cnpmf.embrapa.br](mailto:apmatos@cnpmf.embrapa.br)

#### ABSTRACT

The availability of healthy and vigorous planting material is usually a problem faced by pineapple growers every planting season. In order to overcome that situation, this work was carried out at the Universidade Federal do Tocantins, campus de Gurupi with the aim of evaluating the potential production and development of ‘Pérola’ pineapple plantlets, obtained by the stem section technique. Stem sections from the basal, intermediate and apical parts of selected plants, and stem sections from suckers as well, were evaluated for pineapple plantlet production. Stem sections from the basal part of pineapple plants, selected after harvest yield more plantlets with more vigor than do stem sections from suckers.

#### INTRODUCTION

Production of pineapple planting material by stem sectioning technique has been known for long time. The development of pineapple axillary buds into plantlets is influenced by several factors such as cultivar, stage of stem development, size of stem section, cultural practices during growing season, among others. Usually sprouting of axillary buds is not affected by the stem size. However, their further development is influenced by stem section size. Smaller sections take a much longer to develop plantlets of adequate size for planting in the field than larger ones. This and also the higher density of axillary buds are the major reasons why stem sections from ‘Smooth Cayenne’ produce more plantlets than those from ‘Pérola’ (Castro & Kluge, 1998).

Pineapple planting material is affected by pests and diseases all over the world. Among them, the fusariosis, caused by *Fusarium subglutinans* f. sp. *ananas*, has been the most serious one for the pineapple crop in Brazil, since this pathogen is able to infect all the plant parts including the planting material, which is the major means of dissemination of that disease among production areas (CARVALHO et al., 2005). This problem increases in importance since usually it takes long for an infected slip or sucker to express fusariosis symptoms, reducing the effectiveness of visual selection of planting material (MATOS, 2003). Plantlets produced from stem sections has been a good approach to get planting material of good quality for pineapple growers (Giacomelli & Py, 1981 cited by Castro & Kluge, 1998). The objective of this work was to evaluate the potential production and development of ‘Pérola’ pineapple plantlets obtained by the stem section technique.

#### MATERIAL AND METHODS

‘Pérola’ pineapple plants, selected in a commercial orchard located in the municipality of Alvorada, State of Tocantins, Brazil, were removed from the soil one month after harvest. Their leaves were pruned leaving the sheath attached to the stem. Suckers from those plants had their leaves pruned in the same way. Pineapple stems were then brought to the experimental field at the Federal University of Tocantins, campus of Gurupi, where the roots were removed. The stems were cross sectioned, 8 cm apart, and sections were separated in three groups according to their position on the stem (upper, intermediate and lower). Each section was longitudinally sectioned twice to obtain four stem sections. Stems from suckers were longitudinally sectioned only once. Stem sections were then treated with pesticides and allowed to dry before planting.

In a randomized block design with five replications the following treatments were evaluated: T1 – Sections from lower stem part; T2 – Sections from intermediate stem part; T3 – Sections from upper stem part; and T4 – Sections from suckers. Each plot consisted of 20 stem sections planted in four rows, 10 cm apart, and 5 cm spacing from section to section within the row. Stem sections were planted horizontally on sawdust, with the cut surface downwards. The experiment was carried out in a screen house with 50% shade. Water was supplied periodically to keep desirable humidity of the substrate.

Evaluations consisted of determining the number of axillary buds and the number of sprouts at least 0.5 cm tall at 38, 53 and 68 days after planting the sections, as well as the number, height and diameter of plantlets obtained per treatment at 53 and 68 days after planting. Data were analyzed by the SISVAR software and treatments compared by the Tukey's test at the 5% level of significance.

## RESULTS AND DISCUSSION

The percentage of stem sections with sprouts and the number of axillary buds that originated plantlets increased with time from planting to final evaluation date. At 38 and 53 days after planting stem sections from suckers and from the apical part of selected plants had the lowest percentages of sprouts which were statistically similar to each other. On the other hand, stem sections from the basal and from the intermediate parts of selected plants gave rise to higher percentages of sprouts and were statistically different from stem sections from suckers. Similar results were obtained for the evaluation at 68 days after planting (Table 1). Higher percentages of sprouts in stem sections obtained from basal plant parts may be due to the fact that the basal part of the stem has more nutritional reserves (Reinhardt et al., 1999). No statistical difference was observed regarding to number of sprout out.

The number of sprouts per stem section increased with time from planting and was higher in basal stem sections (Table 2). Sucker stem sections generated thinner and taller plantlets than those obtained from plant stem sections. These results suggest that plantlets from sucker stem sections have less vigor than those from plant stem sections.

## CONCLUSIONS

Sections from basal stem part of 'Pérola' pineapple plants, obtained after fruit harvest, produce more and more vigorous plantlets than sections from the intermediate and upper stem parts. Sucker stem sections produce less vigorous plantlets than plant stem sections.

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Table 1. Percentage of sections with sprouts and number of axillary buds per section of 'Pérola' pineapple plants that originated plantlets at 38, 53 and 68 days after planting.

Stem section source	38 days		53 days		68 days	
	Sections with sprouts (%)	Buds per section	Sections with sprouts (%)	Buds per section	Sections with sprouts (%)	Buds per section
Lower	35 a*	1.052	75 a	1.522	84 a	1.550
Intermediate	25 a	1.066	65 a	1.286	74 a	1.418
Upper	22 ab	1.074	62 ab	1.360	71 b	1.358
Sucker	15 b	1.216	48 a	1.316	69 b	1.146
CV (%)	18.73	13.66	12.20	21.81	8.20	19.01

\*Data followed by the same letter in the same column did not differ by Tukey's test at the 5% level.



Table 2. Number of sprouts per 'Pérola' pineapple stem section, plantlet stem diameter and plantlet height<sup>z</sup> at 53 and 68 days after planting of the stem sections.

Stem section source	Sprouts per section		Diameter (mm)		Height (mm)	
	53 days	68 days	53 days	68 days	53 days	68 days
Lower	4.6 Ba <sup>z</sup>	8.6 Aa	20.82 Aa	19.41 Ba	51.43 Aa	48.43 Aab
Intermediate	2.0 Bb	6.2 Ab	21.14 Aa	18.89 Ba	50.42 Aa	52.82 Aa
Upper	2.2 Bb	5.4 Ab	20.47 Aa	18.91 Ba	57.96 Aa	47.06 Ab
Sucker	1.4 Bb	6.0 Ab	19.14 Aa	16.74 Bb	55.20 Aa	53.14 Aa
VC (%)	28.86	15.58	6.80	3.10	17.50	5.91

<sup>z</sup> Data followed by the same small letter in same column, and by the same capital letter in the same row did not differ by Tukey's test at 5% level.

## Evaluation of Pineapple Genotypes For Resistance to the Pineapple Mealybug Wilt-Associated Virus

Neide Moura dos Santos<sup>1\*</sup>, Paulo Ernesto Meissner Filho<sup>2</sup>

<sup>1</sup>Graduate student, Agricultural Microbiology, Federal University of the Recôncavo da Bahia (UFRB)  
[neid.moura@hotmail.com](mailto:neid.moura@hotmail.com).

<sup>2</sup>Virologist, PhD, Senior Researcher, Embrapa Cassava & Fruits

The pineapple, due to its qualities, has been one of the main tropical fruits consumed all over the world. This crop is present in many tropical countries of Asia, Americas, Africa and Oceania. In most of the cultivated areas the mealybug associated wilt, caused by the *pineapple mealybug wilt associated virus* (PMWaV), is considered by growers one of the major constraints. Infected plants show a reddish color and a curling down and drying of the leaf borders starting at their apex. As the disease progresses the plant tends to stop growing, probably due to the viral infection. This work aimed at selecting mealybug wilt symptomless pineapple genotypes from the Pineapple Active Germplasm Bank at Embrapa Cassava & Fruits.

Data were collected from March to May 2010. Genotypes available in the Pineapple Active Germplasm Bank were submitted to visual inspections for the presence of symptoms of the pineapple mealybug wilt. Data obtained were compared with those from evaluations performed in previous years.

Several genotypes (Table 1) did not express mealybug wilt symptoms after growing for eight years in an experimental area where this disease is present in high intensity on plants of susceptible genotypes. It was hypothesized that symptomless genotypes may have some kind of resistance to the pineapple mealybug wilt causal agent and research is under way to check this hypothesis.

Table 1. Genotypes from the Pineapple Active Germplasm Bank, located at Embrapa Cassava & Fruits, grown for eight years in a mealybug wilt infested field, which did not show symptoms of that disease in evaluations carried out in 2002, 2008 and 2010.

Pineapple accession	Species
Pérola	<i>Ananas comosus</i> var. <i>comosus</i>
FRP 493	<i>Ananas comosus</i> var. <i>comosus</i>
FRP 684	<i>Ananas comosus</i> var. <i>comosus</i>
Pseudananas x Rondon	<i>Ananas</i> sp.
FRP 292	<i>Ananas</i> sp.
ARM 955	<i>Ananas</i> sp.
FRP 13	<i>Bromelia</i> sp.
FRP 354	<i>Bromelia</i> sp.
FRP 355	<i>Bromelia</i> sp.
FRP 481	<i>Bromelia</i> sp.
FRP 288	<i>Bromelia balansae</i>
LC 4101	<i>Bromelia goeldiana</i>
FRP 319	<i>Dickia</i> sp.
FRP 353	<i>Pseudananas saganarius</i>
FRP 43	<i>Tillandsia</i> sp.
FRP 294	<i>Tillandsia</i> sp.
FRP 326	<i>Tillandsia</i> sp.
FRP 370*	<i>Bilbergia</i> sp.
FRP 377*	<i>Ananas</i> sp.
FRP 746*	<i>Ananas comosus</i> var. <i>comosus</i>

\*Genotypes evaluated only in 2002 and 2008.

## Development of 'Pérola' Pineapple Plantlets from Stem Sections in Different Recipients

Cledivone Soares da Silva<sup>1</sup>; Susana Cristine Siebeneichler<sup>2</sup>; Aristoteles Pires de Matos<sup>3</sup>

<sup>1</sup>Undergraduate Student, Federal University of Tocantins, Gurupi, TO; <sup>2</sup>Professor, DSc, Soil Sciences, Federal University of Tocantins, Gurupi, TO, [susana@uft.edu.br](mailto:susana@uft.edu.br); <sup>3</sup>Plant Pathologist, PhD, Senior Researcher, Embrapa Cassava & Fruits, Cruz das Almas, BA, [apmatos@cnpmf.embrapa.br](mailto:apmatos@cnpmf.embrapa.br)

### ABSTRACT

The pineapple stem sectioning technique is a viable method for production of good quality plantlets from both an agricultural and a sanitary perspective. This work aimed at evaluating the development of 'Pérola' pineapple plantlets obtained from stem sections and transferred to three growing recipients: seed beds, tubetes and polyethylene pots. Plantlet growth was determined at 20 day intervals from 20 to 140 days after planting based upon leaf number and height and stem diameter. In addition, at 140 days the dry weights of leaves and roots and root volume were determined for five plantlets per experimental pot. Plantlets grown in seed beds were taller, but with their root system widely spread in the soil. On the other hand, plantlets grown in tubetes were smaller and had a confined root system, which facilitated planting under field conditions.

**Key words:** *Ananas comosus* var. *comosus*, volume of root system, vegetative growth.

### INTRODUCTION

Pineapple is grown commercially all over Brazil. In the state of Tocantins, ranking ninth among the pineapple producing states of Brazil, there were 2,273 ha cultivated in 2010, with a production of 71,530 metric tons (IBGE, 2010). Slips are the most commonly used planting material for 'Pérola' pineapple crops in Brazil due to their large availability. Despite the feasibility of that practice, pineapple planting materials can be contaminated by pests and diseases, especially the fusariosis, caused by the fungus *Fusarium subglutinans* f. sp. *ananas*, which incites high yield losses to the pineapple crop in Brazil.

The stem sectioning technique enables production of pineapple plantlets free of pests and diseases, thus constituting an economically feasible alternative to reduce fusariosis dispersal. The objective of this work was to evaluate the development of pineapple plantlets, obtained from the stem sectioning technique, and transplanted to three distinct pot types.

### MATERIAL AND METHODS

The experiment was carried out at the campus of the Federal University of Tocantins, located in the municipality of Gurupi, at 11°43'S and 49°04'W, 280 m above sea level. Plantlets obtained by stem sectioning technique, 4.5 cm to 6.0 cm tall, were used in this work.

The experiment was carried out in a randomized blocks design with three replications, each plot containing 10 plantlets. Three growth recipients were tested for plantlet development: Tubetes (145 cm<sup>3</sup> of substrate); polyethylene pots (180 cm<sup>3</sup> of substrate); and seed beds. Before planting the plantlets were weighed, their diameters measured, and the number of leaves counted. All recipients were filled with the same substrate - a mixture of soil and carbonized rice residue (1:1; v:v) supplemented with single superphosphate, 0.6 g/L of substrate. Treatments were evaluated at 20 day intervals, from 20 to 140 days after planting, based upon the following variables: plantlet height, diameter and leaf number. In addition, at 140 days after planting were determined leaves and roots dry weights (after drying at 70° C for 48 hours), root length and volume, for five plantlets per plot. Data were submitted to variance and regression analyses. Average treatment values were compared by Tukey's test at 5%.

### RESULTS AND DISCUSSION

There was no significant effect of treatments studied on plantlet height up to 100 days after planting (Fig. 1). From then on plantlets in seed beds showed to be taller with a significant difference to the other treatments at 140 days after planting. This difference may be due to the fact that the seed bed plantlets could explore a larger soil surface and volume.

For the stem diameter no significant differences were observed among treatments (Fig. 2). This may be probably related to the partition of photo assimilates, directing nutrients to leaves and roots, important pineapple

organs at that stage of plantlet development, while the importance of the pineapple stem increases from the period in which the plant starts storing photo assimilates.

Similar to stem diameter, no significant statistical difference was observed for number of leaves (Fig. 3), even though the number was always a little lower for seed bed plantlets. In general, from 100 days plantlets started showing necrosis of basal leaves, together with a slight reduction of average leaf number, probably due to the size of the containers (polyethylene pots and tubetes) interfering on the nutritional imbalance.

As shown in Table 1, at 140 days after planting the root length of plantlets grown in seed beds was significantly longer than those of plantlets grown in polyethylene pots and in tubetes. These results suggest that root length of pineapple plantlets may be affected by the design of the container and by the volume of substrate in it. On the other hand, root volume was the largest for plantlets grown in tubetes, with a higher root density. It seems that due to the restriction for root length growth more roots were emitted in tubetes, thereby increasing the volume of the root system.

Leaf dry weight of pineapple plantlets transplanted to seed bed was significantly higher than those of plantlets grown in tubetes and in polyethylene pots, but there was no significant difference for stem and root dry weights.

Plantlets grown in seed beds showed better shoot development, but presented a root system spread in the soil substrate in a way that makes transplantation to the field rather difficult. On the other hand, pineapple plantlets grown in tubetes showed lower shoot development, but their root systems were less spread and denser, thereby facilitating plantlets transplanting to field conditions.

#### ACKNOWLEDGEMENT

This work was granted by the Federal University of Tocantins and the project CECT/CNPq number 61.0083/2006-6.

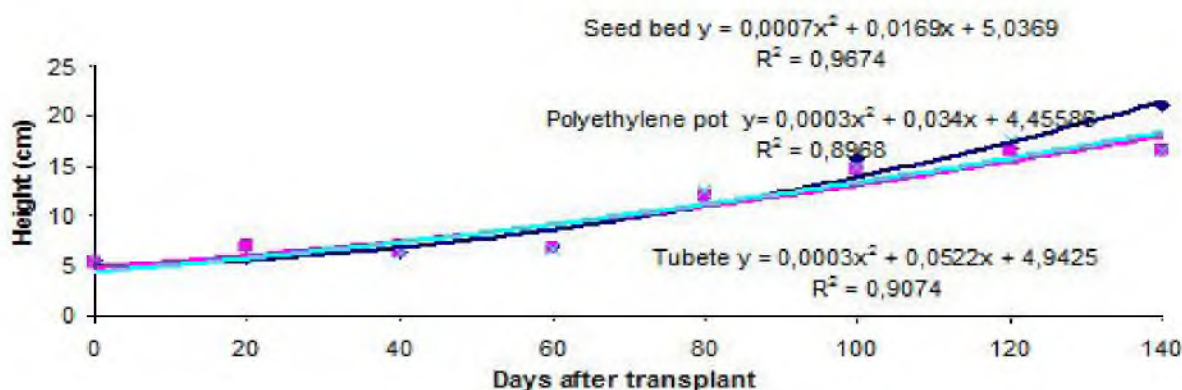


Figure 1. Growth in height of 'Pérola' pineapple plantlets grown in tubete (—), polyethylene pots (—) or beds (—), after transplanting.



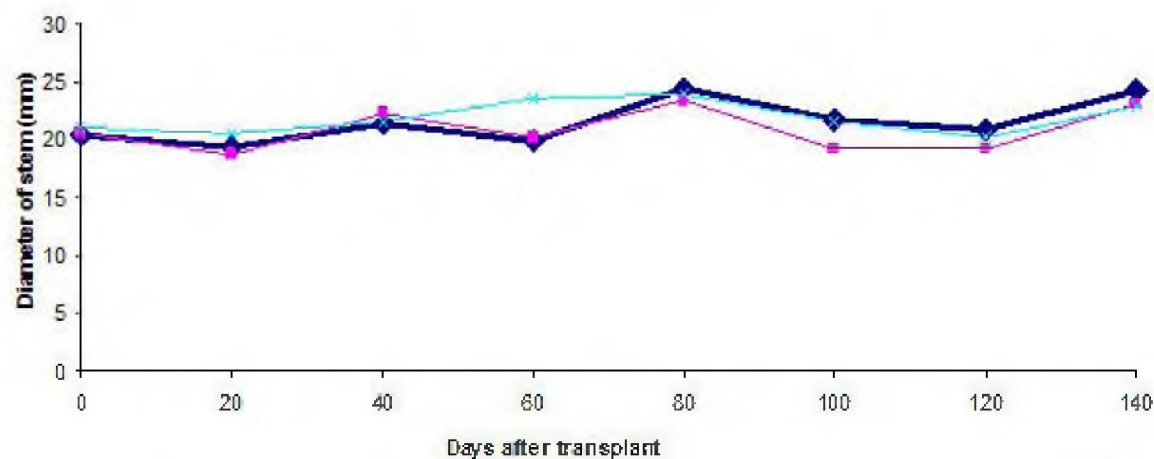


Figure 2. Growth in stem diameter of 'Pérola' pineapple plantlets after transplanting into tubetes (—), polyethylene pots (—) and seed beds (—).

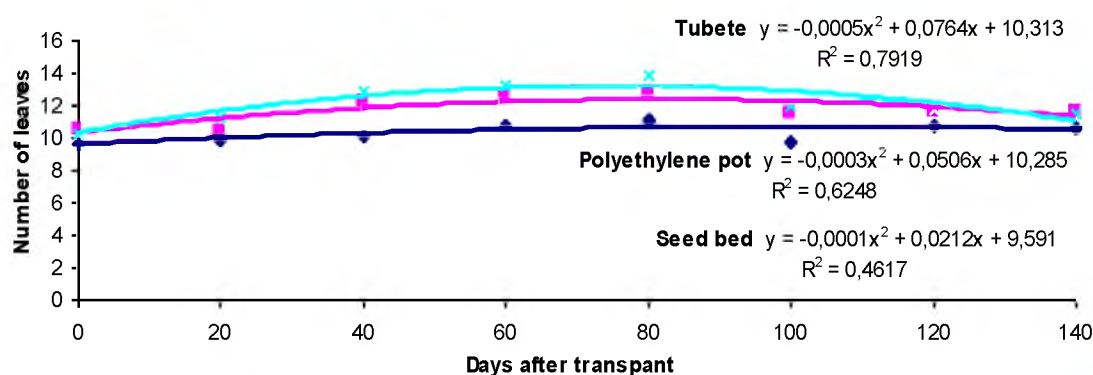


Fig. 3. Leaf number of 'Pérola' pineapple plantlets along 140 days after planting to tubetes (—), polyethylene pots (—) and seed beds (—).

Table 1. Leaf, stem and root growth of 'Perola' pineapple plantlets at 140 days after planting in seed beds, polyethylene pots and tubetes<sup>z</sup>.

Treatment	Height (cm)	Stem diameter (mm)	Leaf number	Root length (cm)	Root volume (cm <sup>3</sup> )	Dry matter (g)		
						Leaves	Stem	Roots
Seed bed	21.1 a	24.3 a	10.6 a	29.0 a	15.0 b	36.4 a	3.12 a	2.94 a
Polyethylene pot	16.6 ab	23.1 a	11.6 a	22.4 b	14.7 b	24.6 b	3.27 a	3.64 a
Tubete	16.5 b	23.0 a	11.5 a	13.5 c	19.3 a	24.3 b	3.47 a	3.20 a
CV (%)	8.72	2.36	7.81	6.96	9.07	13.5	9.61	11.9

<sup>z</sup> Data followed by the same letter in the column did not differ according to Tukey's test at 5 %.

## New Species of Insect Predators of Pineapple Mealybugs and Related Pests

Mark Paul Culik and José Aires Ventura

Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural – INCAPER

Rua Afonso Sarlo 160, CEP 29052-010, Vitória, Espírito Santo, Brasil

markculik3@yahoo.com, ventura@incaper.es.gov.br

Pineapple mealybugs and other scale insects are major pests of pineapple and other crops worldwide and control of these pests depends on the integration of appropriate management methods (integrated pest management). Unfortunately, there is a lack of information on beneficial insects (parasites and predators of pests) that may be of importance for integrated management of pests of agricultural crops, including pineapple, in many areas such as Espírito Santo, Brazil. Therefore, studies have recently been conducted to identify parasites and predators of scale insects in Espírito Santo during which several new species of insect predators of pineapple mealybugs and other pests were found.

A new species of fly, *Rhinoleucophenga capixabensis*, was collected from a pineapple heavily infested with pineapple mealybugs in Espírito Santo, Brazil, in 2008. A description of this new species of potential predator of pineapple mealybugs was published in 2009 (Culik and Ventura 2009). This beneficial insect belongs to the family Drosophilidae (fruit flies), genus *Rhinoleucophenga*, a little known group whose larvae are believed to prey on scale insects. Adult *R. capixabensis* have an appearance similar to small house flies, with red eyes and dark brown body about 2- 3 mm long (Figure 1A).

In addition, several other new species of predators belonging to the fly family Cecidomyiidae (more commonly known as gall midges), Figure 1B, were also collected in association with pineapple mealybugs and other scale insects in crops including coffee as well as pineapple in Espírito Santo in 2008 (Culik et al 2009a). Descriptions of these new species have been submitted for publication but unfortunately remain unpublished.

These and related results (Culik et al 2009b) indicate the great potential of natural enemies of pineapple mealybugs and similar pests that remain unknown in areas such as Espírito Santo, and the great need for additional research to more completely identify and better understand the value of such beneficial insects for integrated management of pests of pineapple and other crops.

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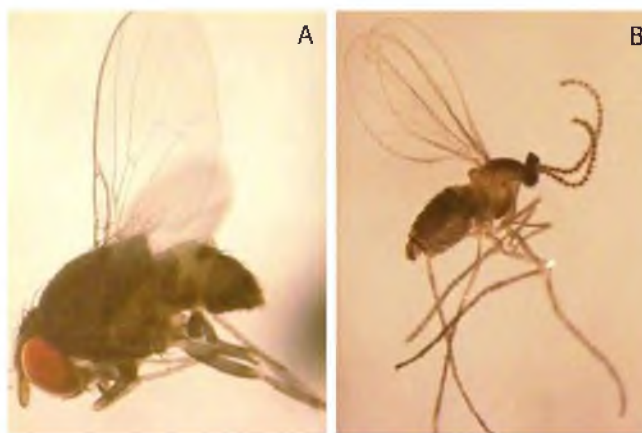


Figure 1. Predatory fly species that feed on pineapple mealybugs: *Rhinoleucophenga capixabensis* Culik & Ventura (A); *Diadiplosis* species (B).

## Pineapple Germplasm Bank at Embrapa Cassava and Fruit Crops

Cristina de Fátima Machado<sup>(1)</sup>, Fernanda Vidigal Duarte Souza<sup>(1)</sup>, José Renato dos Santos Cabral<sup>(2)</sup> e Everton Hilo de Souza<sup>(3)</sup>

<sup>(1)</sup> Senior researcher at Embrapa Cassava and Fruit Crops, Cruz das Almas, BA State, CP 44380-000. E-mail: cmachado@cnpmf.embrapa.br, (Contact author), fernanda@cnpmf.embrapa.br

<sup>(2)</sup> Agronomic Engineer and Master. E-mail: jrenatocabral@hotmail.com

<sup>(3)</sup> Agronomic Engineer and Doctoral student at CENA (Centro de Energia Nuclear na Agricultura) Universidade de São Paulo (USP), São Paulo, CEP: 13400-970. E-mail: hilosouza@gmail.com

### ABSTRACT

Genera the source of breeding programs of this crop. This work aimed to search information about characterization and agronomic evaluation in CNPMF pineapple gene bank in order to organize the documentation of this collection and to establish the current state of the art. Out of 616 conserved accessions, 197 have been characterized morphologically, 79 using molecular markets, 217 were evaluated for *fusarium* resistance, 35 cytogenetically and 84 to identify the ornamental potential in the collection. This source information made it possible make a correct diagnostic and to organize the documentation of the data generated in the last 30 years.

**Index terms:** *Ananas*, Germplasm collection, genetic resources, documentation

### INTRODUCTION

Brazil is one of the most important centers of origin and diversity of the genus *Ananas* and has the responsibility to preserve this germplasm as an important source of genetic variability to breeding programs of this important crop (Cabral and Souza, 2005).

Embrapa Cassava and Fruit Crops has one of the largest collections of pineapple in the world as a result of many collection activities during this last 30 years. Currently this Germplasm Bank has more than 600 accessions, most of them belonging to the genus *Ananas*, conserved under field conditions (Cabral, 2000).

However the genetic conservation of the accessions is not useful if these materials are not characterized and the generated information well documented and available to breeders. Since its creation many characterizations and evaluations have been done with several accessions in the genebank.

The morphological characterizations that have been done were based on morphological descriptors development for pineapple by International Board for Plant Genetic Resources (IBPGR, 1991). Molecular marker techniques, cytogenetic techniques, agronomic evaluations and assays of resistance to *fusarium* were some of the studies carried out in the last 30 years with accessions in the collection in order to generate useful information that could be used in genetic breeding programs.



This work aimed to search and organize all the available information and to establish the state of the art of pineapple gene bank in order to facilitate the management and use of this important genetic resource.

## MATERIAL AND METHODS

The pineapple gene bank is maintained under field conditions in an experimental area at Embrapa Cassava and Fruit Crops located in Cruz das Almas city in the State of Bahia-Brazil. This collection comprises more than 600 accessions with at least five plants/accession.

The data searching was done by consulting scientific documents (published papers and also unpublished documents). Original data has been organized and processed and has given rise to an upcoming paper (Machado et al., 2011). The morphological, cytogenetic and molecular characterizations were taken into account, including the physicochemical evaluation and tests for resistance to *fusarium*. The results were organized in tables, figures and graphics to facilitate the view of the current state of the art of the Pineapple Germplasm Bank.

## RESULTS AND DISCUSSION

The current distribution of the botanical varieties and related species in the bank (Figure 1) is dominated by *A. comosus* var. *comosus* and some pictures from these materials can be seen in Figure 2. However, of the 616 conserved accessions, only 197 were characterized morphologically, 79 using molecular techniques, 217 were evaluated to *fusarium* wilt and 35 were characterized cytogenetically (Table 1).

It was expected that *Ananas comosus* var. *comosus* would comprise the greatest percentage of performed characterizations considering the importance of this information to development of new varieties of pineapple fruit. The commercial pineapple belongs to this botanical variety. Fusarium wilt is the most important disease in Brazil and to find several resistance sources was fundamental to the breeding program. On the other hand such traits as sugar content, brix, fiber and pulp color are also very important to improve fruit quality. The results of these characterizations were the development of several hybrids resistant against fusarium wilt considering that three, Imperial, Vitoria and Ajuba, are already in commercial phase.

However in the last years a new ornamental focus has been explored in this collection. The wild varieties present a spectacular potential to be explored for ornamental plants and a breeding program with this aim has been carried out at Embrapa since 2003. Out of 616 accessions 84 genotypes were characterized with potential to develop potted plants, cut flowers and plants for gardens and squares. Some of them were selected as parents, several crosses were performed and new hybrids developed (Souza et al., 2009). Currently these hybrids are under regional and field evaluation to commercial validation.

Finally an important loss of some accessions was detected due mainly to lower adaptation capability of some of them to climate conditions of Cruz das Almas and after a long period of cultivation under field conditions. The majority of lost accessions originated from the Amazonia region, which has very high humidity and specific ecosystems. This situation was detected a few years ago, which led to the creation of an in vitro gene bank like a security copy to guarantee the conservation of these accessions (Souza et al., 2006). Currently this in vitro collection has 130 conserved accessions. Another 80 accessions were introduced recently under in vitro condition but they are still under the indexation phase to detect the presence or not of mealybug wilt-associated virus (PMWaV). A new methodology has been developed to clean the infected accessions (Andrade et al., 2010).

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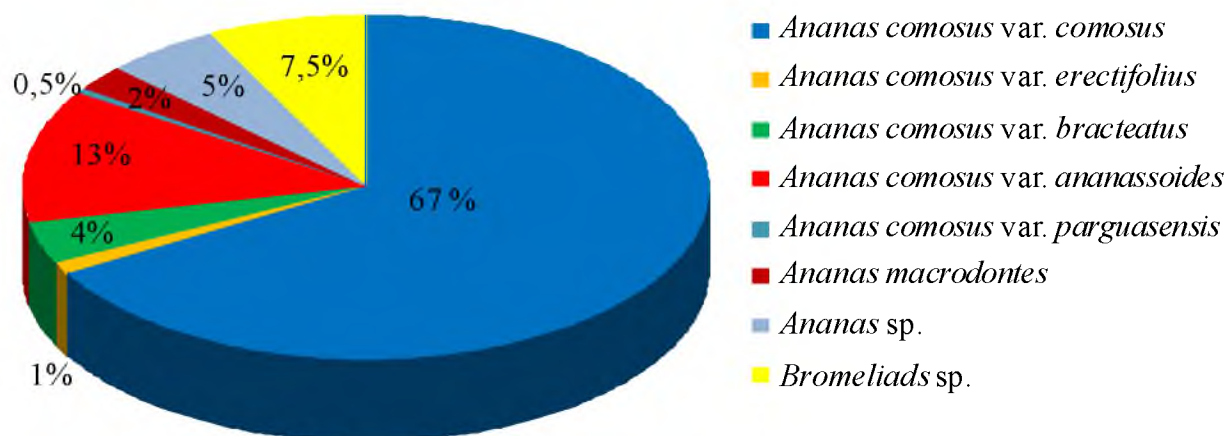


Figure 1. Percentage composition of the genus *Ananas* and related species in the Active Germplasm Bank of pineapple maintained by Embrapa Cassava and Fruits, Cruz das Almas, Bahia, Brazil.



Figure 2. Botanical varieties of the genus *Ananas* having potential value as ornamentals in the Active Germplasm Bank of Embrapa Cassava and Fruits: (AD) *A. comosus* var. *bracteatus*; (E-H) *A. comosus* var. *ananassoides*, (I-J) *A. comosus* var. *erectifolius*; (K) *A. macrodontes*, (L) *A. comosus* var. *parguasensis*; (M) *A. comosus* var. *comosus*. Cruz das Almas, Bahia, Brazil.

Table 1. Botanical varieties and species of pineapple (*Ananas (A.)*) and bromeliads characterized and stored in the Active Germplasm Bank of Embrapa Cassava and Fruit. Cruz das Almas, Bahia, Brazil. Sources are ACC (*A. comosus* var. *comosus*), ACA (*A. comosus* var. *ananassoides*), ACE (*A. comosus* var. *erectifolius*), ACB (*A. comosus* var. *bracteatus*), ACP (*A. comosus* var. *parguasensis*), AM (*Ananas macrodentes*), As (*Ananas* sp.), Bs (*Bromeliads* sp.).

Source	Accessions characterized (% of total within a species)					Accession conservation		
	Morphologica l	Molecular	Fusarium test	Cytogeneti c	Ornamenta l	<i>In vitro</i>	Field	Lost*
ACC	194 (47.20)	78 (18.98)	180 (43.80)	35 (8.52)	8 (1.95)	98	411	128
ACA	1 (1.28)	0	6 (7.69)	0	29 (37.80)	14	78	35
ACE	0	0	3 (50.00)	0	3 (50.00)	3	6	7
ACB	0	0	9 (37.50)	0	24 (100.00)	8	24	0
ACP	0	0	0	0	2 (66.67)	0	3	9
AM	0	0	2 (12.50)	0	4 (25.00)	0	16	2
As	2 (6.45)	1 (3.22)	6 (19.35)	0	13 (41.94)	6	31	2
Bs	0	0	11 (23.40)	1 (2.13)	4	47	10	
TOTAL	197	79	217	35	84	130	616	193

\* Lost accessions from 1979 to 2008.

## Stem Section Plantlet Development Influenced by Size at Transplanting

Ivivo Alves Milhomem<sup>1</sup>; Susana Cristine Siebeneichler<sup>2</sup>; Aristoteles Pires de Matos<sup>3</sup>

<sup>1</sup>Undergraduate Student, Federal University of Tocantins, Gurupi, TO; <sup>2</sup>Professor, DSc Soil Sciences, Federal University of Tocantins, Gurupi, TO, susana@uft.edu.br; <sup>3</sup>Plant Pathologist, PhD, Senior Researcher, Embrapa Cassava & Fruits, Cruz das Almas, BA, apmatos@cnpmf.embrapa.br

### ABSTRACT

Production of plantlets from pineapple stem sections has been a viable method for getting healthy planting material. This technique has been especially useful in Brazil where slips and suckers are the main vectors of interregional dissemination of *Fusarium subglutinans* f. sp. *Ananas*. The objective of this work was to determine the best height of pineapple plantlets obtained from stem sections for their transplanting. Plantlets were transplanted to tubetes at heights of 1, 2, 3, 4 and 5 cm. At 140 days after transplanting 4 and 5 cm plantlets grew faster as measured by plant height, stem diameter and leaf emission.

### INTRODUCTION

Pineapple, *Ananas comosus* L. (Merrill) var. *comosus* Leal & Coppens, is susceptible to several pathogens that cause severe yield losses. *Fusarium subglutinans* f.sp. *ananas*, the causal agent of the pineapple fusariosis in Brazil, infects the whole plant, including the planting material. Infected vegetative propagules are responsible for the spread of fusariosis from one pineapple growing area to others (Matos et al., 2009). Once infected planting material is introduced in a given growing region, the pathogen is disseminated by wind, rain splash and insect vectors, as well as by the movement of infected propagation parts (Matos, 2003). It is estimated that 15 to 20% of pineapple planting material is discarded during visual pre planting selection but this does not assure that the planting material brought to the field is *Fusarium* free (Ventura & Costa, 2002).

Healthy planting material reduces the initial inoculum level and is the first measure to be done as part of the integrated management of the pineapple fusariosis (Matos, 2003). In addition, using pathogen free planting material also prevents the accidental introduction of that disease in pineapple growing areas.

The stem sectioning technique has been adapted as a good tool for the multiplication of valuable mother plants and to obtain healthier planting material. However, there are still a few aspects of this technique to be improved, especially for the 'Pérola' pineapple plant, which has been less studied than the well-known 'Smooth



Cayenne' cultivar. The objective of this work was to determine the best height for transplanting pineapple plantlets obtained from stem sections.

## MATERIAL AND METHODS

This work was carried out at the campus of Gurupi, Federal University of Tocantins. The experimental design was a randomized block with three replications with X plant per replicate. Plantlet heights at transplanting were 1, 2, 3, 4 and 5 cm. Selected mother plants obtained at 30 days after harvest from a commercial 'Pérola' pineapple plantation were used for plantlet production by the stem sectioning technique. Stems cross-sections 10 cm long were then longitudinally sectioned twice to generate four stem sections. After treatment with fungicide to prevent fungal infection, the stem sections were placed on seed beds to allow axillary buds to develop into plantlets.

Pineapple plantlets were transplanted to tubetes filled with 300 cm<sup>3</sup> of substrate, a mixture of soil and cattle manure (1:1; v:v), supplemented with 0.6 g super phosphate per liter. Foliar spray of nutrient solution was started three weeks after transplanting and was performed at weekly intervals. The nutrient solution contained 1% N, 1% KCL, 0.005% B, 0.012% Ca, 0.0048 %Mg, 0.0002% Cu, 0.005% Fe, 0.005% Mn, and 0.0005% Zn. Watering by micro sprinkler irrigation was done twice a day.

Evaluations of plantlet height stem diameter and number of leaves were made at 20-day intervals from 20 to 140 days after transplanting. Plantlets were removed from the tubetes and washed in running water. Leaves, stems and roots were removed and dried separately at 70 °C for 48 hours and their dry weights were determined. Data were analyzed by regression analyzes using the SISVAR program.

## RESULTS AND DISCUSSION

There was a positive quadratic response for height, stem diameter and leaf number along the experimental period (Figures 1, 2 and 3). Increases in height, stem diameter and number of leaves were directly proportional to the plantlet size at transplanting (Fig 1, 2, 3). These results suggest that the greater leaf area and nutritional reserves of larger plantlets at transplanting time determined their improved shoot development. In addition, smaller plantlets at time of transplanting may also be more sensitive to stress than larger ones, resulting in slower growth.

Pérola plantlets 4 and 5 cm tall at the time of transplanting had greater plant height, diameter of stem and number of leaves than did plantlets that were 1, 2 and 3 cm tall. Plantlets transplanted when 3 cm tall had larger diameter of stem than those transplanted when 1 and 2 cm tall. Plantlets transferred when 1, 2 and 3 cm tall were of similar height and had the same number of leaves.

Considering that the average size of pineapple planting material to be brought to the field is around 30 cm (Matos et al., 2009), it is clear that pineapple plantlets produced by stem sectioning should be at least 4 to 5 cm tall at time of transplanting.

Leaf, stem and root dry weights obtained at 140 days after transplanting the plantlets increased with plantlet size at transplanting time (Table 2). Plantlets 5 cm tall at transplanting had greater leaf, stem and root dry weights than did 4 cm plantlets, showed the best results regarding to these parameters, however stem dry weights of the two groups of plants were not different. These results confirm those observed for the shoot development shown in Table 1. The differences were especially great for root development.

## CONCLUSIONS

Plantlets obtained from 'Pérola' pineapple stem sections should not be transplanted before reaching a height of 4 cm to 5 cm, as they present a much faster shoot and root growth than that observed for smaller plantlets at transplanting date.

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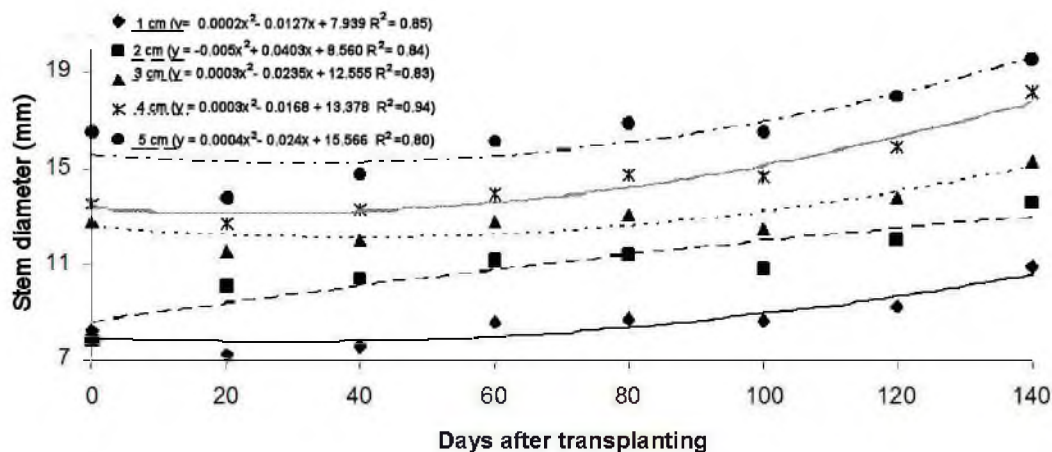


Figure 1. Growth in height of plantlets ranging from 1 to 5 cm tall at time of planting.

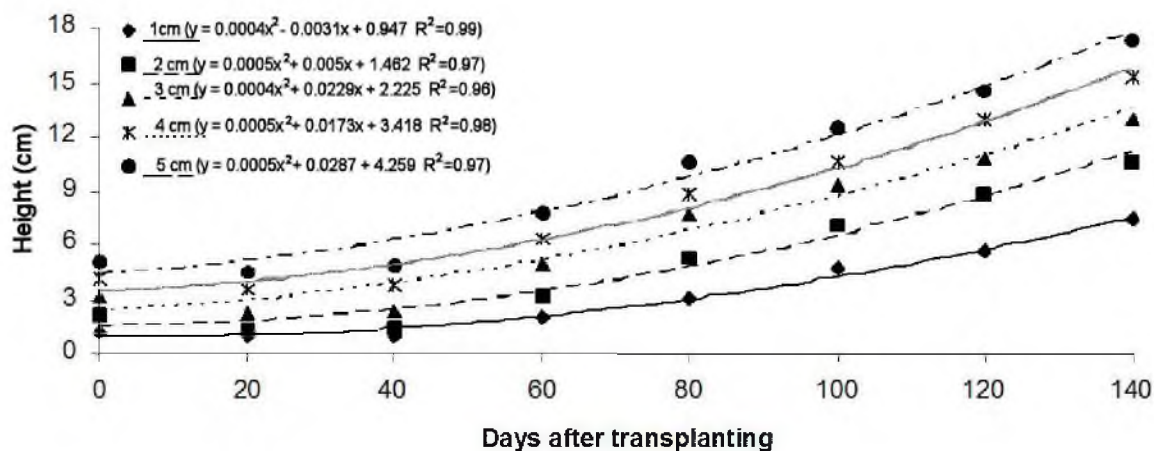


Figure 2. Increase in stem diameter over time for plantlets ranging from 1 to 5 cm tall at time of planting.

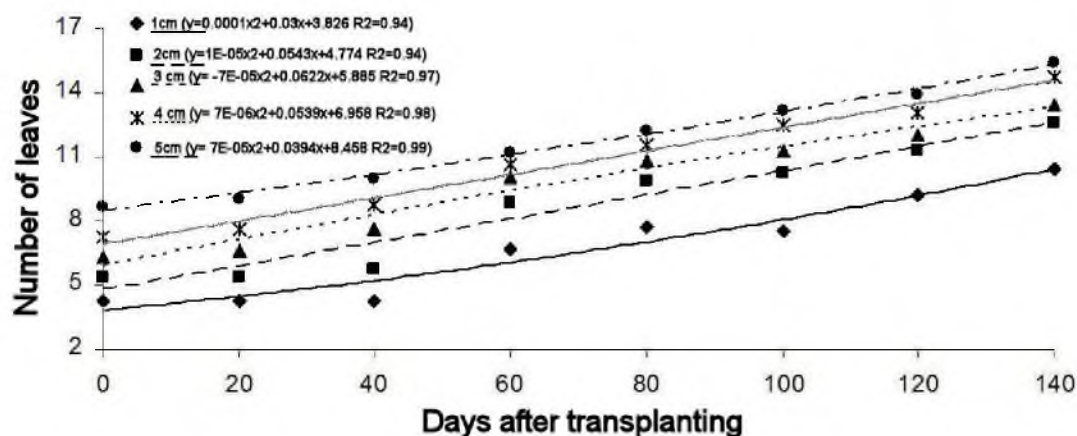


Figure 3. Increase in leaf number over time for plantlets ranging from 1 to 5 cm tall at time of planting.

Table 1. Height, stem diameter and number of leaves of 'Pérola' pineapple plantlets at 140 days after transplanting, as affected by the size of the plantlets at time of transplanting. Gurupi, TO, Brazil, 2010.

Plantlet height	Height (cm)	Stem Diameter (mm)	Leaves/plant
1 cm	7.42 d <sup>z</sup>	10.87 d	10.40 c
2 cm	10.54 c	13.65 c	12.55 b
3 cm	12.0 bc	15.33 b	13.42 b
4 cm	15.3. ab	18.19 a	14.79 a
5 cm	17.35 a	19.57 a	15.37 a
VC (%)	7.6	3.3	3.4

<sup>z</sup>Data followed by the same letter in the same column did not differ according to Tukey's test at 5%.

Table 2. Effect of height of 'Pérola' pineapple plantlets, at time of transplanting to tubetes, on dry weights of leaves, stem and roots, at 140 days after transplanting. Gurupi, TO, Brazil, 2010.

Plantlet height	Dry matter (g)		
	Leaf	Stem	Root
1 cm	0.74 d <sup>z</sup>	0.04 d	0.05 d
2 cm	1.78 d	0.09 cd	0.10 d
3 cm	3.02 c	0.13 bc	0.16 c
4 cm	4.12 b	0.16 ab	0.30 b
5 cm	5.20 a	0.22 a	0.37 a
CV (%)	12.5	18.04	10.26

<sup>z</sup>Data followed by the same letter in the same column did not differ according to Tukey's test at 5%.

## News from China

### Integrated Effects of Ethylene Absorbents on Flower Forcing of Yellow Mauritius Pineapple

Liu Sheng-hui<sup>1,2</sup>, Zang Xiao-ping<sup>1,2</sup>, Zhang Xiu-mei<sup>1,2</sup> and Sun Guang-min<sup>1,2</sup>

<sup>1</sup>South Subtropical Crops Research Institute of CATAS, Zhanjiang, Guangdong 524091, China.

<sup>2</sup>National Center of Important Tropical Crops Engineering and Technology Research, Haikou, Hainan.

#### INTRODUCTION

Pineapple (*Ananas comosus* [L.] Merril) is one of the tropical fruits most fit for organic cultivation because of few pests and diseases during the growth period. In China, pineapple flowering usually takes place in spring through the low temperature and short photoperiod in winter. However, the flower period can be controlled and adjusted by external hormones, which is called forcing. Pineapple forcing technique was widely studied all over the world in order to provide an all-year balanced commodity supply.

Ethylene is one of the gas hormones which can induce all the Bromeliaceae plants, and it is the most effective in pineapple's forcing. However, its solubility in water is very low (0.0149g) and needs a special pump to supply high pressure, so it is hard to manipulate for mountain cultivation and is only fit for the mechanized pineapple plantation. Ethephon is the most popular forcing agent with low price and facility. However no chemical reagents are permitted in organic cultivation, including forcing agent. It was reported that cooling plants by adding ice or shading could force some cultivars. However, several applications are required and it only works with sensitive cultivars. Therefore, it is not a very practical method.

Activated carbon (AC), also called activated charcoal or activated coal, is a form of carbon that has been processed to make it extremely porous and thus to have a very large surface area available for adsorption of chemical reactions. Zeolite is a microporous aluminosilicate mineral commonly used as a commercial adsorbent. The pore diameter is at the molecular-level and only the material with molecular diameter less than the pore diameter can be absorbed by the zeolite. Hence, zeolite was used as a solid absorbent in the chemical industry to dry, purify, separate or reclaim gas and liquid. It can be regenerated after use. Both AC and zeolite were widely used as carriers in the absorption of waste gas and for the clarification of gas. In this study, AC and zeolite were used as ethylene carriers, which were then used to force "Yellow Mauritius" pineapple, 200mg/L ethephon as control, to reveal the effect of ethylene absorbents on the forcing of pineapple.

#### MATERIALS AND METHODS

The experiment was conducted in South Subtropical Crops Research Institute of China Academy of Tropical Agriculture Science in 2010. Pineapple (*Ananas comosus* cv. Comte de paris) plants 14 months old were selected for flower forcing. No urea was applied before forcing. A 5A zeolite was supplied by Shanghai Molecular Sieve Limited Company and 40% ethephon was supplied by Shanghai Pengpu Chemistry Factory. The experiment was repeated three times in different blocks. Foshan Meisaier Gas Company supplied 99% ethylene gas. Ethephon forcing was done using a solution containing 400 mg/L ethephon and 2% urea and 50 ml was applied into the shoot apex (Liu, 2009). A 2% urea solution was applied to the control plants.

The molecular sieve and active carbon used for forcing were prepared by filling a vessel with the respective adsorbent. Ethylene gas was then flowed slowly into the vessel through a pipe until no further uptake occurred.

Forcing of the plants was done at 6 pm in June. Zeolite and active carbon which had absorbed the ethylene were put into the heart of the pineapple plant. Each plant was treated with, in g, 0.1, 0.2 or 0.3 of the respective product. Control plants were treated with 30 ml of a 200 mg L<sup>-1</sup> of ethephon solution. There were 20 plants for each treatment with 3 replications.

Data on inflorescence emergence time and rate were collected and then fruits were harvested when one-third of fruitlets were yellow. Fruit weight, fruitlet number, total soluble solids, titrable acids and vitamin C were measured. The data were statistically analyzed using DPS software system.



## RESULTS

Flowering rate was the important index of whether the pineapple forcing technique was successful or not, and directly influenced the yield of pineapple. Results showed both 0.1 to 0.3 g ethylene-saturated zeolite and ethephon solution induced 100% of Yellow Mauritius flowering (Table 1). The maximum flowering rate of plants treated with ethylene-saturated active carbon was only 60%, which is not satisfactory for the needs of commercial cultivation. There was no significant difference in flowering date among all treatments. Yellow Mauritius is very sensitive to flower forcing by ethylene, however, a high percentage of forcing was not achieved with the amounts of ethylene-saturated activated carbon used in this research.

The forcing effect of active carbon was related to amounts applied to some extent. And, with the increase of absorbent, the fruitlet number and weight decreased but showed no significant difference. There were no significant differences in total soluble solids, total titrable acids and vitamin C among all the treatments.

## DISCUSSION

Both activated carbon and zeolite have been widely applied in the storage and conservation of fruits and vegetables. For example, adding some  $\text{KMnO}_4$  to the active carbon helps to clear the ethylene released by fruits and vegetables. It is also reported that active carbon added to ethephon or  $\text{CaC}_2$  solution can improve forcing of pineapple. However, no studies were found on the direct application of ethylene-saturated active carbon on pineapple forcing.

Li et al. (2001) reported that the ethylene-absorbing character of activated carbon III was much better than that of 5A zeolite. However, in this experiment, the former showed the lower flowering rate than the latter in pineapple forcing, maybe it was because the ethylene in the activated carbon was much easier to be desorbed and then released in the air, so the pineapple plants couldn't absorb it in time.

Liu (2009) reported that there was an optimal ethephon concentration for "Pearl" pineapple forcing. When the ethephon concentration exceeds the best one, the fruitlet numbers and fruit weight will be reduced. In this trial, the forcing effect of activated carbon was related with the amounts applied to the plants. There was no significant difference in fruitlet number as the quantity of zeolite applied increased. Therefore, Yellow Mauritius, forcing with 0.1g of ethylene saturated zeolite was the best treatment.

In this paper, 0.1-0.3 g of ethylene saturated 5A zeolite not only induced 100 percent flowering of Yellow Mauritius. Because a zeolite granule weighs an average of 0.05 g, only 2 granules are needed to force pineapple. Furthermore, no water is needed for forcing. In China, the main pineapple producing area are located in the mountain area with poor water supply. Hence, this method definitely is a very convenient way to force pineapple. What is more important is that zeolite is environment-friendly and can meet the requirement of organic pineapple production.

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Table 1 Effects of different forcing agents on the flowering rate and flowering time of Yellow Maritius pineapple.

Treatment	Flowering rate (%)	Days to inflorescence emergence
Ethephon control	100	35
Active carbon 0.1g	30	37
Active carbon 0.2g	50	37
Active carbon 0.3g	60	37
5A Zeolite 0.1g	100	37
5A Zeolite 0.2g	100	36
5A Zeolite 0.3g	100	36

Table 2 Effects of ethephon (CK) and ethylene-saturated activated carbon (AC) or zeolite (Z) on the characteristics of Yellow Mauritius pineapple fruits.

Treatment	Fruitlet number	Fruit weight (g)	TSS (%)	TA (%)	VC (mg/100gFw)
CK	118 $\pm$ 5 c	1145 $\pm$ 90 a	14.5 $\pm$ 0.2 a	0.32 $\pm$ 0.08 a	32.3 $\pm$ 1.2 a
AC 0.1 g	133 $\pm$ 5 a	1187 $\pm$ 90 a	14.3 $\pm$ 0.3 a	0.31 $\pm$ 0.05 a	31.8 $\pm$ 1.1 a
AC 0.2 g	130 $\pm$ 8 ab	1174 $\pm$ 94 a	14.35 $\pm$ 0.2 a	0.32 $\pm$ 0.04 a	31.9 $\pm$ 1.4 a
AC 0.3 g	126 $\pm$ 6 ab	1165 $\pm$ 106 a	14.6 $\pm$ 0.2 a	0.32 $\pm$ 0.03 a	32.4 $\pm$ 1.2 a
Z 0.1 g	132 $\pm$ 4 ab	1186 $\pm$ 108 a	14.3 $\pm$ 0.38 a	0.31 $\pm$ 0.07 a	31.9 $\pm$ 1.2 a
Z 0.2 g	129 $\pm$ 5 ab	1179 $\pm$ 80 a	14.4 $\pm$ 0.2 a	0.33 $\pm$ 0.09 a	32.1 $\pm$ 1.3 a
Z 0.3 g	127 $\pm$ 3 ab	1154 $\pm$ 89a	14.3 $\pm$ 0.2 a	0.31 $\pm$ 0.08 a	32.0 $\pm$ 1.4 a

Means within the same column followed by same letter are not significantly different at  $p < 0.05$  (lower case) or  $p < 0.01$  (upper case).

## News from Costa Rica

### Physiological Alterations and Phytopathological s Causes of the "Sunken" Damage in Pineapple Fruits

C. Demerutis, O. Aguirre, E. Rodríguez. Universidad EARTH, Las Mercedes de Guácimo, Limón, Costa Rica. E-mail: [cdemerut@earth.ac.cr](mailto:cdemerut@earth.ac.cr)

In order to evaluate the cause of "Sunken" damage in 'MD-2' pineapple fruit (Figure 1A, B) after harvest, we evaluated whether the levels of dehydration, mechanical damage and phytopathological tests can contribute to the occurrence of such damage on the fruit. For the different treatments we used fruit produced by companies located in Najera Pococí, Lemon and Finca el Tremedal located in San Carlos, and then were transported to the Laboratory of Food Processing and Natural Sciences located on the campus of the University EARTH, where treatments were evaluated, each in their different timelines as indicated by the methodology of this work in 2010.

The results indicated that in the presence of Sunken in mechanical damage, it increases with the passage of days after harvest, just as in fruit harvested from the field, as in fruit packed, so it is not considered a influential factor in the emergence of Sunken. On their part the result of dehydration show that there is a relationship between the occurrence of damage and loss of weight from dehydration, and that the more weight they lost the fruit, damaged fruit count was higher. Finally, the assessment notes that the fungus Sunken injury affects only the skin of the fruit without causing internal damage, likewise found that the fungi are saprophytic, *Fusarium* being the most common, and that they should be taking advantage of the injuries caused by damage to stay in them. In conclusion the result, the Sunken damage is attributed to the three possible aspects evaluated.

### Nutritional Status of Pineapple (*Ananas comosus*) as a Possible Cause of the "Sunken" Fruit Disorder

C. Demerutis, P. Alvarez, C. Moreno. Universidad EARTH, Las Mercedes de Guácimo, Limón, Costa Rica. E-mail: [cdemerut@earth.ac.cr](mailto:cdemerut@earth.ac.cr)

In order to identify the causes of the "Sunken" (Figure 1A,B) appearance in 'MD-2 pineapple (*Ananas comosus*), we studied the nutritional status of fruit to identify nutritional deficiencies as a possible cause of this symptom. For this, we sampled on two farms, each sample was subjected to laboratory analysis to study levels of calcium and silicon, this applied to field samples collected at different physiological ages. Another part of the samples were stored for a period of fifteen days to follow up the symptom in the postharvest stage, five, ten and fifteen days of stored fruit. For purposes of this evaluation used the "Equator Limited Method." In obtaining the results of nutritional status and incidence of symptoms of both farm was tested them. The concentrations of calcium and silicon in fruits produced by the farm with the highest incidence of the disorder were lower than fruits from the farm with a lower incidence of symptoms. In this way we can infer that the causes of the "Sunken" disorder are due to nutritional status of the fruit. A higher nutritional content, a lower incidence of symptoms. For both farms, it was observed that the fruit does not absorb nutrients as fast as it grows, and this is a limitation and a possible cause of symptom onset.



Figure 1A, 1B. Photos of “Sunken” fruit disorder in ‘MD-2’ pineapple fruits.

Note: The above abstracts were translated from the originals in Spanish with the assistance of <http://www.spanishdict.com/> with additional help from Glorimar Marrero and Prof. Anne Alvarez, Dept. of Plant and Environmental Protection Sciences, Univ. of Hawaii, Honolulu, HI 96822.



## News from France

### Pineapple Systemic Resistance, an Ecological Control of Nematodes?

Soler A. ([alain.soler@cirad.fr](mailto:alain.soler@cirad.fr)) and Marie-Alphonsine P.A., CIRAD Martinique, Quénéhervé P., IRD Martinique, PRAM. Tel (596) 596 42 30 92.

#### INTRODUCTION

Pineapple monoculture and the use of pesticides reduced the biodiversity in agrosystems and increased the imbalance between pathogenic and beneficial organisms. Plant natural defenses may contribute to an ecologically based IPM as an alternative to pesticides. These plant defenses may be induced by chemical elicitors or non pathogenic micro-organisms (Ahmed *et al.*, 2000; Vallad and Goodman, 2004; Avis *et al.*, 2008; Backman and Sikora, 2008; Chaves *et al.*, 2009). They are called Induced Systemic Resistances (ISR) when they are the result of interrelationships of plants with non pathogen micro-organisms. They are called Systemic Acquired Resistances (SAR) when they are the result of a 'primary' attack of pathogens (Jourdan *et al.*, 2008).

Chemical elicitors such as methyl-jasmonate (Met-jasm), salicylic acid (SA) and  $\beta$  1-3 glucans (LAM) are commonly used in systemic resistance experimentations. After elicitation, these molecules are produced by the cell itself and act as part of the signaling system that transfers the information towards the different part of the plant. As elicitation simulates a moderate stress, the early physiological reaction of the plant includes changes in enzymatic activities involved in the classical "oxidative burst" and in lipoxigenase and phenylalanine ammonia lyase pathways as part of the defenses of the plant (Mittler, 2002; Apel and Hirt, 2004; Gunes *et al.*, 2007).

We showed that pineapple plants ('MD-2') can produce a systemic resistance against nematodes after application of the elicitors Met-jasm, SA and LAM. We measured *Rotylenchulus reniformis* populations and the evolution of enzymatic activities linked to plant defenses (oxidative enzymes such as catalase (CAT) and superoxide dismutase (SOD), and lipoxigenase (LOX) and phenylalanine ammonia lyase (PAL) as key enzymes for signaling molecules, Met-jasm, and SA. These enzymatic activities were tested as potential markers of physiological changes after 3 elicitor treatments.

#### MATERIAL AND METHODS

'Smooth Cayenne' (SC) and 'MD-2' were grown in 1 L pots for 2 months. Elicitor treatments were soil applications of 50 mL of  $10^{-4}$  M Met-jasm,  $10^{-3}$  M SA, or 37 g/L LAM, 3 times at one week intervals. Plant fresh weight at treatment was  $201.6 \pm 42.7$ g. One week after the last elicitor application pots were inoculated with 5000 *R. reniformis* larvae. Nematode populations development was evaluated 45 days after inoculation. The CAT, SOD, PAL and LOX activities were measured on roots of treated but non-inoculated plants. Enzyme activities were expressed in units/g fresh weight of root in the crude extract, 1 unit CAT = 1 dDO/min at 240 nm ( $\text{H}_2\text{O}_2$  consumption) and 1 unit SOD = 1% inhibition of  $\text{O}_2^-$  production by xanthin oxidase activity on xanthin, dDO at 234 nm, 1 unit LOX = 1 dDO/min at 234 nm on linoleic ac and 1 unit PAL = 1 dDO/h at 420 nm on phenylalanin. Standard deviations at  $\alpha=0.05$ .

#### RESULTS

Here, we will mainly focus on the data obtained after Meth-jasm treatment.

**1- Nematode populations:** 'MD-2' is more tolerant to nematodes than SC (Soler *et al.*, 2009) and set up efficient defenses against nematodes (Fig 1) while SC did not (data not shown). The nematode population growth was slowed by Met-jasm and SA (Fig. 1) and *p* values were 0.003, 0.021 and 0.111 respectively for Met-jasm, SA and LAM. Most of the decreases ranged from 30 to 70%. Fecundity was particularly affected with reduced egg numbers (data not shown).

**2- Biochemical markers:** Met-jasm and LAM induced similar and significant decreases in CAT and PAL activities while SA treatment did not (Fig 2 and 3). Meanwhile LOX activity increased significantly, especially in 'MD-2', and a slight increase in SOD was also observed. Globally, both varieties underwent similar physiological changes after the elicitor treatments at least as far as the enzymatic activities we measured were concerned.

## DISCUSSION

Pineapple set up efficient defenses against nematodes after elicitor treatments reducing both number and fecundity of nematodes, but tolerant and sensitive varieties did not react equally. Is the 'MD-2' response a Systemic Resistance? Similar experiments using a split-root system are intended to confirm this (Fig 4; data to be published).

The enzymatic activities measured here can characterize physiological changes in the plant but these were similar in both cultivars. LOX controls the Met-jasm pathway, while PAL controls the SA and phenylpropanoids pathways. The LOX activity increase may reflect an enhanced biosynthesis of the signal molecule Met-jasm, characterizing Induced Systemic Resistance (ISR) set up. The decrease of PAL activity is less clear as it means lower levels of phenylpropanoids are involved in plant defense. It may also reflect a necessary balance between SA and Met-jasm pathways (Beckers and Spoel, 2006).

After the Methyl-jasm treatment, we also observed a slight increase of SOD activity, the first line of defense against  $O_2^{2-}$ , a decrease of CAT activity (Fig 2). This could lead to an increase of  $H_2O_2$  concentration in root cells. In turn, the  $H_2O_2$  could have played a direct role in defense against eventual pathogens or as part of the signaling system through the plant (Mittler, 2002; Apel and Hirt, 2004) possibly contributing to set up a systemic resistance.

Although the physiological changes we observed were similar for both cultivars, it appeared that an efficient ISR against nematodes could take place only on the more tolerant variety 'MD-2'. This indicates that some of the mechanisms set up by the two cultivars after elicitor treatments are general responses but the efficient ones against nematodes are specific for 'MD-2' and probably linked to its genome.

## CONCLUDING REMARKS

Based on the available literature on this topic, we assume that non-pathogenic endophytes (Rhizobacteria and fungi) would have induced plant natural defenses in pineapple more efficiently (long lasting effect and maybe stronger) than the chemical elicitors used here (Backman and Sikora, 2008; Jourdan *et al.*, 2008; Chaves *et al.*, 2009). It is our further objective to investigate this research area.

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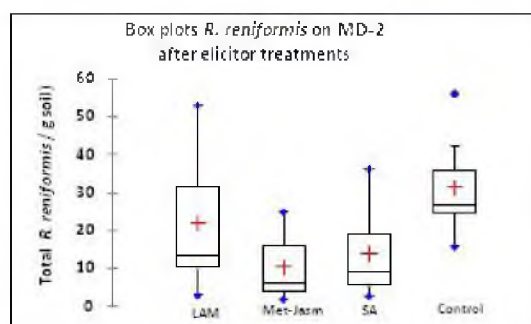


Figure 1 : Box plots for data on the total\* nematode population after elicitor applications on the root system of MD-2. \* (Total stands for “adults + pre adults + eggs”).

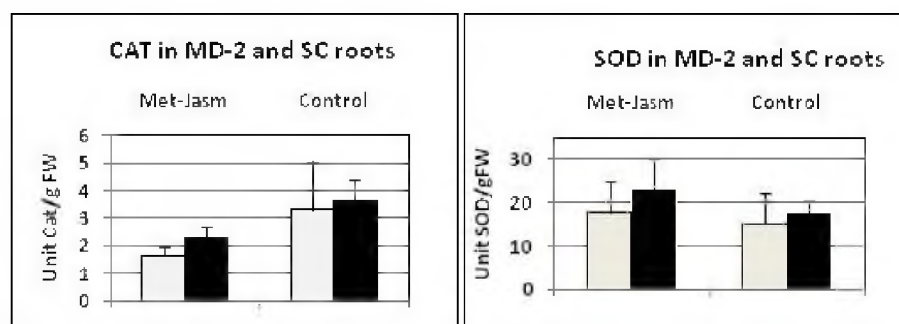


Fig 2 : Oxidative enzymes controlling toxic reactive oxygen species (catalase – CAT and superoxide dismutase – SOD; Black bars = MD-2, Grey bars = Smooth Cayenne (SC)).

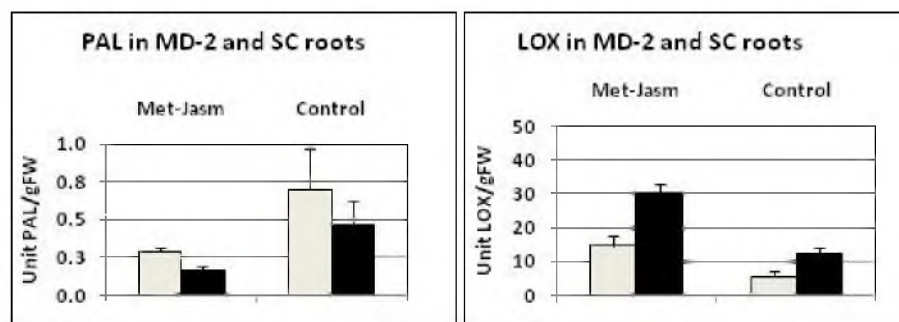


Fig 3: Enzymes controlling the Signaling molecules pathways (PAL – phenylalanin ammonia lyase and LOX – lipoxigenase; Black bars = MD-2, Grey bars = SC).



Fig 4: Tissue cultured 'MD-2' plants grown in split-root system culture to demonstrate the systemic effect of elicitor application on nematode population growth. (Experiment in progress).

## Leaf Margin in Pineapple

Geo Coppens d'Eeckenbrugge<sup>1</sup> and Garth Sanewski<sup>2</sup>

<sup>1</sup>CIRAD, UMR 5175 CEFV, 1919 Route de Mende, 34293 Montpellier, France. [geo.coppens@cirad.fr](mailto:geo.coppens@cirad.fr)

<sup>2</sup>DEEDI, Maroochy Research Station, 47 Mayers Rd, Nambour, Queensland Australia  
[garth.sanewski@deedi.qld.gov.au](mailto:garth.sanewski@deedi.qld.gov.au)

Leaf margin form is an important descriptor in pineapple and is one of the distinguishing characteristics of cultivars and botanical varieties. More importantly it can be a key distinguishing feature for intellectual property protection such as Plant Breeders Rights or Plant Patent. Six leaf margin types were originally described by Collins (1960), spiny, spiny tip, scallop, smooth, piping and sandpaper. Loison-Cabot (1990) described six leaf margin types (considering the large spines of *Ananas comosus* var. *bracteatus* as a distinct type and omitting scallop).

It is known that the environment can influence the expression of spines in some genotype backgrounds and this, together with some misunderstanding, can result in erroneous varietal descriptions. A brief description is given here of six leaf margin types and is mostly intended for varietal description purposes. A summary of the most well accepted understanding of the genetic determinism for leaf margin is also given. This follows Collins (1960). Kinjo (1993) presented segregation ratios similar to those predicted by Collins (1960) but presented a slightly different interpretation. Usberti-Filho et al. (1995) also modified Collins' hypothesis, to explain particular segregation ratios in three of their crosses, but did not distinguish between the different smooth leaf phenotypes.

The six leaf types described here are spiny, partial spiny, spiny tip, piping, smooth and sandpaper. Scallop has been omitted as Collins (1960) gives little description and the phenotype has not been observed by the authors.

### Spiny

Spiny leaf margin can be considered the usual condition in pineapple. It is easily identifiable, as the entire leaf margin on all leaves is completely spiny. Spine shape, size and density are highly variable between cultivars. They are generally relatively fragile, small and dense on spiny cultivars such as 'Queen' and 'Pérola'. They are much larger, stronger, and more spaced in rare primitive cultivars as well as in *Ananas comosus* var. *bracteatus* (Figure 1), *A. comosus* var. *parguazensis* and *A. macrodontes*. In the latter three, part of the spines may present a retrorse orientation. Spine size is variable in the common wild form *A. comosus* var. *ananassoides*, however most representatives of this botanical variety exhibit short but strong spines disposed at a low density along the leaf margin.



Leaf spininess is controlled by a single pair of recessive alleles (Collins and Kerns, 1946). Collins and Kerns (1946) named the allele coding for spiny leaves 's'. Collins (1960) believed *A. comosus* var. *bracteatus* contained a different gene for leaf spininess which was dominant but this has not been validated by any other studies. Also, the existence of a smooth mutation in *A. comosus* var. *bracteatus* indicates that the spiny condition is recessive in this variety.

Interestingly, the same genetic mechanism has been observed in *Aechmea*, another genus of the Bromeliaceae, where the "wild" allele determining the spiny phenotype is recessive to the spine suppressing mutation (Vervaeke et al., 2003).

### Partial spiny

The partial spiny phenotype is characterised by irregularly spaced spines usually present at the tip and intermittently along the margin on most leaves. The cultivar 'Red Spanish' (in fact 'Española Roja') exhibits partially spiny leaf margins (Figure 2).

Collins (1960) considered the partial spininess of 'Red Spanish' an environmental response. However, clonal selection has shown that clones of both 'Red Spanish' and 'Singapore Spanish' with very few spines can be obtained (Wee, 1974; Pérez et al, 1997), indicating the trait is under genetic determinism. Furthermore, when 'Red Spanish' is crossed with spiny tip and spiny cultivars, an appreciable proportion of plants are partly spiny. The transmission of the trait to part of the progeny suggests a similar inheritance as for spiny tip, the trait being possibly controlled by another allele in the spiny series. Alternatively, partial spininess may result from the incomplete penetrance<sup>1</sup> of an 'S' or 'Se' allele (smooth margins), as indicated by observations from the Brazilian hybrid breeding programme (Cabral et al. 1997).

### Spiny tip

'Smooth Cayenne' and 'MD-2' exhibit leaves with a spiny tip (Figure 3) that are otherwise smooth. 'MD-2' and some 'Smooth Cayenne' clones will occasionally have a small number of spines at the leaf base but are still considered spiny tip. Spiny tip cultivars will sometimes develop some spines further along the leaf margins on some plants if subjected to very unfavourable growing conditions (Collins and Kerns, 1946). Spiny tip clones will also readily mutate to complete spiny and this is often seen in a percentage of plants derived from meristem culture.

The dominant form of the 's' gene, 'S', codes for spiny tip. The heterozygous 'Ss' and homozygous 'SS' genotypes both produce the spiny tip phenotype. The spiny tip phenotype is explained as the production of leaf spines only in the early stage of leaf growth. Most 'spiny tip' cultivars such as 'Smooth Cayenne', are 'Ss' heterozygotes.

### Piping

Piping leaf margin is usually explained as a folded leaf margin that is visible to the naked eye as a white/silvery margin (Figure 4). It is usually attributed to a thin strip of the lower leaf epidermal surface extending onto the upper surface. The white margin arises from a greater density of trichomes (Collins and Kerns, 1946) and possibly a lighter epidermal colour. Closer examination of some piping leaf cultivars can reveal a lack of folding despite an obvious wide, white leaf margin. While the white leaf margin appears consistent on piping leaf plants, the folding can be present or absent such that it might be seen only on a short section of some leaves or even not at all. In the absence of a definite fold there is sometimes a shallow valley. The 'fold' can be absent, present as a shallow valley or strongly developed as a deep fold (Figure 5). The fold is therefore not an identifying feature of the piping leaf margin but dependant on modifying endogenous or exogenous factors. Some cultivars such as '73-50', which have a thin white margin and are devoid of spines, do not appear to develop a deep fold but are still usually considered as piping.

Collins and Kerns (1946) demonstrated that the piping trait is controlled by a dominant 'P' allele of a distinct gene, with epistatic effect on the 'S' gene. This means that, whatever the genotype for the 'S' gene, all bearers of the 'P' gene present a piping phenotype. Some cultivars such as 'Primavera' are homozygous for the 'P' gene (Cabral et al. 1997). However, the dominance of the 'P' allele over the recessive 'p' is not absolute.

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<sup>1</sup><http://science.irank.org/pages/38469/penetrance.html>

Collins (1960) indicates a dosage effect, where 'PP' homozygotes (100% P) show a wider piping than 'Pp' heterozygotes (50% 'P').

### Smooth

The smooth leaf margin is not common in commercially grown fresh or processing cultivars. The botanical form *A. comosus* var. *erectifolius*, including the ornamental clone 'Selvagem 6', is characterised by a smooth leaf margin. This leaf margin type is smooth without a white upper margin or folding (Figure 6) and is not considered piping. A few isolated spines are sometimes observed, particularly towards the leaf base but not at the leaf tip.

A smooth mutant of the ornamental variegated cultivar of *A. comosus* var. *bracteatus*, 'Tricolor' is cultivated in Côte d'Ivoire for the cut flower market.

Collins recognized the distinct nature of the smooth leaf margin and investigated its inheritance. According to Collins (1960), and hybrid progenies observed in Martinique, when *A. comosus* var. *erectifolius* is crossed with *A. comosus* var. *comosus*, the progeny segregation follows either a 2 piping : 1 smooth : 1 spiny (when crossed with a piping cultivar) or a 2 smooth : 1 spiny tip : 1 spiny (when crossed with 'Smooth Cayenne'), or 1 smooth : 1 spiny (when crossed with a spiny cultivar). These ratios indicate that the smooth phenotype is controlled by a third allele, the 'Se' allele, which is dominant over 'S' and 's'.

### Sandpaper

To our knowledge, the sandpaper phenotype was first described by Collins (1960) from a spontaneous amphidiploid found in the hybrid progeny of 'Pernambuco' and 'Monte Lirio'. Loison-Cabot (1990) referred to this type as 'samba', but the original name given by Collins (1960) is more descriptive. Collins' description holds for the Peruvian cultivar 'Samba', whose leaf margins appear smooth without an obvious white margin but have very small spines not visible to the naked eye usually towards the tip (Figure 7). These small spines feel raspy or like sandpaper when the finger is run along the leaf margin.

The observations of Collins (1960) on the sandpaper phenotype of the 'Pérola' x 'Monte Lirio' amphitetraploid are interesting. Given that 'Pérola' genotype is ppss and the 'Monte Lirio' genotype is Ppss, the amphitetraploid genotype can only be Pppp ssss (25% 'P'). In this case, the sandpaper phenotype may thus correspond to a dilution of the 'P' allele effect. Similarly, the sandpaper phenotype observed in diploid cultivars such as 'Samba' probably corresponds to a weak expression of the 'P' allele. Sandpaper phenotypes have commonly been observed among hybrid progenies of piping cultivars in the Martinique breeding programme, giving another indication that it simply corresponds to a very weak expression of the piping genotype. The Hawaiian variety '53-116' also has minute spines towards the tip but exhibits an obvious white margin and most likely would be considered as piping. A variety can therefore exhibit key identifying features of both sandpaper and piping phenotypes.

### Phenotypic stability of leaf margin types

The key identifying features of leaf type are the presence or absence of spines, the size, shape and colour of spines, the position of spines along the margin, and the presence or absence of a white margin. The consistency of the characteristics across all mature leaves on the plant and between different plants is important. The temporal effect of environment and permanent mutations affecting only part of some leaves also need to be considered (Figure 8). Your attention is drawn to the significant affect of environment on gene penetrance. This can be temporal or in some genotypes persistent. The uniformity and stability of the leaf margin type are critical to varietal description.

In this respect, it must be emphasized that the piping condition appears very stable, as no case of reverse mutation to (or temporal expression of) the spiny condition has been reported. The piping trait is much more desirable in selection than the spiny tip phenotype of 'Smooth Cayenne' and 'MD-2', which frequently mutates to complete spiny. The sandpaper phenotype appears to be equally stable and useful for cultivar description. The spiny condition appears equally stable. No environmental stress leads to the loss of spines, and mutations to smooth or partly spiny leaves are very uncommon.

Phenotypic expression for the S and Se alleles appears less stable, being affected by environmental stress. In addition, the mutations (1) from smooth to partly spiny or spiny, (2) from spiny tip to partly spiny or spiny, and (3) from partly spiny to spiny have all been observed. However, as far as varietal identification is concerned, this

should not be a problem. Indeed, the control of environmental variations is part of the cultivar validation process. Thus, permanent variants (stable mutants) should be discarded from the cultivar to be validated.

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Figure 1. The large, widely spaced spines of *Ananas comosus* var. *bracteatus*. *Ananas macrodontes* spines are similar but basal spines may point towards the base of the leaf.

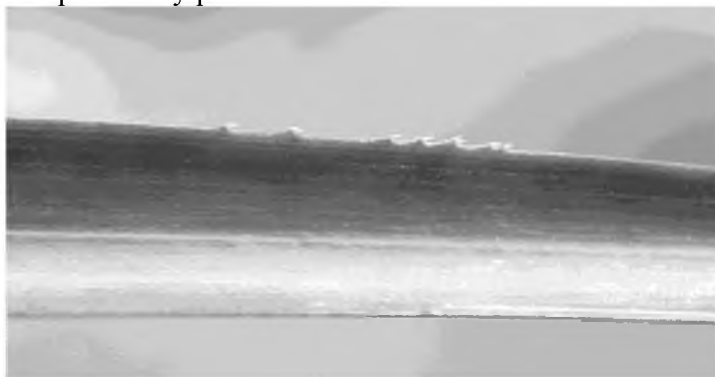


Figure 2. The partial spiny leaf margin on 'Española Roja'.

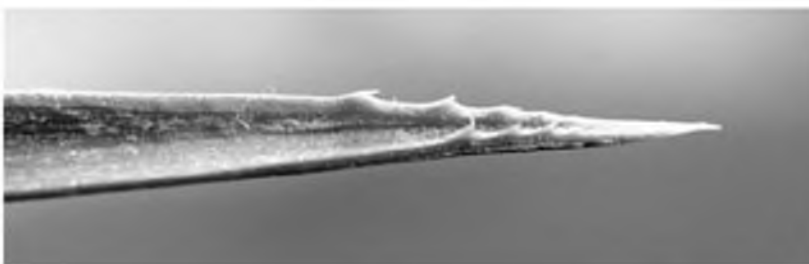


Figure 3. Spiny tip on 'Smooth Cayenne'.



Figure 4. The white leaf margin characteristic of the piping leaf type shown on the cultivar 'Manzana'.

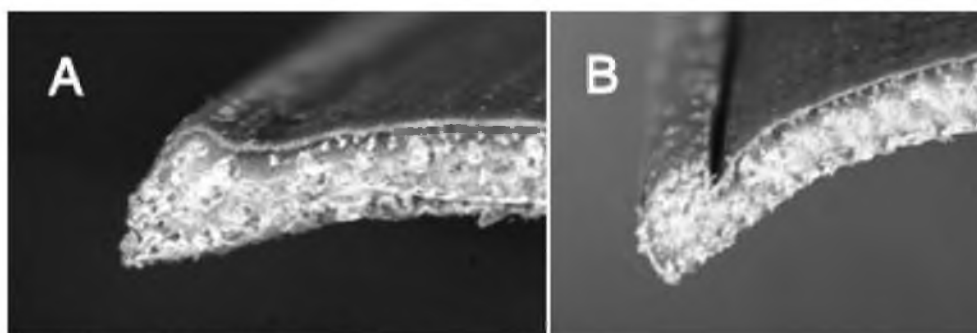


Figure 5. Piping with no fold (A) and with an obvious fold (B).

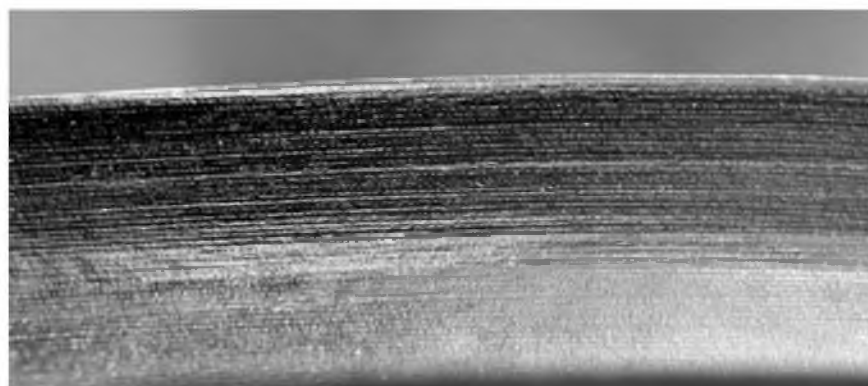


Figure 6. Smooth leaf margin of a *A. comosus* var. *erectifolious* hybrid.



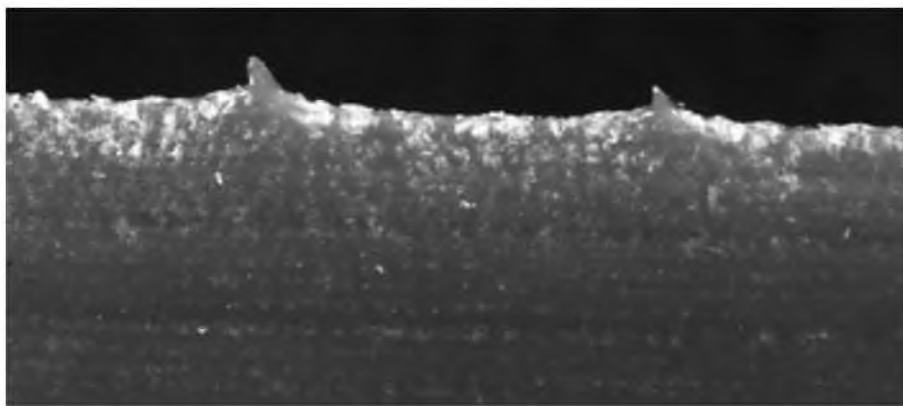


Figure 7. The small spines of the sandpaper leaf margin of '53-116' shown here at high magnification (x50).

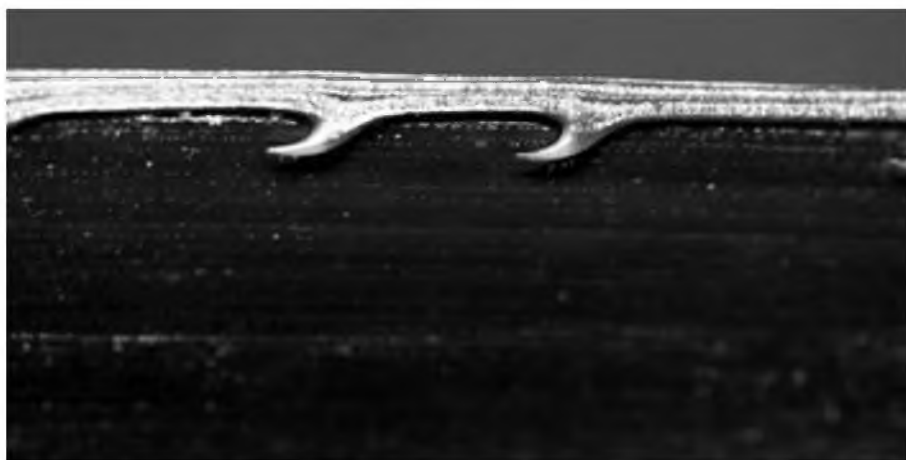


Figure 8. An unusual phenotype showing significant spine development on a piping margin confined to only one leaf margin on some leaves of an otherwise smooth plant.

## News from Malaysia

### Production of Pigments from Bacteria Grown in Solid and Liquid Pineapple Waste

A. Wan Azlina and Y. Nur Zulaikha. Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia  
Email: [azlina@kimia.fs.utm.my](mailto:azlina@kimia.fs.utm.my)

**Keywords:** *C. violaceum*; *S. marcescens*; Textile dyeing

#### INTRODUCTION

Safety problems with many artificial synthetic colourants (Dziezak 1987; Francis 1987, 1989; Kim *et al.*, 1995) have results in wide interest in the production of pigments from natural sources. Natural colours are friendlier to the environment because they can be more biodegradable (Kamel *et al.*, 2005). One promising source of biopigments is microbes because they have a high growth rate and can be produced in bioreactors (Zhao *et al.*, 1998). However, one limitation to the use of natural pigments is that they yield only a few grams of pigment per kg of dried raw material. The result is a current market price of about \$1/g, which limits their application to high-value-added natural-coloured garments (Mapari *et al.*, 2005). There is a need to develop a low cost process for the production of pigments that could replace the synthetic ones (Babitha *et al.*, 2006).

In recent years, there has been considerable research on converting agricultural waste, which is renewable and abundantly available, into value-added products. A cheaply available substrate can make biopigment production economically feasible (Babitha *et al.*, 2006). The aim of this study was to examine the potential of pineapple waste materials as substrates for the production of violet and red pigment by *C. violaceum* and *S. marcescens* and to test their potential to colour fabrics.

#### MATERIALS AND METHODS

Active cultures of *S. marcescens* and *C. violaceum* were prepared by inoculating 25 ml of nutrient broth with a loopful of bacterial cells. The cultures were incubated at 25 °C with agitation at 200 rpm using an orbital shaker (Certomat®R, B.Braun) for 12 hours.

Liquid pineapple waste (LPW) waste was obtained from the downstream process at Lee Pineapple Manufacturing Industry, Tampoi. Prior to using the LPW, it was filtered through muslin cloth and then centrifuged (SIGMA 4K-15, B.Braun) at 7000 rpm for 5 minutes to remove the solid waste. To kill endogenous microorganisms in LPW, ethanol, 5% (v/v) (HmbG® Chemicals) was added and then neutralized using NaOH (Nordin, 2006). An active culture (10 ml) of *S. marcescens* was then transferred into a 1 L Erlenmeyer flask containing LPW medium. The flask was shaken at 200 rpm at 25 °C for 24 hours.

The solid pineapple waste (SPW) consisted of pineapple peels and ground core. Three g of SPW was transferred into a 500 ml Erlenmeyer flask and sufficient distilled water was added to obtain a final working volume of 50 ml. L-tryptophan was used as a precursor for pigment production by *C. violaceum* (Vasconcelos *et al.*, 2003). The solution was thoroughly mixed, the pH was adjusted to 7 with 0.1 M NaOH and the flask was inoculated with 10% (v/v) active culture of *C. violaceum* and shaken at 200 rpm at 30 °C for 24 hours.

The liquid in the bacteria-SPW suspension was filtered and the violet pigment in the liquid portion was extracted using ethyl acetate at a ratio 1 ml (ethyl acetate):5 ml of the growth medium. The solid portion (1g) was extracted with 10 ml methanol. The ethyl acetate and methanol fractions were placed in separate vials and evaporated to dryness at room temperature. The pigment was dissolved in methanol for the UV/Vis analysis.

The red pigment in LPW was extracted with ethyl acetate at a ratio 1 ml (ethyl acetate): 5 ml of the growth medium. The ethyl acetate fraction was placed in a vial and evaporated to dryness at room temperature. The red pigment was dissolved in ethyl acetate prior to UV/Vis analysis. The  $\lambda_{\max}$  of the pigments was determined by scanning from 800-200 nm with a Shimadzu UV 1601PC spectrophotometer. Methanol was used as the blank. Further analysis of the pigments was done using FT-IR spectroscopy. Dried pellets (1 mg) of each pigment were finely ground with 200 mg KBr (Scharlau). The mixture was molded under pressure to form a disk that was immediately recorded using FT-IR spectrophotometer (FT-IR 8300, Shimadzu) in the range of 4000-400 cm<sup>-1</sup>.

The dyeing potential of the red pigment was tested using cotton, silk, polyester, acrylic and polyester microfiber. Dyeing tests with the violet pigment were conducted using silk, pure rayon, jacquard rayon, cotton and silk satin while the synthetic fiber used was polyester.

Dyeing was by boiling 1 g of fabric with 20 ml of solution containing bacterial cells grown in either SPW or LPW. Mixtures containing natural fibers were heated for one hour while the synthetic fibers were heated at 130 °C for 1.5 hours. The dyed fabrics were washed with cold water. Fabrics dyed with the red pigment were mordanted while dyeing (simultaneous mordanting) with a 4% (w/v) tamarind solution (Chua, 2007). Alum (70 g/L) was used as the mordant for dyeing fabric using the violet pigment. Alum powder was dissolved in water, the mixture was stirred for 5 min, the precipitates were left to settle and the clear solution was used as a mordant.

Fabrics dyed with the violet pigment were mordanted after dyeing by immersing the fabric in mordant solution for 15 min at room temperature followed by washing and drying at room temperature.

The dyed samples were tested according to ISO specific tests MS ISO 105-X12-2001, colour fastness to rubbing/crocking; MS ISO 105-A05-2003, colour fastness to washing; MS ISO 105-E04-1996, colour fastness to perspiration; and MS ISO 105-B02-2001, colour fastness to light (Salmiah, 2006).

## RESULTS AND DISCUSSION

The bacteria produced pigment in both solid (*C. violaceum*) and liquid (*S. marcescens*) medium containing pineapple waste (Figure 1a, 1b). In the SPW medium, pigment was produced in the solution and in the solids fraction, which can serve as an anchorage site for the cells (Pandey, 2000). The bacterial growth and pigment production was supported by sucrose, glucose, fructose and other nutrients in the pineapple waste (Sasaki *et al.*, 1991; Krueger *et al.*, 1992).

UV-Vis analysis showed that the  $\lambda_{\max}$  of the crude violet pigment extract in methanol was 585.72 nm (Fig. 2), identical with that of violacein-containing butanol (Blosser and Gray, 2000). The  $\lambda_{\max}$  of the crude red pigment in ethyl acetate-acetone was at 531.5 nm (Fig. 3), which was similar to the maximum absorbance of pure prodigiosin at 535 nm (Anuradha *et al.*, 2004).

These pigments owe their colour to the presence of chromophore groups (C=C or C=O), which are responsible for electronic absorption. The carbonyl groups absorb intensely at the short wavelength end of the spectrum but carbonyl groups have less intense bands at higher wavelength owing to the participation of n electrons (Mohan, 2007). The presence of carbonyl and alkene groups were confirmed by IR data.

FTIR analysis of the violet pigment (data not shown) indicated that the functional groups present were OH, N-H amide, C=O amide, C=C, C-O phenol and C-N. For the red pigment, the FTIR spectrum (data now shown), indicated the functional groups present were N-H, C=C, C-O, C-N and C-H *sp*<sup>3</sup>.

The violet (Fig. 4) and red (Fig. 5) pigments dyed both natural and synthetic fibers. Different types of textile fibers require different kinds of dyes (Vickerstaff, 1954) and the fabrics intensely dyed with violet (violacein) pigment were rayon, rayon-jacquard and silk-satin. Cotton, silk and polyester were less intensely dyed with violacein. With the red pigment acrylic was intensely coloured compared to polyester microfiber, polyester and silk while cotton fiber was poorly dyed.

The different colour shades obtained during dyeing (Fig. 4, 5) were due to different rates of adsorption between dyes and fibers. In the fabric dyeing process, the chemical nature of different textile materials is important as this will determine the exact mechanism by which dye is adsorbed onto particular reactive groups on the fabrics (Vickerstaff, 1954).

Solid and liquid pineapple waste can be used as substrates for the production of natural dyes that can be used to dye natural and synthetic fabrics.

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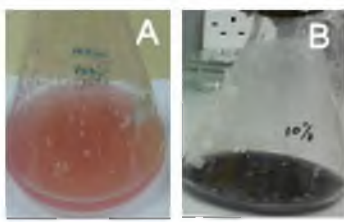


Figure 1. Pigment production by A) *S. marcescens* in liquid pineapple waste and B) *C. violaceum* in solid pineapple waste.

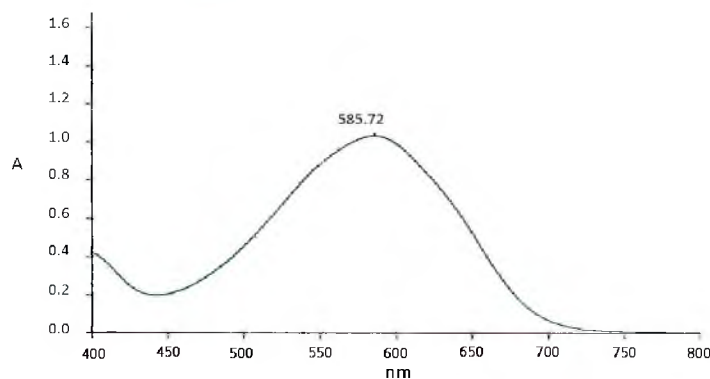


Figure 2. UV/Vis spectrum for methanolic extract of violet pigment.

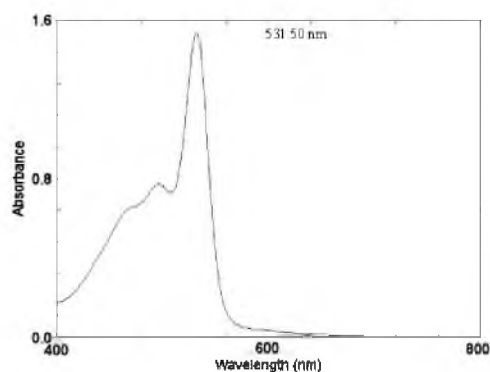


Figure 3. The UV/Vis spectrum for pigment extracted using acetone and ethyl acetate.

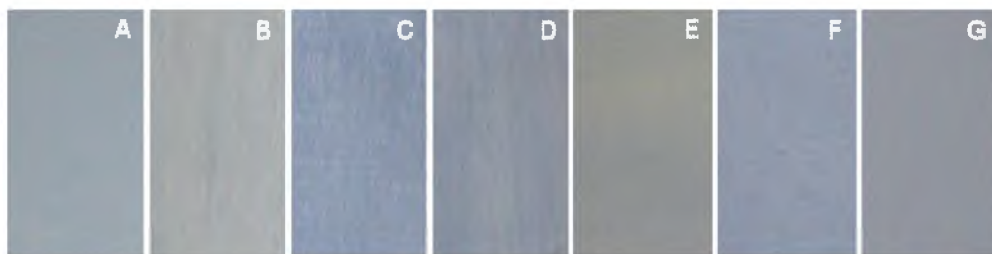


Figure 4. Dyeability of violacein on different fabrics using alum as mordant; A) pure cotton, B) pure silk, C) pure rayon, D) rayon jacquard, E) silk satin, F) cotton and G) polyester.

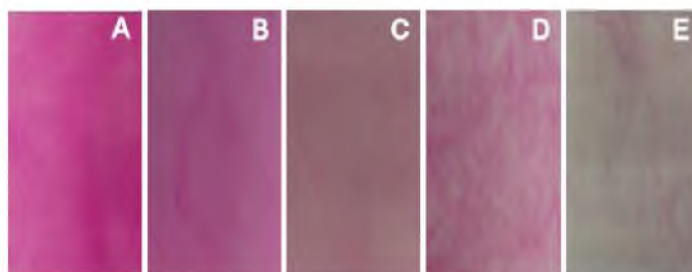


Figure 5. Dyeability of red pigment on different fabrics using alum as mordant; A) acrylic, B) polyester microfiber, C) polyester, D) silk and E) cotton.

**Ed. Note:** The full version of this paper will be published in the proceedings of the 7<sup>th</sup> International Pineapple Symposium.

## The Quality of Cookies Made From Pineapple Agro Waste, a Novel Functional Food

H. Chek Zaini, and I. Suzila. Faculty Science and Technology, Universiti Sains Islam Malaysia, Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan. Malaysia E-mail: chek\_zaini@usim.edu.my

### INTRODUCTION

Pineapple juice is a byproduct of the production of canned pineapples and the out-flowing juice, pulp from the peel and core are the starting materials for juice production. Pineapple waste is a by-product of the pineapple processing industry and consists basically of the residual pulp, peels, and skin. It is not considered attractive as an animal feed, since it contains, on a dry matter basis, a high fiber content and soluble carbohydrates, as well as low protein content (Correia et al., 2001). This residue, as well as many other agricultural wastes, can cause serious environmental problems, since they accumulate in agro-industrial yards without having any significant industrial or commercial value. Solid-state bioprocessing consists of the utilization of water-insoluble substrates for microbial growth and it is usually carried out in solid or semi-solid systems in the near absence of water (Zheng and Shetty, 1998). Biological conversion of fruit processing wastes into value-added products through solid-state bioprocessing (Zheng and Shetty, 2000) offers an opportunity to reduce the waste stream volume and produce value-added products. Here we report the production of cookies that are high in fiber utilizing material recovered from pineapple cannery waste.

### MATERIALS AND METHODS

Cookies were prepared using ingredients purchased at the local supermarket and pineapple decanter wastes obtained from Pineapple Board Sdn.Bhd Johor Bahru Johor. Pineapple decanter wastes were dried overnight in a food dehydrator and then ground and sieved to a powder. Cookies were prepared using blends of different levels of pineapple solids using methods similar to those of others (Ajila *et al.*, 2007; Gisslen, 2009; Leelavathi and Haridas Rao, 1993).

Cookie texture, expressed as breaking strength (kg force), was measured using the triple beam snap (three-point break) technique of Ajila *et. al.* (2007) and Gaines (1991) using a Texture Analyzer (Shimadzu Autograph AGS-J Series, Japan). Average values were reported.

A one (dislike extremely) to nine (like extremely) hedonic scale was used by 60 judges to evaluate the overall acceptance of a cookie sample. The results were subjected to ANOVA to determine if significant treatment differences existed. Means were ranked using Tukey's Test.

The cookies were analyzed for crude fiber using the Fibertherm and for crude protein using the Kjedahltherm (Gerhardt, Germany). Cookie fat content was analyzed using the Soxtherm (Gerhardt, Germany). All samples were analyzed in triplicate.

### RESULTS AND DISCUSSION

Cookie diameter decreased and thickness increased as the percentage of pineapple solids incorporated into the cookies increased (Figure 1, 2). The decrease in diameter with the addition of pineapple solids may be

due to the dilution of gluten (Ajila *et al.*, 2007) and the increase in thickness could be due to the nature of pineapple solids, which are not as fine as the commercial flour used. The net result was a gradual decrease in the spread ratio as the pineapple fiber content was increased. While a higher spread ratio is considered desirable (Kirssell and Prentice, 1979), it is not unusual for the spread ratio to increase when other fiber sources were substituted for wheat flour (Chen *et al.*, 1998; Patel and Rao, 1996; Sharma and Chaulan, 2002; Singh *et al.*, 1996). Hooda and Jood (2005) reported a reduction in spread ratios with fenugreek flour substitution, and Sudha *et al.* (2007) with wheat, rice, oat and barley bran. Reduced spread ratios of the cookies produced in this research may be due to the fact that composite flour apparently forms aggregates with increased numbers of hydrophilic sites available to compete for the limited free water in cookie dough (Hooda and Jood, 2005; McWatters, 1978). The addition of pineapple solids to the cookies markedly increased their percentage of fiber (Figure 3). All cookies that included pineapple solids were significantly different from controls ( $p \leq 0.05$ ).

The addition of pineapple solids in cookies resulted in a decrease of protein, compared to the control (Figure 4). Rath *et al.* (2004) added depigmented pearl millet to biscuits and protein decreased with the higher incorporation of other solids. The authors suggest that the lower protein values may be attributed partly to hydrolysis and partly to the leaching of protein. Protein would also decrease as the proportion of fine flour in the recipe decreased. Fat content in the cookies was inconsistent (Figure 5) but the variation was small. The result is not unexpected because the amount of fat (butter) added was constant in all formulations.

The masticable quality of a food product is equal to the product *hardness* x *elastic quality* x *cohesive quality*. The addition of pineapple solids increased the hardness of the cookies and mastication energy increased from 0.72 in the control 7.02 N.mm with the maximum level of pineapple fiber incorporation in the cookie. The increase in hardness of cookies may be due to the relatively higher water content in pineapple solids incorporated into the dough. This is because doughs having higher water content produce an extensive gluten structure, which results in harder cookies (Ajila *et al.*, 2007). The breaking strength of cookies also increases with the addition of cereal bran (Sudha *et al.* 2007).

Hedonic testing was done on cookie formulations that had the highest fiber content with acceptable taste. The perception of taste and flavor of the cookies improved on incorporation of pineapple solids because the cookies had pineapple flavor. With 40% fiber incorporation, there was an undesirable tooth-stick residual of grainy particles after chewing. However, there was a significant preference by the taste panel for this cookie relative to the control (Figure 6). Preference for the control cookie was not significantly different from preference for cookies with 30% pineapple fiber. When considering appearance, texture and flavor attributes, the cookie with 30% pineapple fiber was optimum.

## CONCLUSION

Substitution of 40% pineapple solids for flour in cookies produced highly acceptable sensory scores (mild Pineapple taste, appealing crust colour and appearance, and crunchy mouth feel texture), and excellent storage quality. We conclude that pineapple decanter waste can be utilized for the preparation of cookies and is a suitable bakery ingredient in other food products with improved functional and nutraceutical properties.

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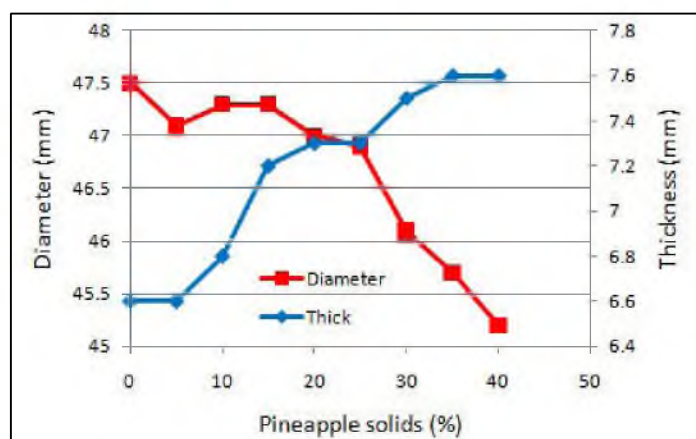


Figure 1. Diameter and thickness of cookies made with increasing amounts of pineapple solids.

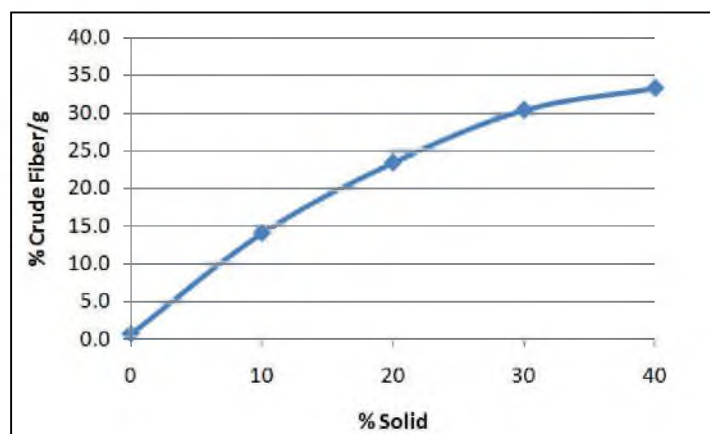


Figure 2. Percentage of crude fiber in cookies containing variable amounts of pineapple fiber.

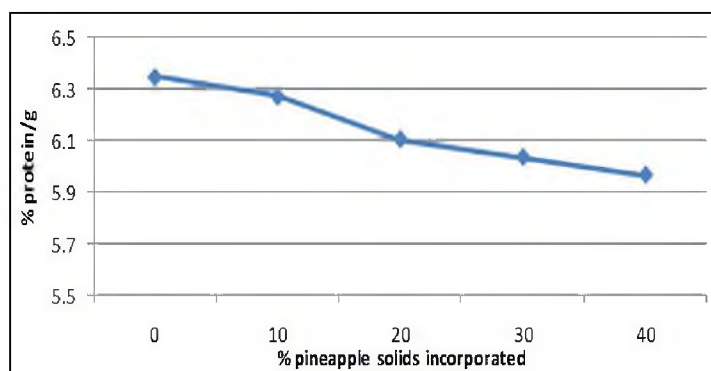


Figure 3. Percentage of protein in cookies containing variable amounts of pineapple fiber.

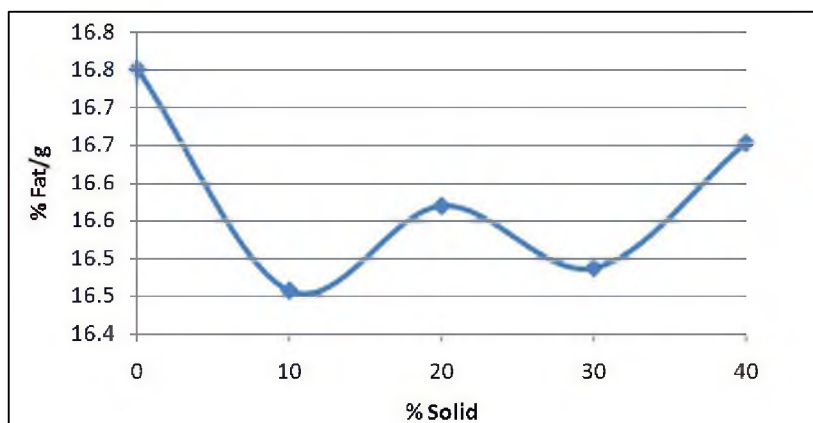


Figure 4. Percentage of fat in cookies containing variable amounts of pineapple fiber.

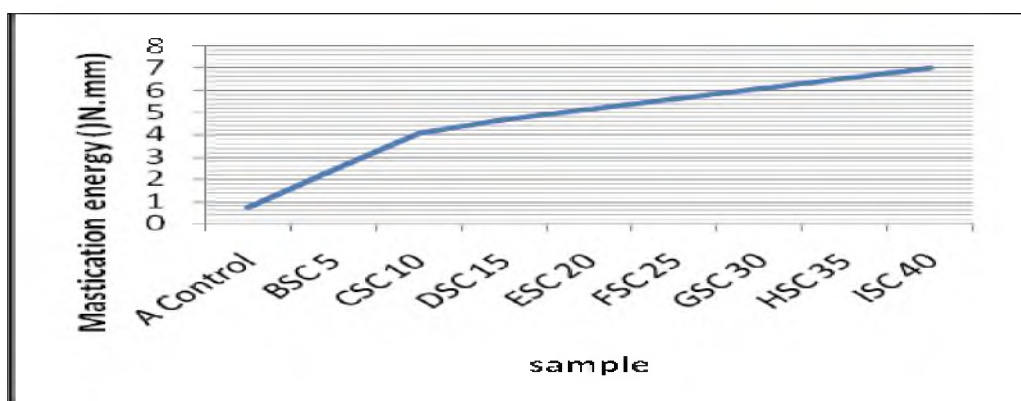


Figure 5.

Mastication energy of pineapple cookies containing 5 to 40% pineapple fiber.



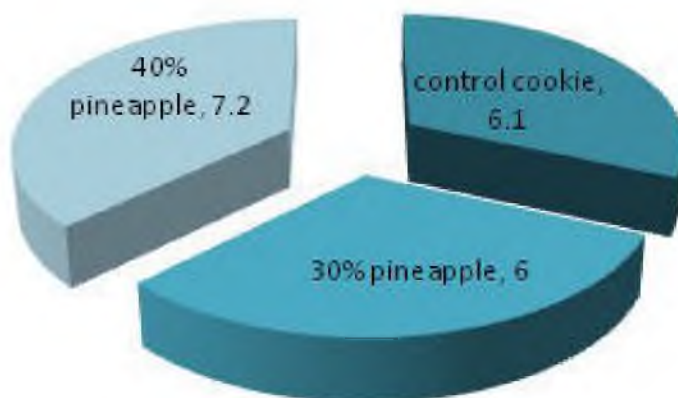


Figure 6. Mean Value of Overall Acceptability of Cookie Formulation.

## News from Mexico

### The Cascajal Block: Another Testimony of the Antiquity of Pineapple in Mexico?

Geo Coppens d'Eeckenbrugge<sup>1</sup>, Daniel E. Uriza Ávila<sup>2</sup>, Andrés Rebolledo Martínez<sup>2</sup>, and Laureano Rebolledo Martínez<sup>2</sup>

<sup>1</sup> CIRAD, UMR 5175 CEF, 1919 Route de Mende, 34293 Montpellier, France. [geo.coppens@cirad.fr](mailto:geo.coppens@cirad.fr)

<sup>2</sup> Campo Experimental Cotaxtla- SE Papaloapan, INIFAP, Veracruz, México. [uriza.daniel@inifap.gob.mx](mailto:uriza.daniel@inifap.gob.mx);

[rebolledo.andres@inifap.gob.mx](mailto:rebolledo.andres@inifap.gob.mx); [rebolledo.laureano@inifap.gob.mx](mailto:rebolledo.laureano@inifap.gob.mx)

Coppens d'Eeckenbrugge and Duval (2009) recently reviewed the evidence for the evolution of pineapple under cultivation and suggested that the domestication process, which produced the large-fruited variety (*A. comosus* var. *comosus*), was initiated at least 6000 years ago. Among other arguments, they considered the antiquity of the pineapple in the Peruvian Coast and Mexican inland, with archaeobotanical remains dated 3200-2800 BP and 1300 BP respectively (Callen, 1967; Pearsall, 1992), and linguistic information indicating that pineapple has been significant to Mesoamerican peoples for more than 2500 years (Brown, 2010). These regions are distant and climatically distinct from the Guianese centre of origin, so the domesticated pineapple had to have been introduced and adapted there well after the first phases of domestication and diversification suggested by available genetic evidence. Thus these data are particularly interesting in providing a minimal time frame for the spread of the fully domesticated pineapple in all the Neotropics, and, indirectly, for the domestication process, which necessarily predated it by a few thousand years.

Here we suggest that other evidence of the ancient presence of the pineapple in Mexico is provided by the oldest written document known in the Americas, the stone block known as the Cascajal Block. The latter was discovered near the Olmec archaeological site of San Lorenzo, in the Mexican state of Veracruz. It was associated with the Olmec civilization and dated 2800-3000 years BP (Rodríguez-Martínez *et al.*, 2006; see also Skidmore, 2006). With the kind permission of Stephen D. Houston, we present here a drawing of the Cascajal inscriptions, numbered as in the original paper (Figure 1).

The Cascajal text's 62 glyphs reveal a signary of 28 distinct elements, several of which clearly correspond to natural organisms or products. Thus sign 4, present in glyphs 1, 23 and 50, represents an insect; sign 17, present in glyphs 7 and 40 represents a maize cob; sign 6, of glyphs 9, 27 and 54, seems to represent an animal skin, while sign 21, of glyphs 13 and 42, has been interpreted as a fish, and sign 18, of glyphs 11 and 55 a clam shell (Rodríguez *et al.*, 2006).

Among the clearly vegetal icons represented, we cannot avoid recognizing our pineapple in sign 2, present four times on the block, as glyphs 16, 45, 53 and 59. If we are right, this constitutes another indication of the antiquity of pineapple in Mexico and its considerable importance for the early Mesoamerican agricultural societies.

That pineapple was important to the Olmec civilization, often considered a "mother culture" in the Mesoamerican cultural area, should not surprise us. Indeed, there are clear indications of very ancient interrelationships between Middle and South America. Among the many cultural traits shared between the two regions by direct diffusion, are particular pottery techniques common to the coasts of Ecuador and Peru on one hand, and the Pacific coast of Guatemala on the other hand, at least as early as 3500 BP. The close homologies between the two areas strongly suggested maritime trade (Coe, 1960). As the pineapple was present on the coasts of South America during the same epoch, pineapple could easily have been introduced by the same route, and the Olmec, who maintained an important trade with most other Mesoamerican cultures, would have embraced such a marvellous fruit. Obviously, this scenario does not discard the possibility of a more ordinary diffusion of the pineapple through the Panamerican isthmus and Central America, as proposed for many South American crop plants (e.g. tobacco, cacao, cashew, achiote, peach palm) by Brücher (1987). For example, an even earlier diffusion of the South Amazonian manioc has been supported by recent data indicating that it had reached Panama by 7000 BP (Zeder *et al.*, 2006). Whatever the route taken by the pineapple to Mexico, its diffusion to Mesoamerica and its probable rapid adoption by local people was only one early step, repeated many times in its highly successful conquest of a pantropical distribution.

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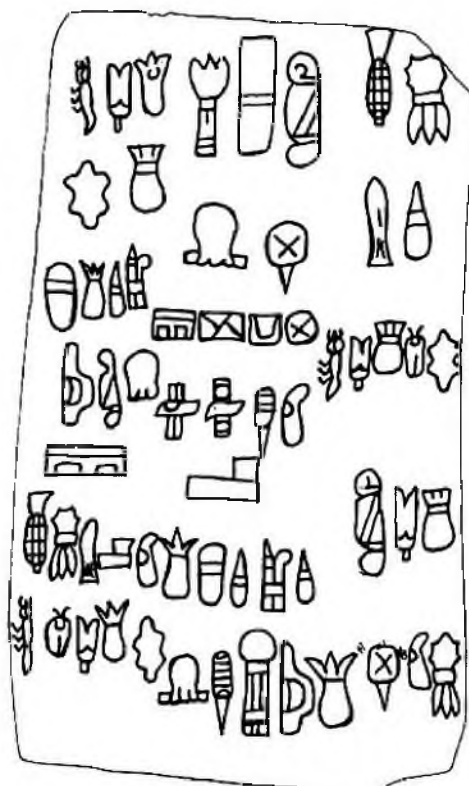


Figure 1. Epigraphic drawing of the Cascajal Block, by Stephen D. Houston, with permission.

## News from the Philippines

### ‘Ulam’, a Novel Pineapple Cultivar

Juan C. Acosta, Romeo Lledo and Noriel L. Agulay

JC Acosta Corporation, 2F JCA Bldg., Manolo Fortich, 8703 Bukidnon, Philippines Telefax No. : +63 88 2409

Email: [jacorp@yahoo.com](mailto:jacorp@yahoo.com)

#### INTRODUCTION

According to the study made by the Department of Agriculture’s Agribusiness Marketing Services, the prospects for fresh pineapple are bright. The domestic demand over the next 10 years was estimated to grow by an average of 4-7 percent every year. In 2004 to 2008, pineapple production expanded with an annual growth of 4.88%. Because of the opportunities presented by the growing fresh fruit industry, a breeding program was set up to improve the existing pineapple cultivars.

#### MATERIALS AND METHODS

Collection of germplasm for breeding was done. Wild species *A. bracteatus*, cultivars and hybrids were used as parents (Figure 1).

The cross of Cayenne x *A. bracteatus* produced vigorous hybrid plants having intermediate fruit sizes. The F1 cross of *A. bracteatus* and Smooth Cayenne were segregating (Figure 2). The F1 hybrids were re-crossed to recombine their desirable characteristics and selections were taken (Figure 3). Reselections were made and an outstanding plant was subjected to meristem propagation.

#### RESULTS

There were many variations in the plants produced by meristem propagation. An elite selection was made and is named “ULAM”, which is a Filipino word for viand. After more than 30 years of breeding since 1972, Ulam was re-evaluated. The Philippine Department of Agriculture personnel conducted the Distinctiveness, Uniformity and Stability (D.U.S) testing. A certificate of Plant Variety Protection was issued on November 27, 2009. Trial evaluation plantings in Davao, Cavite and Batangas showed encouraging results.

**Technical Description:** Plant Characteristics (Figure 4A). The mature plant is 1.1 to 1.8 m in height with high slipping and suckering. The stem is club-shape and the peduncle is short, 25-30 cms. The leaves are spiny along the margins, light green in color, and form a dense rosette. The leaf blades are usually narrow and enlarged at base. Adventitious roots are moderately dense. Ulam is tolerant to heart and root rot.

Fruit Characteristics (Figure 4B, C). The fruit weight ranges from 1.1 to 1.8 kg., is semi-tapered, has small eyes (fruitlets), and a long fruit neck. The crown height ranges from 10 to 14 cm. The fruit has a pleasing taste, is very sweet with a °Brix of 18-22, and has a good aromatic flavor.

#### CONCLUSION

‘Ulam’ is a fruit with a Heavenly Taste. Ulam is the emerging sweetest pineapple variety ever developed from a cross of a wild spiny Brazilian species and a standard non-spiny Hawaiian hybrid. Apart from its unparalleled sweetness, Ulam Pine which (as the name implies) can be a viand, is deliciously crunchy down to its core.

Ulam is a product of more than 30 years of hard work and passion from the foothills of Mt. Kitanglad. This Champion pineapple is projected to capture a big share of both the local and international markets. ‘Ulam’ is ready to “take off” and solicitations are welcome from interested buyers. The production target within three years is 60 ha and in 6 years, it will be 500 ha.





Figure 1. Two varieties of the wild species *Ananas bracteatus* (Variegated and Green leaves).



Figure 2. Segregation of progeny of the 'Smooth Cayenne' x *A. bracteatus* cross.



Figure 3. A. Selection with early big fruit. Note long narrow leaves, which have strong fibers that may be good for the weaving industry. B. Population of mass-selected plants from crosses (note variations).



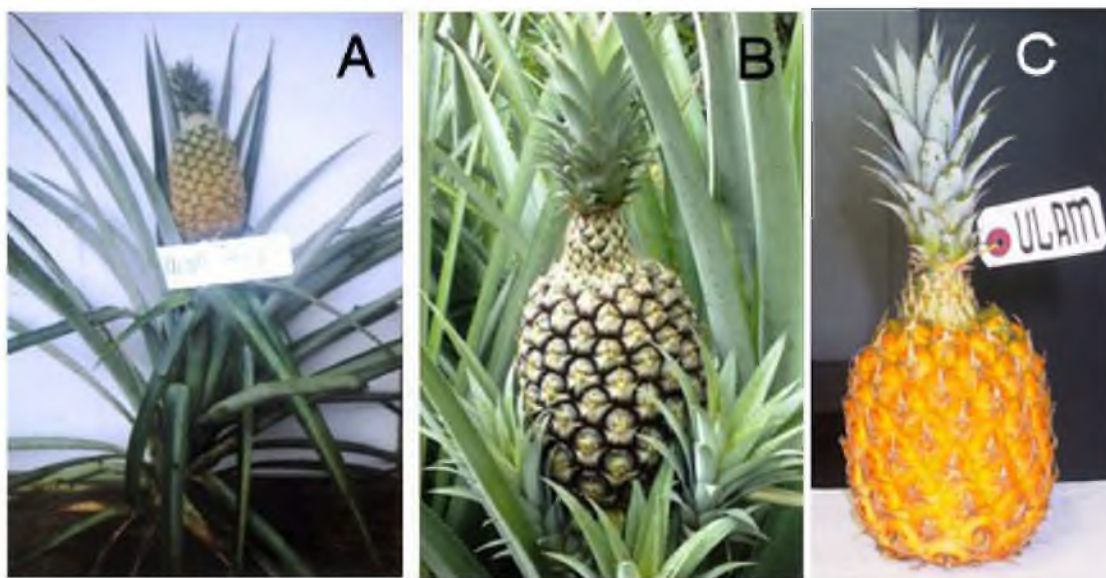


Figure 4. A new premium hybrid, a Novel Cultivar.



Figure 5. Top. Harvesting and packaging the green mature fruits for Export Trial (M.F., Bukidnon, 2009) . Bottom. Right, ripe fruits in the field. Left, newly harvested ripe yellow fruits from Tanaun, Batangas in April 2009 were for sale in the Auction Sunday Markets at the Lung Center in Quezon City. As of today our cooperator, Mr. Raymond Pamplona has planted three (3) hectares.

## News from South Africa

### South Africa Pineapples – Status Report

Dave Murray. Pineapple Growers Association, Bathurst, South Africa. E-mail: [info@pga.org.za](mailto:info@pga.org.za)

**Climate:** The years since 2004 have seen our region on the SE coast generally receiving about 60% of our normal 700 mm of rainfall each year. Rainfall has been gentle and seldom exceeds 10 mm at a time with 3-4 month dry periods interspersed. The winters have been longer but are generally warmer while temperature max/min would appear to be much more extreme at present. Summer solar radiation arrives later and departs sooner as well. The first cool days/nights started 25 March this year. Last year saw this area run out of potable water for 6 - 7 Months but 200mm (Nov/Dec 2010) and 75 mm (March 2011) have brought our catchment dams back to approx 25% of capacity. I don't know if its age but the wind seems to blow more often as well.

To a large extent Elmarie Rabie (ARC, Hluhluwe, Zululand) is experiencing similar abnormal climate. Genesi Langwenya (Swazican, Swaziland) has also been experiencing long dry winters with either frost or hail damage on a much more regular basis. His usual rainfall is generally late and torrential so his grey hairs are creeping up above sideburn level. Above average rainfall has been registered in a broad band running from Namibia (top west) down through Johannesburg and into the Free State and down the length of the northern escarpment. Hence our national maize surplus of 4m tons/low prices etc.

**Pineapple:** The regular small showers and increased humidity from the sustained easterly winds are generally acceptable to established plants with canopy cover. However the smaller / younger plants (0 - 2 yrs/50,000/ha) have taken strain recently. We got to stages when soil moisture was totally absent below 50 mm in the young plants and they were probably subsisting solely on foliar sprays. Growth however has improved with the good rains mentioned earlier. The improved rainfall trend needs to continue. Over the years (1971 - 2011) I have noticed that whatever climatic abnormality/extreme befalls the Australian eastern seaboard (drought/floods) invariably reaches us here (on a reduced scale) about 6 - 9 months later....without fail!! As I write, we are repairing all contours/storm drains on the research farm.

Of course the extreme temperatures (high/low) the longer winters and the shorter summers are having some plant physiological repercussions. Young plants take longer to reach normal maturity standards and on the colder, southern aspects, the loss of apical dominance due to what I call "partial fertilizer burn" is at present a problem. This damage causes the partial/total loss of apical dominance and the plant to sprout multiples of ground suckers from below the damage site which occurs at or slightly above the soil surface (Figure 1). It would appear that this damage occurred approx May each year (only in young 10-12 month old plants established on cool/cold slopes; their compatriots on the warmer northern aspects show significantly less damage/faster growth). This problem was first observed in the 1900's but severity has snowballed since then.

Commencing early 2010, with the help of a local producer, I laid out a fairly large boom-sprayed trial on a high risk site in our inland area where the severity has been the highest (250-400 m above sea level and drier/colder than the coastal zone). I also chose one of the more shallow soils with a flat to gentle south aspect. On a repeated tank for tank basis, we applied his normal foliar program (incl. K, Fe, Zn, Mg) but evaluated Urea HB/Urea LB/Black Urea/Urea and Ammonium Sulphate mix. All the water was sanitized with Chlorine. A control section, which received no sprays until after winter, was attached to the end of the trial area. All foliar sprays were at 900-1000 L of water per hectare. Higher spray volumes (1400-2500 L/ha) which are more representative of this industry, have also shown heart damage potential in the past.

Well, the trial revealed in excess of 70% heart damage throughout. However random plants showed immunity as well as superior growth and colour. One of our Cayenne selections, CM94 which originated from within our field-run gene pool is exhibiting a high degree of resistance to this problem. The control (no sprays) until after winter and the warmer northerly aspect revealed little/no effect. Remember this is not a historical problem. In the past 20 yrs, mainly due to production cost pressures, commercial pineapple production has become concentrated into fewer hands. These are traditionally the more efficient/less speculative producers with their own well-proven production strategies, which resulted in an enviable track record of yield, consistency etc. During the same 20 years cost pressures have encouraged some degree of swing towards "convenience"

production planning, i.e. pro-mechanization layouts, concentrating plant age groups in one section irrespective of aspect/slope, lower ridges to accommodate planting machines, higher plant densities, and reduced foliar fertilizer application passes. Over the same period nitrogen application levels have been reduced by 30% to around the 360 kg N/ha/crop level without any corresponding drop in fruit yield, shape or quality. The last point that must be made here is that this is only happening here at 34°s latitude. This loss of apical dominance in 10-11 month old pineapple plants established on the more shallow soils on cooler aspects seems to be peculiar to the E. Cape, South Africa - even Zululand, Swaziland and Mozambique (a few degrees to the north) have no unexplained incidence of this problem.

Bearing in mind all the detail above, I am hopeful that this might provoke a few ideas/suggestions from the global Pineapple Family. For the time being, I am of the opinion that this heart damage is correlated to nitrogen spray concentrations and spray scheduling in excess of the plants requirements on the cooler aspects/slopes. However given the gradual increase in incidence, is this climate change or just climatic extremes? Is this a warning that climate shift is:

- Revealing inadequacies in our plant gene-pool? (Random unaffected plants, CM94 resistance) cold seems to be the trigger.
- Revealing an obscure trace element deficiency in our soils after over 120 years of pineapple cultivation i.e. molybdenum? Plant nutrient status is in decline generally over our June-Nov winters.
- Revealing just how severe the drought has been i.e. diminished root capacity/foraging?
- Revealing that some of our soils which are inherently marginal in terms of depth, organic matter levels, temperature are now becoming even more marginal?

It would be heartwarming to have a response / ideas from the Family (contact via E-mail at the above address).

**Biological pineapple:** The aforementioned input cost inflation as well as the mounting environmental -food safety lobby, has prompted the first tentative steps by a small band of producers down the biological path. To some extent the move has also been sparked by results from my Trash Incorporation and Organic pineapple findings here on the Bathurst ARC/ITSC research unit.

The first step was taken some 15 years ago when the local industry commenced with pineapple plant reincorporation at the end of every 4-year 2-crop cycle. The second step now involves alternative biological nematode control. The new approach aims to promote aerobic trash decomposition, more beneficial/complex biological systems which operate in comprehensively amended soils. A preplant (in conversion) phase of approximately 12 months preceeds pineapple reestablishment. All actions/operations/amendments are dependant on the results of a series of soil mineral, pathogen, nematode and biolife audits.

Apart from the above topic, the local industry has recently focused its thrust on the production of juice / juice concentrate plus a few non-pineapple products for local / export markets. At the same time pleasing progress is being made with various avenues of pineapple waste beneficiation. In the short term South African Pineapple volumes (incl. Queens) are expected to return to the 150 000 ton pa level. The strong local currency (Rands) is curtailing both fresh / processed pineapple export volumes.

**Floral hybrids:** Some of the latest floral hybrids can be seen in Figure 3.

**Personnel changes:** Anni Macleod, the feisty Pineapple Growers Association secretary for the past eight years has moved on to manage her brothers super yacht factory near Cape St Francis. She stays in contact with us and sends regards to all those in the Family that, when caught with an early soothing ale, abide by the rule "At any given moment its 12 "clock somewhere in the world" Today, our "friendly voice" at the PGA belongs to Lee Botha ([info@pga.org.za](mailto:info@pga.org.za)).





Figure 1. Left, a plant that lost apical dominance and is regrowing from a basal sucker. Right, injury associated with the loss of apical dominance.

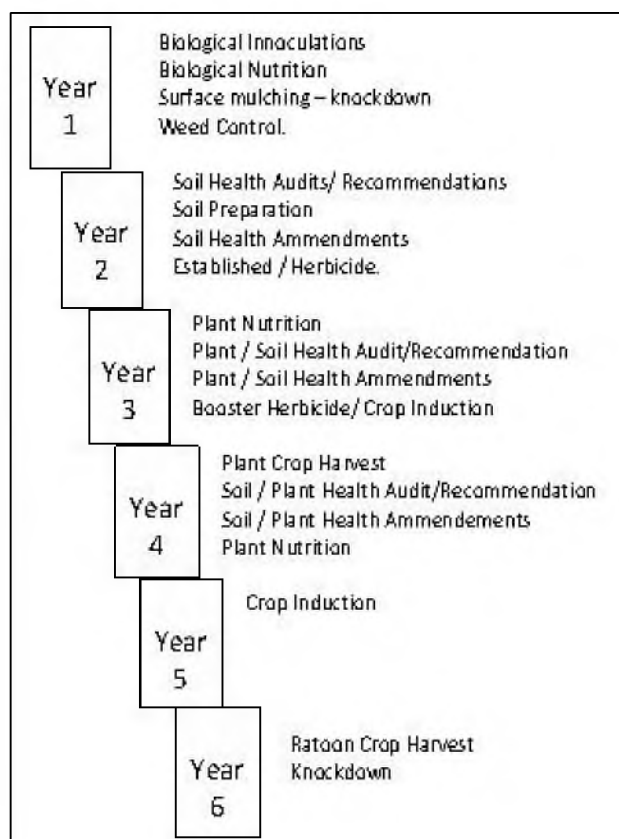


Figure 2. Biological pineapple cultivation program.



Figure 3. A peek at some of the latest floral hybrids.



## News from the USA (Hawaii)

### Global Studies on Control of Black Spot Disease/Fruitlet Core Rot Caused by *Fusarium guttiforme* in South Africa and Panama

Glenn Taniguchi, University of Hawaii, 3190 Maile Way, St. John's Hall 303, 3190 Maile Way, Honolulu, Hawaii, 96822, USA. [gtani@hawaii.edu](mailto:gtani@hawaii.edu)

In countries where susceptible pineapple cultivars are grown, Black Spot Disease (BSD)/Fruitlet Core Rot (FCR) causes serious economic losses as high as 80% during certain months of the year (Barker, 1926; Petty et. al. 2006). Both BSD and FCR are synonymous with the causal agent involved being *Fusarium guttiforme*. In South Africa the most susceptible cultivar is 'Queen' and in Panama 'Manzana' is the most susceptible cultivar.

In 2006 and 2010, studies in South Africa were centered on survey and detection methods, fungicide screening, and ethephon application to inhibit flower opening. Survey and detection studies were successful with the use of a selective media which eliminates or slows the growth of unwanted organisms and allows *Fusarium* sp. and some other fungi to grow. *F. guttiforme* was detected from leaf washings in South Africa and Panama.

Fungicide studies with Procure® (triflumizole), were field tested after laboratory bio-assays in Hawaii with 8 different fungicides indicated that this fungicide was the top candidate for field testing. However results indicated that Procure® did not prevent the disease in 'Queen' or in 'Manzana'.

Reassessing the etiology of *F. guttiforme*, a possible novel method using ethephon to prevent flowers from opening was proposed (Lim and Lowings, 1979; Pinto da Cunha and Pires de Matos, 1987). Speculation is that infection takes place via the open flowers (Barker, 1926; Bolkan et. al. 1979; Johannsson, 1934; Petty et. al. 2006; Mourichon, 1997) so preventing flowers from opening would prevent transfer of spores of *F. guttiforme* into the open flowers. Development of methodology was done in Hawaii, which consisted of spraying plants during anthesis with ethephon at 1000 or 2000 ppm every 3-7 days to keep all flowers on the inflorescence cone from opening, thus in theory preventing fungal spores from entering the open flowers.

The results from this study showed that ethephon at 2000 ppm was very effective in keeping the flowers from opening while 1000 ppm was less effective. However preventing flower opening did not inhibit the disease. In South Africa and Panama the disease incidence was greater at 2000 ppm ethephon than in controls (Table 1). This results imply that infection does not necessarily occur thru open blossoms as suggested in numerous publications, but rather from inoculum present in the heart water as discovered in the early survey and detection studies. It is speculated that the developing inflorescence is immersed in inoculum and the spores of *F. guttiforme* are trapped or enter the developing inflorescence and become imbedded in the blossom prior to anthesis. Thus disease becomes symptomatic under favorable conditions and with susceptible varieties. Consequently efforts to control this disease will need to be centered around fungicides that offer curative properties rather than protective properties as is the case with Procure®.

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Table 1. Mean percent incidence of frutlet core rot on whole fruit (mean of 3 replicates).

Treatment	Trial 1	Trial 2
	South Africa: 'Queen'	
Control	35.6	34.6
20 ppm	21.4	22.1
1000 ppm	35.2	42.9
2000 ppm	54.4	54.9
	Panama: 'Manzana'	
Control	15.2	0*
20 ppm	10.0	3.1
1000 ppm	21.0	2.5
2000 ppm	30.1	4.0

\*Treatment replicates consumed by cattle.

## Hawaiian Strains of *Erwinia chrysanthemi* (*Dickeya* sp.) Associated With Pineapple Heart Rot Disease

Glorimar Marrero<sup>1</sup> and Anne M. Alvarez<sup>2</sup>

Dept. of Molecular Biosciences and Bioengineering<sup>1</sup> and Plant and Environmental Protection Sciences<sup>2</sup>, University of Hawaii at Manoa, Honolulu, HI 96822, U.S.A.

E-mail: [glorimar@hawaii.edu](mailto:glorimar@hawaii.edu)

Pineapple heart rot disease was first observed in pineapple fields of Hawaii in December 2003 and the causal agent was identified as *Erwinia chrysanthemi* (Vine et al., 2005; Kaneshiro et al., 2006). Bacterial strains were collected from infected pineapples from repeated surveys on the islands of Oahu and Maui from 2003 to 2011 (Alvarez, et al., 2007; Kaneshiro et al., 2008; Marrero et al., 2010 and unpublished data). Phenotypic and genetic traits of pineapple strains were compared with reference strains from a French collection of well-characterized *E. chrysanthemi* strains that were recently reclassified as six distinct *Dickeya* species. Phylogenetic relationships between reference strains and the Hawaiian pineapple strains indicated that the pineapple heart rot strains should be placed into a new *Dickeya* sp. (Marrero et al., 2009; Marrero, 2010). *Dickeya* species from pineapple in Hawaii were most closely related to an unclassified *Dickeya* sp. isolated in 1961 from pineapple in Malaysia.

In the early disease outbreaks in Hawaii strains were isolated from pineapple planting stocks originating in Central America, whereas in later outbreaks we also recovered strains isolated from planting stocks originating in the Philippines. Genetic traits of the Hawaiian pineapple strains were compared to strains isolated from local water sources and locally grown ornamentals and field crops to determine their relationships.

Genomic studies were undertaken to find molecular markers for rapid identification of bacterial strains (Schneider et al., 2009, 2011). Multiple genetic markers were used in an analysis that showed that there were at least two introductions into Hawaiian pineapple fields: one with the imported stock from Central America and a second from the stock imported from the Philippines. The bacteria associated with these introductions were phylogenetically distinct from each other and coincided with the time of the arrival of the planting stocks in Hawaii. The spatial distribution of bacterial strains with different genetic characteristics indicated that the disease had spread to several fields and some fields had bacterial populations of two different genetic types. The pineapple strains were distinct from strains

isolated from irrigation channels during the initial outbreak in 2003-2005. *Dickeya* strains with genetic characteristics similar to the pineapple pathogen were subsequently recovered from an ornamental nursery, a taro germplasm collection in Waimanalo, Oahu, Hawaii and most recently from corn hybrids showing severe symptoms of corn stalk rot. The genetic relationships between the pineapple heart rot pathogen and these *Dickeya* strains from alternative hosts are now being analyzed.

Pathogenicity tests were conducted on pineapple cultivars 'CO-2' (73-50) and 'MD-2' (73-114) to characterize strains with different levels of virulence. In all tests 'CO-2' was unaffected while 'MD-2' developed typical heart rot symptoms. Based on different virulence ratings and rep-PCR fingerprinting patterns of the *Dickeya* strains monoclonal antibodies were produced for use in an immunodiagnostic test (Peckham et al., 2009, 2010; Luu, 2010). The immunodiagnostic test is best used to confirm the presence of the pathogen in a mixture of many saprophytic bacteria, which are also associated with pineapple heart rot. The initial test should be followed by a genomic method to identify the specific genetic population (s) associated with different fields.

In Hawaii, the knowledge generated about disease spread from infected sites has impacted plant quarantine regulations as well as industry practices. Based on results of a thorough disease survey on Maui and Oahu, commercial plantations on Maui were declared disease-free. *Dickeya* strains with a genotype identical to Oahu strains was detected in irrigation channels but no disease was observed in commercial plantings. On this basis, movement of planting stocks from Oahu to Maui was prohibited and the disease outbreak has been restricted to Oahu. The results will eventually have impact on the pineapple industries worldwide where the pathogen is prevalent and measures to control the disease are enhanced by the ability to rapidly identify and destroy the major sources of infected materials. The methods we have generated will enable scientists in pineapple growing countries to answer epidemiological questions.

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## Services

The listings below are provided 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. No effort was made to confirm/update the list in the past year so some material may not be current.

### 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](mailto:mandava@compuserve.com)
- Centro de Bioplasmas. Dr. Justo L. Gonzalez Olmedo, Director of Foreign Affairs Office, Centro De Bioplasmas. Universidad De Ciego De Avila, Carretera a Moron Km 9. Cp69450. Cuba. Centro De Bioplasmas offers certificates of authenticity for pineapple material propagated in their tissue culture facility. Web site: <http://www.Bioplasmas.cu>
- LAMERSA, Dole's meristem laboratory in Honduras. Contact John T. Mirenda PhD, Dole Fresh Fruit International Ltd., San Jose, Costa Rica. Phone: 506 287 2175. Fax: 506 287 2675. E-mail: [Jmirenda@la.dole.com](mailto:Jmirenda@la.dole.com). The laboratory can produce meristematically-derived plants of pineapple as well as banana and other crops.
- Thai Orchids Lab, Dr. Paiboolya Gavinlertvatana. Horticulture/ agriculture/ forestry tissue culture laboratory with exports to Australia, U.S.A., Africa, and Asia. MD2 pineapple available (open to acquiring additional varieties) or confidential exclusive contract propagation. Phone: +1 510 931 7865 Fax: +66 2510 9452 Website: <http://www.tolusa.com/> E-mail: [info@tolusa.com](mailto:info@tolusa.com).
- Vitropic, Zone d'Activités Economiques des Avants, 34270 Saint Mathieu de Tréviers France; Tel: + 33 (0)4 67 55 34 58; Fax: + 33 (0)4 67 55 23 05. E-mail : [vitropic@vitropic.fr](mailto:vitropic@vitropic.fr). Web site: [www.vitropic.fr](http://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](mailto: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 Rica, Guatemala, Ecuador).
- Ing. Alejandro Chavarria. APDO 4437-56 Pital, San Carlos. Alajuela, Costa Rica. Tel: (506) 88-20-79-55 / (506) 24-73-40-00, [alechava@hotmail.com](mailto: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](mailto:markculik3@yahoo.com). Experience: PhD in Entomology with more than 25 years of agricultural pest management experience in crops ranging from apples to papaya and pineapple, identification of pests and beneficial arthropods ranging from Collembola to fruit flies, and current work on scale insects with emphasis on pineapple mealybugs. Areas of specialization: Entomology, Insect and Pest Identification, Integrated Pest Management.
- Dr. Francisco Gomez (E-mail: [fgomez1@cablecolor.hn](mailto:fgomez1@cablecolor.hn)) and Jose R. Vasquez, MBA (E-mail: [jrva46@excite.com](mailto:jrva46@excite.com)). Golden Pacific Ag Services, PO.Box 15088, Lomas Miraflores, 4a. Calle, 1a Avenida # 4326, Tegucigalpa, Honduras. Phone: 504 230 1120; 504 969 5568.
- Experience: Pineapple and melon production, from seed propagation-planting-field maintenance-forcing-harvesting-post-harvest management and commercialization.
- Mr. L. Douglas MacClure. 360 Hoopalua Dr., Pukalani, Hawaii, U.S.A. E-mail: [norfolkldm@aol.com](mailto: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](mailto: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](mailto: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](mailto:ventura@incaper.es.gov.br); Tel.: 55-27-31379874. [www.incaper.es.gov.br](http://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](mailto: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

- Adapting to market changes project (The Queensland Pineapple Industry: [http://www.dpi.qld.gov.au/26\\_19326.htm](http://www.dpi.qld.gov.au/26_19326.htm).
- Research Team Receives Patent for Method of Genetically Engineering Soybean Plants to Control Destructive Parasite (nematode) <http://www.k-state.edu/media/newsreleases/nov10/patent110810.html>.
- Promoting plant growth with Compost Teas: [http://www.ctahr.hawaii.edu/sustainag/news/articles/V7-Radovich\\_etal-CompostTea.pdf](http://www.ctahr.hawaii.edu/sustainag/news/articles/V7-Radovich_etal-CompostTea.pdf)

## New References on Pineapple

The list below includes papers related to various aspects of pineapple culture, physiology, processing, preservation or byproducts that were published or located since the last issue of the newsletter was printed. Some papers may seem relatively unrelated to pineapple but the list follows the principle of inclusion to provide the widest possible content. Often, abstracts of the papers listed below can be found on-line and of course all abstracts of paper published in *Acta Horticulturae* are available from [info@ishs.org](mailto:info@ishs.org).

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All readers of Pineapple News are invited to contribute articles to this newsletter. The scope of contributions includes:

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