

# Pineapple News

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

### From the Editor

Dear Colleagues:

Welcome to issue 21 of Pineapple News. Initially few articles were available for inclusion in this issue of the newsletter. However, there were several very interesting submissions that came in recently so I delayed publication of the newsletter so they could be included. I hope readers find interesting contributions worth waiting for.

### On-line Pineapple Extension

D.P. Bartholomew ([duaneb@hawaii.edu](mailto:duaneb@hawaii.edu)) and G. Taniguchi ([gtani@hawaii.edu](mailto:gtani@hawaii.edu))

Over the years I have responded to quite a large number of inquiries from growers for help in diagnosing or understanding problems encountered when growing pineapple. When I received a request for help with a problem I had not seen or heard of before, I had two valuable resources available to me. One resource was my colleagues in the Pineapple Working Group (PWG) who responded to my email request for help in diagnosing or suggesting solutions to grower problems.

More recently I have been working closely with Glenn Taniguchi, an expert on the pests and diseases of pineapple. Glenn is a particularly valuable colleague because he is a keen observer who has many years of in-field experience working on control of the pests and diseases of pineapple. Glenn has also traveled to foreign locations to conduct research on pineapple disease problems and shared his expertise with Pineapple Working Group members through papers presented at International Pineapple Symposiums. When neither of us has an answer to a grower's problem, we send messages to specialists known to us or I request help from members of the Pineapple Working Group.

Both Glenn and I enjoy the unique challenges presented to us by growers. If you have a problem with no obvious solution, send it to me or Glenn, or both, and we'll do our best to come up with an answer, hopefully the correct one. If we are stumped by your problem, we'll ask the members of the Pineapple Working Group to help us. Below are some of the interesting problems that came to us in the past year.

### Peduncle Cracking

Below is a summary of responses from PWG members to an inquiry from a pineapple grower in Florida who wanted to know what caused cracking of the the pineapple fruit peduncle (stem) of one the pineapple cultivars he was growing. Thanks to Add Dolmans who called my attention to Broadley, R.H., Wassman, R.C.I., and Sinclair, E. (1993. Pineapple Pests and Disorders. Queensland Department of Primary Industries, Brisbane; out of print) where the disorder is illustrated on Plate 88 and a brief description (it occurs mainly on plants induced (forced) in early spring and appears to be the result of a change from slow to rapid fruit stalk elongation during hot, humid weather) on p. 44. Nothing else on the subject was found in the published literature. Below is a summary of the observations of the problem received from PWG members. It appears that some cultivars are particularly susceptible to the problem and it seems to be associated with a rapid increase in growth of the developing peduncle and fruit. A collage of the photos showing the symptoms is included below.

- Miss Szu-Ju Chen, a PhD student and researcher in Kaohsiung District Agricultural Research and Extension Station, Taiwan. Peduncle cracking is common especially in cultivar 'Tainon 17', a major pineapple cultivar in Taiwan. The disorder frequently occurs in pineapple fields with excessive nitrogen fertilization before forcing. Plants with dark green leaves before forcing are especially prone to peduncle cracking. Growers in Taiwan have been advised to stop N application 1.5 months before forcing and keep leaves slightly yellowish green to reduce the disorder. Boron application during forcing can also help reduce the symptom. There are some environmental factors before bloom, such as extreme day/night temperature shift, continuous raining conditions, etc. may also induce fruit cracking.
- George Choisis, Columbia. I have seen this type of symptom in Smooth Cayenne plants in the Far East Colombian Plains (Llanos Orientales) COLOMBIA. The symptoms were exactly at the middle of the

peduncle where there is a node. I consider the symptom could be a deficiency of potash and/or Calcium. Applications of both nutrients in the leaves at their intersection with the stem should avoid future problems.

- Col Scott, Queensland, Australia. The disorder is quite common in Queensland and both Smooth Cayenne and Queen can be affected. To date, it hasn't been seen in the hybrids. The cause is unknown but it seems to be related to high humidity and rapid growth flushes.
- Grant Joyce, Antigua. Antigua Black pineapple, a Queen type, from time to time has cracked stalk approaching 5% of the crop. It occurs during rapid elongation of the peduncle (red heart to flowering), and we feel it is brought about by some environmental factor, most probably high rainfall during peduncle elongation. We see it during summer and winter, so temperature doesn't appear to be a factor. It always results in misshapen and smaller fruit.
- Prof. Le Van Be, Vietnam. 'Tainung 4' is susceptible but the problem is not seen in 'Queen'. The problem occurs when the peduncle is elongating during flowering and most often occurs at noon when the radiation level is highest. Covering the peduncle with grass can control the problem.
- Juan Luis Morales, Costa Rica. The majority of varieties are susceptible to a greater or lesser degree. For example 'MD-2' is more susceptible to heart fruit splitting when there are very strong and sudden changes in temperature between day and night and such changes will cause the stem splitting in other varieties.
- Liu Sheng-hui, Zhanjiang, China. The symptom was observed on our plantation several years ago. One side of deformed fruits was normal, the other side was abnormal with unfertile fruitlets. Only the cultivars, Yellow Mariutus (Queen) and Taiwan hybrid (Tainung No.6 and No.17) had this problem since 2006. Stem splitting was observed on 'Queen, the main cultivar in Zhanjiang and Hainan province, only in plants forced in summer. Experienced growers, who usually don't observe the problem until the deformed fruits can be seen, think the problem is caused when soil humidity (Ed. Note: probably soil water) increases dramatically during flower differentiation and formation.
- International Tropical Fruits Network (<http://www.itfnet.org/contents/fruit/fruitInfo/html/trdLevel746.html>). Original source not provided. Split peduncle is prevalent in the 'Queen' but not in 'Smooth Cayenne' or 'Spanish'. A horizontal split occurs at a node of the peduncle about 1-2 cm from the base of the fruit during early fruit development. At maturity the fruit is smaller than average and tends to bend towards the side of the split. Plants that flower naturally tend to have much lower incidence than those forced into flower. The defect is related to late application of fertilizer and to copper deficiency.
- de Silva, A.E., Kadir, M.A., Aziz, M.A., and Kadzimin, S. (2008. Callus induction in pineapple (*Ananas comosus* L.) cv. Moris and Josapine. International Journal of Agricultural Research 3:261-267) report that 'Moris' is highly susceptible to developing split peduncles "which is associated with copper deficiency".



Figure 1. Peduncle cracking and fruit splitting.

### **Environmentally Friendly Way to Control Mealybugs in Planting Material**

A grower in South Africa wrote to ask if any studies had been done on environmentally friendly ways, for example Neudosan, an insecticidal soap, of treating pineapple planting material for mealy bugs. Nandwani et al., (Nandwani, D., Cabrera, I.T., and Attao, D. 2011. Pineapple production in the Commonwealth of Northern Mariana Islands. *Acta Horticulturae* 902:87-91) reported that neem leaf extract and soap solutions were being used by growers in the Islands but no procedure was provided. I suggested that malathion (LD50 for Rats: 5,500 mg/kg) was much less toxic than the dimethoate (LD50 for Rats: 387 mg/kg) currently being used. Vásquez Jiménez ([jvasquez@proagrocr.com](mailto:jvasquez@proagrocr.com)) wrote to say that a key to controlling mealybugs was to control ants in the field with hydramethylnon baits. Ant infestations can be discovered using peanut butter on a stick or a small amount of tuna fish. Only pesticides registered for the crop can be legally used to control pests.

### **Crown Damage**

A grower in Israel wrote that fruit crowns had been injured, possibly by 2-(3-Chlorophenoxy) propionic acid sprayed to enlarge the fruits, and appeared to be rotting in the center. The growth regulator is not registered for use in the United States. G. Taniguchi fielded that problem and stated that since the rot had not penetrated living tissue, it was not pathogenic and no treatment was required.

### **Stunted Plant Growth Due to Equipment Malfunction or Operator Error**

The right photo came from a grower in southern Africa who wrote to ask what might have caused the stunting of single rows of plants in a typical two-row bed system. The cause of stunting in the left photo was known to have resulted from improper installation of irrigation tubing. It is impossible to diagnose the problem in the right photo without having information about what fertilizer, pest and disease control practices were used on the farm. Stunting of plant growth can be caused by numerous soil, pest and disease problems. However, when entire two-row beds or single rows are stunted, it is always due to problems with equipment (clogged openings, broken drive chain of shaft) or to operator error. As G. Taniguchi says, “the plants don’t lie.” Such examples illustrate why it is necessary to always carefully check all equipment before spraying fertilizers and pesticides and injecting fumigant. The very late discovery of the problem in the field on the right could result in loss of half the fruits in the field if the fruits are too small to be of economic value.



### **What factors control °Brix (TSS)?**

Below is a summary of what was found in the literature in the Pineapple Reference Database regarding the factors demonstrated to have some influence on pineapple fruit total soluble solids (TSS; a.k.a. °Brix). In searching the above database of 9,505 references, °Brix or TSS, or both, was mentioned in 286 papers. However, most papers that included information on fruit sugars had no information on the factors that might be controlling their accumulation in ripening pineapple fruits. Below are excerpts or quotations from papers that provided some information on the factors that affect total soluble solids levels in pineapple fruits. In the interest of saving time, most of the information was obtained from the abstracts of papers..

It is hard to draw more than broad conclusions about the factors controlling the accumulation of TSS in fruits but it seems clear that high solar radiation levels, for example in summer, result in higher TSS levels than do low levels that occur in cool and cloudy seasons. Though the information on water availability is limited, there

is evidence that some degree of water stress will increase fruit TSS. By analogy, some nitrogen (N) stress, which would slow vegetative growth and, at least in cooler environments, increase plant carbohydrate reserves, might also increase fruit TSS. However, no data was found to show what level or quantity of N in soil or plant tissue would be required to achieve such a result. The most commonly studied nutrients are N and potassium (K) but no references were found that potassium stress or excess had any effect on TSS levels. However, because K is essential for normal functioning of the stomata, a potassium deficiency would reduce photosynthesis and stunt plant growth. Sufficient K to sustain normal growth rates will assure that ripe fruits will have the highest possible sugar contents.

Sideris, C.P., 1934. The influence of light on the weight of the fruit, the citric acid and Brix content of the juice, and the development of endogenous brown spot. *Pineapple News* 8: 244-248, 327-329.

Plants were grown in shade (0, 20, 50, 66%) at 9 months after planting in September. Fruits on plants receiving greater amounts of light ripened earlier. Plants under 33% light had fruits only half the size of control fruits and fruit size in the treatments was inversely proportional to shade level. Brix was little affected while citrate increased with decreasing light. Endogenous brown spot increased almost linearly from near zero at 0% shade to almost 70% at 66% shade.

Clark, H.E., Young, H.Y., and Louis, L., 1938. Fruit Development in Relation to Organic Reserves. Association of Hawaii Pineapple Cannery (AHPC) Research Report 9: 1-16.

Plants of contrasting growth status grown under recorded environmental conditions were analyzed at intervals during the period of fruit development. Plants grown in a region of lower sunlight (Kahuku) and liberal nitrogen had high nitrate in leaves and fruit while soluble carbohydrates (sugars) and insoluble carbohydrate (starch) were relatively low. Plants grown in a region with higher sunlight and abundant nitrogen (Kunia B) were intermediate in nitrate content and had comparatively high organic nitrogen in leaves and stump while sugars and starch in leaves and stump were quite high. In the interval between red bud and harvest all groups showed decreases in nitrate in all tissues, increases in sugars in stumps and decreases in percentage of starch in leaves and stumps. The data indicated that carbohydrate reserves of leaves and stump were used by the developing fruit along with sugars produced in the leaves.

Clark, H.E. and Cooil, B., 1939. Fruit quality in relation to environment and growth status. AHPC Monthly Report:235-243.

The most pronounced quality difference was higher TSS, higher translucency, and lower acidity in summer fruit. Differences in acidity between winter and summer fruit was much less for fruit from olive-green plants than from black-green plants. It was suggested that temperature differences between winter and summer alone were great enough to account for differences in fruit quality.

Ed Note: No equipment for the measurement of solar radiation was available at the Pineapple Research Station when the study was conducted. Since the radiation minimum in Hawaii is about 50% of the summer maximum, it is probable, that the decrease in TSS was due to reduced solar radiation.

Clark, H.E., 1940. Translocation of organic reserves in relation to fruit quality. Monthly Report 40: 127-129.

The study was conducted at two locations and fruits were harvested in winter and summer. Sugar concentration was higher in summer than in winter fruit and the higher sugars in summer fruit was not associated with consistently greater utilization of carbohydrate reserves in any part of the plant.

The following comments come from PRI Research Report 99 (Gortner, W.A., Spiegelberg, C.H., Dull, G.G., and Krauss, B.H., 1963. Field-fresh pineapple for export. Research Report 99).

It is general knowledge that all fruit in a single shell-color-translucence class do not have the same chemical composition, since there are large variations between fields and seasons. Singleton called these geographical or seasonal variations "base-line" differences, as opposed to "ripeness" differences.

The causes of these base-line differences have not been established. Singleton stated that "even without genetic variability, it has been remarkably difficult to show a large fruit quality effect by the agricultural treatments tested.

Although weather effects on the base-line have not been established, Singleton considered these as promising explanations of variations. The best lead, to date, involves soil moisture. Quoting Singleton (1959), "the fact that we have been unable to find a single environmental factor with great base-line

effects on quality makes the syndrome of related root deficiency-soil deficiency factors attractive as an explanation both of our experimental problems and of the quality variation".

Chan, Y.K., 1997. Performance and stability of new pineapple hybrids in g x e trials in Malaysia. *Acta Horticulturae* 425:201-211.

Fruit size was larger on pineapples grown on peat soils where there was no drought than on mineral soils where there was a distinct 2-3 month drought. As a result, yield was 50 % higher on peat soils; however, fruit TSS % was lower and fruits matured later on peat soils.

Liu, C. and Liu, Y., 2012. Impacts of shading in field on micro-environmental factors around plants and quality of pineapple fruits. *Journal of Food, Agriculture & Environment* 10:741-745.

Field-grown pineapple plants were shaded severely (S1, about 25% of natural sunlight) and slightly (S2, about 75% of natural sunlight) when the fruits were about 6 cm in width in horizon (about 30 days after blossom), respectively. Plants growing under natural sunlight were considered as control (S0). Compared with S0, temperature of S1 was decreased by 2-4.5°C in the whole day, RH of S1 was increased by 0.5% to 6.0%. Temperature of S2 was decreased by 1-1.5°C in the whole day. Compared with S0, TSS and contents of total sugar in the fruits of S1 and S2 declined, especially in S1. There was no significant difference in total acid among the treatments. There were more aroma components detected in the pineapple fruits of shading treatments, but the major added aroma components identified in S1 and S2 were alkenes with higher total relative contents. The total contents of ester aroma components of S1 and S2 were both lower than that of S0. In addition, the characteristic aroma component of pineapple fruits, propanoic acid 3-(methylthio)-methyl ester, was only identified in the fruits of S0, and not in S1 and S2.

## Announcements

Soil erosion in pineapple fields is a serious problem in some soils and in some regions. The International Soil Conservation Organization (ISCO), which met in Medellin, Columbia in 2013, would be a logical place for pineapple growers to meet and learn about managing this important problem. The following announcement was provided to me by Prof. Samir El-Swaify, a faculty colleague for many years at the University of Hawaii and also a founding member of ISCO.

Perhaps companies or other organizations that are doing research on soil erosion in pineapple will attend the meeting to report on their research and also share the results of their work with readers of Pineapple News.

## 18th International Soil Conservation Organization Conference

ISCO 2015. Achieving sustainability through conservation in a changing world.

First Announcement

May 31 - June 3, 2015 El Paso, Texas

### About ISCO

ISCO is an organization composed of conservation professionals from around the globe who meet biennially to share their latest research results. Beginning in 1978, ISCO has met on every inhabited continent and is returning to North America for the first time since 1999. Please mark your calendars and plan on spending the week with us in El Paso.

### Conference Topics

- Soil conservation for mitigation and adaptation to a changing climate: sustainable solutions
- Impacts of soil erosion and conservation on soil health and organic carbon sequestration
- Conservation agriculture
- Basic soil erosion: processes, mechanisms, and modeling
- Socio-economic dimensions of soil conservation
- Sustainable intensification of food production
- Soil degradation: salinization, sodification, and desertification

- Soil conservation in non-agricultural settings: i.e. urban and forestry

### Conference Venue

The University of Texas at El Paso campus between the Franklin Mountains and the Rio Grande is the conference site. UTEP is celebrating 100 years of education and research. El Paso is a multi-cultural city rich in history and culture.



### Important Dates

Aug. 15, 2014 - Call for abstracts

Nov. 15, 2014 - Early registration opens

Feb. 16, 2015 - Abstract submission deadline

### For More Information

For more information please email the chair of the Organizing Committee, Scott Van Pelt at: [Scott.vanpelt@ars.usda.gov](mailto:Scott.vanpelt@ars.usda.gov). The Conference web site is [www.Tucson.ars.ag.gov/isco](http://www.Tucson.ars.ag.gov/isco).

## International Pineapple Organization (IPO)

Will Cavan is co-founder and Executive Director of the organization. The first meeting of the IPO and major stakeholders on the post harvest and marketing side of the equation was held in San Jose, Costa Rica on Friday, November 01, 2013. A second meeting was held on March 20, 2014 in Tampa Bay, Florida. Future conferences are planned in Costa Rica on November 5-6, 2014 and in Columbia in 2015.

According to information in an announcement provided by the Tampa Port Authority, “The International Pineapple Organization (IPO) is a Global Trade Organization (GTO) in Winter Park, Fla., dedicated to addressing critical issues that affect the entire Pineapple Industry Supply Chain.”

I was unable to find a web site for the organization but please find their email address and social media (facebook) information below.

E-mail: [ipineapple.org@gmail.com](mailto:ipineapple.org@gmail.com)

Facebook: <https://www.facebook.com/InternationalPineappleOrganizationIpo>

## News from Australia

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

G. Sanewski  
Convenor, 8<sup>th</sup> International Pineapple Symposium.

The International Horticultural Congress 2014 is now only a few weeks away. The 8<sup>th</sup> International Pineapple Symposium is Symposium 35 (SYM35) of the Congress. There are 52 symposia in total. Over 3,000 registrants are expected for the Congress.

The program of presentations for the Pineapple Symposium is now set. We will occupy room P1 on the Plaza level of the Brisbane Convention Centre. There is information on the venue, The Brisbane Convention Centre, at <http://www.ihc2014.org/venue.html> or <http://www.bcec.com.au/attend-an-event>. You can enter the venue from Grey St., Merivale St. or Melbourne St. if you come across the Victoria Bridge. Please check out where your accommodation is, how to reach the venue and what else is in the area. It is also a good idea to familiarise yourself with the layout of the venue although there will be plenty of guides in the centre.

If you haven't registered and intend to come please register soon. Registration closes on August 1. Accommodation nearby is filling fast. The Pineapple Tour is also almost full with only a few places left.

If you are already registered please take some time to explore the IHC2014 website. It contains a lot of information if you drill down through the menus on the left. Make sure you have a plan of what to attend and when so it isn't too confusing during the event.

- Pineapple Symposium presentations are scheduled for Tuesday (19 Aug) and Wednesday (20 Aug). The pineapple symposium is referred to in the program as SYM35. Oral presentations will be 15 min except keynote addresses which are 30 min. Most days (Mon, Tue, Wed & Fri) will however start with Plenary speakers ([http://www.ihc2014.org/plenary\\_speakers.html](http://www.ihc2014.org/plenary_speakers.html)) in the Great Hall up to 10:00 am.
- Digital posters will be presented in the Exhibition Hall (5 min speed talks) on Wednesday (20 Aug) afternoon after afternoon tea.
- On Monday (18 Aug) afternoon (16:00 pm – 17:00 pm), the Pineapple Working Group will meet in room P1 to discuss the venue for the 9<sup>th</sup> International Pineapple Symposium. See <http://www.ishs.org/pineapple> for information on the Pineapple Working Group of ISHS.

For those that are presenting either oral or poster talks there are instructions at <http://www.ihc2014.org/index.html> on the left under 'Presenter Guidelines'. You should also receive advice from the IHC organising committee.

I hope to see you at the 8<sup>th</sup> International Pineapple Symposium.

## Most *Ananas* are Cultivars

Derek Butcher<sup>1</sup> & Eric Gouda<sup>2</sup>

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<sup>2</sup> Curator, Utrecht University Botanic Gardens; Subject Editor *Bromeliaceae* for *Phytotaxa*. Email: [e.j.gouda@uu.nl](mailto:e.j.gouda@uu.nl)

The naming of cultivated plants had its origins in 1862 when Alphonse de Candolle wrote a letter which was subsequently placed before the International Horticultural Congress of Brussels, 1864. De Candolle wished to reserve Latin names for species and varieties and to use only non-Latin 'fancy' names such as 'Bijou', 'Rainbow', etc., for garden forms. He suggested that this common, traditional and ancient practice should be made the only practice. It was not until 1952 for the ICNCP (International Code of Nomenclature of Cultivated Plants) to be born. It was not until 1979 that the Bromeliad Society produced its first Check-list of hybrids entitled 'International Checklist of Bromeliad Hybrids'. The following year they were appointed International Cultivar Registration Authority for Bromeliaceae but it was not until 1998 did we see the first Bromeliad Cultivar Register (BCR) published, closely followed by the on-line register in 2000 with as many photographs as we could find. It has been totally renewed in 2010 (<http://registry.bsi.org/>), see Lawn, Butcher & Gouda (cont.upd.).

Registration is easy and costs nothing but time. It is voluntary and if you have a hybrid that is distinct and you have several asexual plants then consider giving your progeny a name and register it. You would need a couple of photos and all you need do is contact the Registrar. Pineapples are unique in that they are mainly grown for their fruit and these cultivars are best listed in the Brooks & Olmo (1997), "*Register of Fruit and Nut varieties*". But if your hybrid or selection is primarily an ornamental then registration in the Bromeliad Cultivar Register is recommended.

Now let us look at the definition of a cultivar according to the ICNCP - International Code for Nomenclature of Cultivated plants:

**cultivar:** Produced in cultivation as opposed to one growing in habitat; – an assemblage of plants that has been selected for a particular attribute or combination of attributes and that is clearly distinct, uniform, and stable in these characteristics and that when propagated by appropriate means retains those characteristics.

**cultivated plant:** deliberately selected plants that may have arisen by intentional or accidental hybridization in cultivation, by selection from existing cultivated stocks, or from variants within wild populations that are maintained as recognisable entities solely by continued propagation. These are sometimes referred to as cultigens.

Coppens d'Veckenbrugge & Leal (2003) produced a monumental work on Pineapples in the book "The pineapple: botany, production and uses". They researched deeply and made the decision that *Ananas* was really a genus with only two species. The following are the changes that were proposed from that in Smith & Downs (1979). The major change is that the genus *Pseudananas* becomes one of the only two species in *Ananas*. Because the writers saw little difference in the listed species of *Ananas* these are now treated at varietal level under species *A. comosus*. Examples of three *Ananas* are shown below.

Smith and Downs  
*Pseudananas sagenarius*

Present classification  
*Ananas macrodontes*

*Ananas ananassoides*  
*Ananas nanus*

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

*Ananas lucidus*

*Ananas comosus* var. *erectifolius*

*Ananas parguazensis*

*Ananas comosus* var. *parguazensis*

*Ananas comosus*

*Ananas comosus* var *comosus*

*Ananas monstrosus*

Invalidated by Leal (1990) and treated as a form of *A. comosus*

*Ananas bracteatus*  
*Ananas fritzmuelleri*

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

The big change is in variegates because although treated at varietal level in Smith & Downs (1979) they have not been addressed in this work. We know that variegation is not a trait consistently transferred in sexual reproduction and as such is perhaps better catered for under the ICNCP rules.

This means that *Ananas comosus* var. *variegatus* becomes either *Ananas comosus* var. *comosus* 'Variegatus' or *Ananas* 'Variegatus'. Likewise *Ananas bracteatus* var. *tricolor* becomes *Ananas comosus* var. *bracteatus* 'Tricolor' or *Ananas* 'Tricolor'. These changes only apply to plants currently known by these names. However, if you have lost the label on your variegated pineapple, plants can be linked to 'Variegatus' if the plant is like 'comosus' but the leaves are variegated. They can be linked to 'Tricolor' if the plant is like 'bracteatus' and the leaf blades are variegated with longitudinal stripes. There are already accepted cultivars of these two varieties but, no doubt, there will be other cultivar forms of these and other varieties that will arise from time and time in the future.

Coppens et al (2011) updated their work from that in 2003. In 2013, ten years later, these recommendations have been largely ignored by non-Pineapple specialist botanists where current Binomial lists (Luther 2006) produced by Marie Selby Gardens show seven (7) species, *ananassoides*, *bracteatus*, *comosus*, *fritzmuelleri*, *lucidus*, *nanus*, and *parguazensis* and two variegates and one (1) *Pseudananas*.

On the other hand, Kew Gardens, in the World Checklist of Selected Plant Families (Govaerts et al., cont.upd.), have accepted that *Pseudananas* is really *Ananas* and quote six (6) species, *ananassoides*, *bracteatus*, *comosus*, *lucidus*, *parguazensis* and *sagenaria* and no variegates. Lista de Espécies do Brasil (Reflora, cont.upd.), shows seven (7) species, *ananassoides*, *bracteatus*, *comosus*, *fritzmuelleri*, *lucidus*, *nanus*, and *parguazensis* and no variegates and one (1) *Pseudananas*.

In fact, the only place where the 'Pineapple People's' proposal is accepted at the moment is in the New Bromeliad Taxon List (Butcher & Gouda, cont.upd.). None, of course, show any reasoning for synonyms but does show varying opinions. The only one to make comment, as far as we can trace, is Elton Leme (Filho & Leme, 2007). However, Elton Leme has ignored the existence of two variegates mentioned in Luther (2006) and Smith & Downs (1979) and the ICNCP code which we quoted at the start of this article.

In 2009 Coppens d'Eeckenbrugge and Marie-France Duval presented an excellent paper on 'The Domestication of Pineapple' in Pineapple News Issue No. 16, 2009, which showed the differences between domesticated and wild species. However, the domesticated plants were still identified under species names not cultivar names.

In 2014 in looking at the whole of *Ananas*, we have come to the conclusion that all descriptions in the past have been based on cultivated material with the possible exceptions of *A. ananassoides*, *A. parguazensis* and *A. macrodontes*. These are not known for their edible fruit and would have had minimal selection by the native population. We should not underestimate the influence of man on (semi) natural vegetation, especially when they have nutritional value. Therefore we propose the following:

*Bromelia ananas* L. *Sp. Pl.*: 285 (1743) = basionym of *Ananas comosus* **must be considered a man made cultivar**, which makes *Acanthostachys ananassoides* Baker *Handb. Bromel.* 25 (1889) = basionym of *Ananas ananassoides*, the only real *Ananas* species to be recognized besides *Ananas parguazensis* Camargo & L. B. Smith, *Phytologia* 26: 464, fig. 1. 1968. and *Ananas sagenaria* Schult.f. *Syst. Veg.* vii. 1286 (1830). Accordingly we designate a new lectotype for the Genus *Ananas* as *Acanthostachys ananassoides* Baker. The new cultivars will be *Ananas* 'Bracteatus', 'Comosus', and 'Erectifolius'. One variety of *Ananas ananassoides* is recognized here: *Ananas ananassoides* var. *nanus* L.B.Sm. *Bot. Mus. Leaflet* 7: 79 (1939), which is a small form from Surinam and Brazil and often seen as ornamental because of its size.

Clearly the Pineapple fraternity are more interested in *Ananas* than its relationship within Bromeliaceae as a whole but should still look at the whole picture. Not only do they have the "Pineapple News" but are more likely to read mainstream horticultural journals such as *Scientia Horticulturae* and *HortScience*. But there is a danger they may get further away from the general botanical picture.

Perhaps, the concept of an ICNCP solution could be discussed as part of the international symposium in Brisbane next year as part of the IHC2014.

## ACKNOWLEDGEMENTS

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**Ananas ananassoides**  
Photo of Eric Gouda



**Ananas 'Bracteatus'**  
Photo of Oscar Ribeiro



**Ananas 'Comosus'**  
Photo of Belg Hort 3-125

## The Use of Best Linear Unbiased Predictions (BLUPS) in Pineapple Breeding

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### INTRODUCTION

The cultivars 73-50 and MD-2 are the most successful modern pineapple cultivars and are close to the desired type. MD-2 in particular has revolutionized the fresh pineapple market worldwide (Sanewski, 2007; Sanewski, 2009). Both 73-50 and MD-2 are popular fresh market pineapple cultivars on the Australian fresh market but possess a number of faults that reduce profitability. A modest breeding program is therefore in progress in Australia to improve on these cultivars.

Pineapple is often considered to be very heterozygous (Sanewski et al, 2010). This however might be more related to specific cultivars when used as parents. Other cultivars, particularly late generation cultivars, may not be a significant source of variation. Information on the best parents is of course fundamental to any breeding program and there are various techniques to generate this information. This study looked at the use of Best Linear Unbiased Predictions (BLUPS) of cultivar effects for a number of traits. BLUPS predict cultivar effects, based on their progeny by incorporating pedigree information. This pedigree information is not presented here but was used in the calculations. Seedling or family data is likewise not presented here. Only BLUP data for parent cultivars which is generated from the seedling and family pedigree data is presented.

### MATERIALS AND METHODS

A trial comparing 10 seedling populations derived from different parent combinations and two parent cultivars (A2 & A1) was planted in 2010 to provide parent predictions and trait heritabilities. The trial design was a randomised block with five blocks consisting of 12 plots with eight plants per plot. There were therefore 12 "treatments" (10 seedling families and 2 sets of clones) with 40 plants per treatment.

The seedlings were grown in 100 mm pots until approximately 6 months old with a stem diameter of approximately 20-30 mm, then field planted. There were no obvious treatment differences in the size of plants at this time. The trial was planted at a plant density of 53,000 plants/ ha and was surrounded on all sides by seedlings of a similar size. The plants were induced to flower at approximately 12 months of age. Fruit were harvested when one fourth to one half colour.

Data of a continuous nature from this trial including fruit weight, fruit length, fruit diameter, peduncle length, peduncle width, time to harvest, crown weight, fruitlet number, fruitlet width, core diameter, TSS%, slip number, porosity, flesh colour, and mean fruitlet weight were all analysed using a general linear mixed model incorporating pedigree relationships (going back 2-3 generations). Terms for Blocks and Plots within Blocks were included in the mixed model as random effects and estimation was done using Residual Maximum Likelihood (REML) in ASReml (Gilmour et al 2009).

As there was also some clonal material in the trial, a residual genetic effect was able to be included. This allowed the genetic variance to be separated into additive genetic variance and non-additive genetic variance components and hence both narrow and broad sense heritabilities were calculated (see Table 1). Using the additive genetic effect of each family it is also possible to predict which will make the best parents for a particular trait in future crosses.

There were some traits that could not be analysed on the normal scale as they were binomial (presence or absence) data or rating data on very few categories. These traits were plant sucker number, ground sucker number, crown type (single or multiple), crown slips, knobs and translucence. They were all converted to binomial data (presence/absence) and analysed using a mixed model with a logit link function in ASReml (Gilmour et al 2009). Best linear unbiased predictions (BLUPs) were predicted for all families from these analyses.

## RESULTS AND DISCUSSION

The analyses provided rankings for families for all traits. These predictions give an indication of which parents carry the genes necessary to make improvements in specific traits and which cultivars have a propensity to pass on traits of an undesirable nature.

### Breeding values (BLUPS)

Based on the BLUPS in Table 1, highlights of the data are as follows.

- 21-250 is significantly worse than a number of other cultivars for passing on a high incidence of slips.
- 16-1357 and 58-1184 (pollen parent of MD-2) were also a source of slips while 10-1985, A2, 71-92 and 11-149 were at the lower end of the scale.
- A1 and A2 carry a high genetic propensity for a long peduncle whereas MD-2 was useful for shortening the peduncle.
- 21-250 and 58-1184 were the only source of crown slips in the breeding population studied.
- A2 and 53-116 produced a higher core ratio (smaller core compared to fruit diameter). A1 was a source of a larger core.
- 21-250 was the only source of white flesh whereas 10-1985 was a good source of yellow flesh. None of the cultivars studied appear useful for developing an intense yellow flesh.
- 10-1985, 8-187, 71-92, A1 and A2 were all a source of high TSS.
- 1-12025, 58-1184, MD-2 and A2 had a greater capacity to increase fruitlet width compared to the other cultivars.

**Table 1.** BLUPS for several parent cultivars and traits, together with narrow and broad sense heritabilities ( $h^2$  and  $H^2$  respectively;  $n=40$ ).

Progeny	Number Slips	Peduncle length (mm)	Crown slips*	Core ratio**	Flesh colour***	TSS%	Eye width (mm)	Fruit wt (g)
10-1985	0.3	159	0	5.6	2.8	18.5	22.2	1108
11-149	0.1	146	0	5.8	2.5	16.4	22	1023
1-12025	1.0	174	0	5.9	2.5	13.4	24.7	1003
1-13039	1.4	180	0	5.9	2.6	16.1	21.9	921
16-1357	2.5	178	0	6.1	2.2	14.7	22.8	999
16-320	0.9	178	0	6.3	2.1	16.5	19.7	990
21-250	3.4	188	0.03	5.8	1.6	14.4	23.1	1135
71-92	0.1	162	0	6	2.5	19.1	20.4	965
53-116	1.2	184	0	6.8	2.5	17.9	22.8	1031
58-1184	2.9	147	0.09	5.3	2.6	16.9	24.6	1080
59-443	0.7	164	0	6.1	2.4	15.3	22.5	1035
73-50	1.8	167	0	5.9	2.4	15.1	23.4	1089
8-187	0.5	176	0	6.1	2.5	20.1	20.1	833
MD-2	1.1	137	0	5.6	2.6	15.4	23.8	1054
A2	0.1	219	0	8.4	2.6	17.3	24.1	1150
A1	2.0	216	0	4.8	2.7	18.2	22	850
LSD	2.9	78		1.74	0.8	4.6	4	596
$h^2$	0.39	0.19	0.21	0.25	0.64	0.70	0.60	0.17
$H^2$	0.85	0.43	0.33	0.49	0.64	0.70	0.74	0.65

\* Probability of 1 or more crown slips

\*\* Ratio of core diameter to fruit diameter. A large number means a small core.

\*\*\* 1=white, 2=pale yellow, 3=yellow, 4=dark yellow.

Differences were not seen in flesh porosity, fruit diameter, fruit length, fruitlet number, fruit weight, crown weight, peduncle width, translucency, basal knobs, sucker number or mean fruitlet weight.

Looking at the overall contribution of each progeny, 21-250 is highly undesirable carrying multiple faults that are most likely derived from its seed parent, Monte Lirio. 10-1985 is one of the most preferred parents. MD-2 and A2 were also highly desirable parents for some traits. A1 exhibited a low breeding value when most traits were considered. 58-1184, the seed parent of MD-2, was a source of crown slips and slips.

#### **CONCLUSION**

BLUPS were found to be a very useful approach for comparing the genetic value of parents on a trait by trait basis.

#### **ACKNOWLEDGEMENTS**

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## News from Brazil

### Reaction of Pineapple Genotypes to the Nematode *Pratylenchus brachyurus*

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#### ABSTRACT

Nematodes are considered one of the main factors limiting pineapple cultivation. This work had the objective to evaluate the behavior of different pineapple genotypes in relation to *Pratylenchus brachyurus*. Sixteen treatments (genotypes) were inoculated 60 days after transplanting with a suspension containing 1,000 individuals (eggs and J2) and the plant vegetative development and behavior in relation to the nematode presence were evaluated at 120 days after inoculation. The cv. IAC Fantástico behaved as resistant, whereas the cvs. BRS Imperial, BRS Vitória and BRS Ajubá as moderately resistant, the cvs. Smooth Cayenne, Pérola and Perolera as little resistant and the cv. Gold as highly susceptible to *P. brachyurus*.

Keywords: *Ananas comosus*, nematodes, cultivars, germplasm, resistance.

#### INTRODUCTION

Nematodes are considered one of the main factors limiting pineapple cultivation. These pathogens cause significant damage to plant roots, reducing nutrient absorption and fertilizing efficiency. Plants affected show limited root system, chlorotic, narrow and small leaves and fruits of low weight and reduced commercial value (Arieira et al., 2008).

There are reports of more than 100 nematode species associated with the root system of pineapple plants (Sipes et al., 2005), but the pathogenicity of most of these species is still little known. The nematodes of most negative impacts on pineapple plants are *Pratylenchus brachyurus*, *Meloidogyne javanica*, *M. incognita* and *Rotylenchulus reniformis* (Costa and Matos, 2000; Sipes and Schmitt, 1994, 2000; Sipes et al., 2005).

Plant nematodes have been observed in various production areas in Brazil and especially in the States of Minas Gerais, Paraíba, Bahia, Sergipe, Espírito Santo, São Paulo and Rio de Janeiro.

The general field symptoms of *P. brachyurus* attack are similar to those caused by other nematodes, such as limited root system with necrosis, field flecks with plants exhibiting reduced growth, yellow to reddish colored leaves with drying margins, similar to symptoms of nutritional deficiency and water stress, increased vegetative period of the plant cycle, reduction of fruit weight and production, and in some cases even plant death (Ferraz and Zem, 1982).

Among the nematode control measures – cultural, chemical and biological ones - few are economically viable. There is a demand for new alternatives to the application of nematicides which are toxic to humans and the environment.

The identification and use of genetic resistance or tolerance sources are considered appropriate for the reduction of problems caused by nematodes (Soler and Marie-Alphonsine, 2011). Any genotype identified as a source of resistance or tolerance to nematodes may be recommended as a new cultivar or be used in the development of improved cultivars in order to get an economically and environmentally more sustainable production. Within this context, this work had the objective to evaluate the behavior of different pineapple genotypes in relation to *Pratylenchus brachyurus*.

#### MATERIAL AND METHODS

The following pineapple genotypes were evaluated in this study: the cultivars Pérola, Smooth Cayenne, Gold (MD-2), Perolera, IAC Fantástico, BRS Imperial, BRS Vitória and BRS Ajubá; the Embrapa hybrids PA x PE-01, SC48 x PRI-02, SC x PRI-21, PE x SC-52, PE x SC-60, PE x SC-73 and the accessions of the Embrapa Germplasm Bank, BAG 194 and BAG 344 (Table 1). The planting material of these genotypes was produced from stem sections in a nursery and thereafter transplanted to 3L pots containing a sterilized substrate composed of soil, sand and cattle manure in a 3:3:1 proportion.

The inoculum was obtained from pineapple roots previously inoculated with a population of *P. brachyurus* from the biological collection of the Nematology Laboratory of Embrapa Cassava & Fruits. The nematodes were extracted from the roots according to the methodology proposed by Boneti and Ferraz (1981).

Treatments were distributed in a completely randomized design with eight replications on benches in a greenhouse. At 60 days after transplanting each plant was inoculated by pouring 2 mL of a suspension containing 1,000 individuals (eggs and J2) of *P. brachyurus* into two holes opened on opposite sides of the plant stem base, which had exposed the roots.

Plant vegetative development and behavior in relation to the nematode presence were evaluated at 120 days after inoculation. The following variables were determined: shoot and root system weight, number of nematodes in the roots (Boneti and Ferraz, 1981), number of nematodes in the substrate (Jenkins, 1964), final population of nematodes, nematode reproduction factor, reduction of reproduction factor and reaction of the pineapple genotypes (Table 1). The reproduction

factor was calculated by dividing the final population per pot by 1.000 (Seinhorst, 1967). The behavior of the inoculated genotypes was determined based upon the criteria described by Moura and Regis (1987).

Data were submitted to analysis of variance and average values compared by the Scott & Knott test at 5% using the statistical analysis program SISVAR (Ferreira, 2008).

### RESULTS AND DISCUSSION

A rather broad array of responses was obtained to *P. brachyurus* inoculation among the 16 genotypes studied, with variations from highly susceptible to resistant (Table 1).

The genotypes showed different vegetative development. However, these differences are possibly due to the different growth rates of genotypes, since no correlations were found between vegetative growth and response to nematode.

'MD-2' which has the largest volume of fresh pineapples in the world trade was highly susceptible to *Pratylenchus brachyurus* and also to *Fusarium guttiforme* (data not shown). These susceptibilities are important limiting factors to its exploitation in Brazil.

Pérola and Smooth Cayenne, the cultivars most grown in Brazil, have low resistance, a result that gives more relevance to surveys on incidence and severity of damages caused by this nematode in Brazilian commercial pineapple fields.

The Embrapa hybrids were little to moderately resistant to the nematode, almost similar to their parents Pérola (PA), Smooth Cayenne (SC) and Perolera (PE). The results obtained in this study for Pérola and Perolera agreed with those reported by Dinardo-Miranda et al. (1996) and Costa et al. (1999). The two accessions of the Germplasm Bank (194 and 344) have been selected due to their known resistance to *Fusarium guttiforme* and their reaction to *P. brachyurus* can favor their insertion into the breeding program with respect to obtain nematode resistance.

IAC Fantástico, a cultivar derived from the 'Smooth Cayenne' and the Brazilian variety Tapiracanga (resistant to fusariosis), was completely resistant to the nematode. The latter possibly is the source of the resistance shown to *P. brachyurus*, but this resistance must be confirmed under field conditions.

### CONCLUSIONS

The cv. IAC Fantástico behaved as resistant, whereas the cvs. BRS Imperial, BRS Vitória and BRS Ajubá were moderately resistant, the cvs. Smooth Cayenne, Pérola and Perolera were little resistant and the cv. Gold as highly susceptible to *P. brachyurus*. The presence of resistance in one cultivar gives hope that new sources of genetic resistance to this important nematode should be identified in the rich diversity of accessions present in the Embrapa pineapple germplasm collection.

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Table 1 – Average values of plant vegetative development and reaction of different pineapple genotypes to *Pratylenchus brachyurus* evaluated 120 days after inoculation under greenhouse conditions. Cruz das Almas, Bahia, Brazil, 2014.

Genotype	Shoot weight (g)	Root weight (g)	PL Roots	PL soil	Final PL (soil + root)	RF	RRF (%)	B
Gold	250,6 b	11,3 c	1899,4 a	500,0 a	2399,4 a	2,4	100,0	HS
Smooth Cayenne	197,5 b	8,1 c	413,1 b	212,5 b	625,6 c	0,62	73,9	LR
BRS Imperial	313,8 a	13,1 b	216,9 b	262,5 a	479,4 c	0,48	80,0	MR
BRS Vitória	184,4 b	6,3 c	146,7 b	62,5 b	172,5 c	0,17	92,8	MR
BRS Ajubá	176,3 b	6,9 c	179,0 b	37,5 b	149,4 c	0,15	93,8	MR
IAC Fantástico	232,5 b	11,9 c	131,7 b	0,0 b	98,8 c	0,1	95,9	R
Perola	344,4 a	14,4 b	258,1 b	412,5 a	670,6 c	0,67	72,1	LR
Perolera	329,4 a	21,3 a	424,4 b	362,5 a	786,9 b	0,78	67,2	LR
PE x SC-52	219,4 b	10,6 c	209,3 b	100,0 b	283,1 c	0,28	88,2	MR
PA x PE-01	385,0 a	14,4 b	680,0 b	150,0 b	830,0 c	0,83	65,4	LR
PE x SC-73	389,4 a	20,0 a	424,2 b	112,5 b	430,6 c	0,43	82,1	MR
PE x SC-60	155,6 b	8,1 c	518,1 b	75,0 b	593,1 c	0,6	75,3	LR
SC-48 x PRI-02	339,4 a	21,3 a	1022,5 a	337,5 a	1104,4 b	1,1	54,0	LR
SC x PRI-21	362,5 a	16,9 b	527,9 b	50,0 b	511,9 c	0,51	78,7	MR
BAG 194	189,4 b	5,6 c	273,3 b	75,0 b	177,5 c	0,17	92,6	MR
BAG 344	334,4 a	15,0 b	117,1 b	162,5 b	265,0 c	0,26	89,0	MR

PL – population level; RF – reproduction factor; RRF – reduction of reproduction factor; B - behavior; HS – highly susceptible; LR – little resistant; MR – moderately resistant; R – resistant.

Average values followed by the same letter within columns do not differ by Scott & Knott test at 5%

## News from Costa Rica

### Pineapple Photosynthesis and Leaf Sap pH as a Surrogate of CAM Performance in the Field. A Research Advance

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#### Abstract

The objective of this report is to provide an update on the measurement and the agronomic implications of crassulacean acid metabolism (CAM) photosynthesis in pineapple. We show diurnal courses of CAM and related parameters obtained in greenhouse, pot-grown plants during consecutive days using a portable, LI-6400XT, photosynthesis system. We also report on the use of pH analytical indicator paper to measure pH fluctuations as a surrogate of CAM of plants growing in the field, and as an indicator of photosynthetic performance. The results show CO<sub>2</sub> fixation (A) rates of up to 6 μmol m<sup>-2</sup> s<sup>-1</sup> in greenhouse plants. Intense CAM activity as judged by pH changes was observed in vegetative leaves and crowns of reproductive plants in the field. Despite stomatal closure, transpiration was higher during the day-time. Simple pH measurements allow the tracking of CAM rhythms in the field and constitute a good indicator of photosynthetic performance. The final aim of this work was to produce a physiology-based crop profile for pineapple, which includes the morpho-physiological study of the crop and the understanding of night-time activity of pineapples, to improve the effectiveness of agronomic practices and the sustainability of the pineapple agro-ecosystems.

#### INTRODUCTION

Major gaps in knowledge about the physiology and agronomy of the pineapple (*Ananas comosus*) crop have remained unfilled over the years. For example, the 'MD-2' variety, the preferred pineapple cultivar for fresh fruit export around the world, was launched in 1996, but growers still express concerns about the limited technical information available for this cultivar, and no manual of agronomic practices for MD-2 exists (Bartholomew, 1977, 2012).

Pineapples are constitutive crassulacean acid metabolism (CAM) plants. Spiral leaf arrangement and vertical orientation of this rosette plant allows good distribution of available photosynthetically-active radiation (PAR) in field-grown plants. Planting density is high and intense interplant competition influences crop uniformity (Bartholomew, 1977, 2012). Stomata close during the day-light period and open at night (Figure 1).

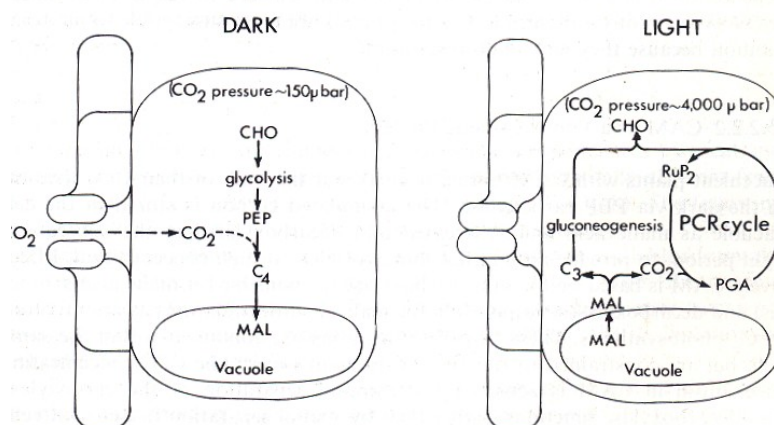


Figure 1. CAM photosynthesis showing the temporal organization of the component processes in the same photosynthetic cell. From: Osmond, Winter and Ziegler. 1982. *Physiological plant ecology. I. Water relations and carbon assimilation.* Springer-Verlag. p. 487.

In pineapple, net photosynthesis is determined primarily by solar radiation (during the day time) and temperature, whose seasonal variation profoundly affects net assimilation rates and dry matter allocation. Plant-environment interactions in pineapple are complex and have a major temporal component, because the environmental effects from the light period are carried-over into the night, and to subsequent daily cycles of CO<sub>2</sub> fixation. During the night, CO<sub>2</sub> diffuses inside the leaves and the first of two CO<sub>2</sub> fixation steps takes place in the cytoplasm of photosynthetic parenchyma by the enzyme PEP carboxylase (Figure 1). The resulting malic acid is stored in large vacuoles whose volumes impact the extent of acid accumulation and crop photosynthesis and productivity. During the next light-day period and with closed stomata, the CO<sub>2</sub> stored in the vacuoles is decarboxylated, and leaf internal [CO<sub>2</sub>] increases greatly, suppressing the oxygenation reaction of Rubisco and photo-respiration. In the chloroplast, Rubisco performs the second carboxylation step, using ATP produced through photosynthetically active radiation (PAR)-driven photo-chemical reactions that power assimilate synthesis in the photosynthetic carbon reduction (PCR) cycle, and their export to carbon-demanding sinks such as roots, expanding leaves, flowers and developing fruits (Osmond 1978, Kluge and Ting 1978, Nobel 1988). CAM responses to temperature are also complex, and the variations in physiological performance affect crop development and growth rates, fruit weight and quality, harvest index, and type and availability of propagules.

Very little information exists about leaf and canopy assimilation profiles and water use efficiency in the field, and only for a few agro-ecological conditions. Major effects of sparse pineapple monocultures on net ecosystem CO<sub>2</sub> exchange are apparent (San José et al., 2007): in early stages of crop development, the energy balance of pineapple fields resemble that of C3 crops. CAM becomes important and pineapple crops become carbon sinks only after full canopy closure. Under current global climate change scenarios, the possibilities to improve carbon fixation, water use and sustainability of the pineapple agro-ecosystems seem numerous.

The objective of this report is to provide a progress report on the measurement and the agronomic implications of CAM photosynthesis in pineapple. Our long-term aim is to produce a physiology-based crop profile for the 'MD-2' pineapple, which includes the morpho-physiological study of the crop and the understanding of night-time activity of pineapples to improve the effectiveness of practices like irrigation, forcing and foliar fertilization. In this preliminary report we characterize CAM photosynthesis of MD-2 pineapples in Costa Rica, and explore whether leaf sap pH serves as a surrogate of photosynthetic performance in the field.

## MATERIALS AND METHODS

We used MD-2 pineapple plants. One generation of this cultivar lasts from 20 to 30 months and one planting may last for 10-15 generations, though it is much less for many growers (Bartholomew, 2012). In pineapple it is common to sample the youngest, fully-expanded "D-leaf" for growth, nutritional status, and other physiological determinations. Young leaves have more active CAM than older leaves. Greenhouse observations were conducted at Fabio Baudrit Experiment Station in Alajuela, Costa Rica (10 N, 840 m). Field work was conducted in commercial plantations at Upala Agrícola, in the northern lowlands of Costa Rica (30 m). CAM was measured by two methods.

In greenhouse-grown, potted plants (10 L containers), diurnal courses of CO<sub>2</sub> fixation (A) and related parameters: photosynthetically-active radiation (PAR), leaf temperature, stomatal conductance (g<sub>s</sub>), transpiration rate (E), and internal CO<sub>2</sub> concentration (C<sub>i</sub>) were measured with a LI-6400XT portable photosynthesis system (Li-Cor, Lincoln, NE, USA). The instruments were set-up to store measurements with a frequency of 10 sec, in order to detect the four phases characteristic of CAM diurnal rhythms. The Li-6400XT cuvette was modified with gaskets, hinge and three-hole shim for thick leaves provided by the manufacturer (Figure 2).

In the field, changes in sap pH of D leaves of large, reproductive plants were monitored over 24-h cycles using paper strips. We used SEOH pH Duotest analytical indicator paper to measure pH in the ranges of 3.5-6.8 and 1.0-4.3. In some cases, the paper strips were surgically inserted radially within the thick leaf blades, in both the upper water-storing tissue and the lower photosynthetic parenchyma (Krauss 1948, 1949). The paper strips remained implanted within the leaves for 10 min, after which they were extracted with forceps and the pH assessed. In most cases the leaves were cut immediately before the pH measurements were performed, and the pH strips treated as described before. Pith pH was also measured in field-grown plants (data not shown).

## RESULTS AND DISCUSSION

### CAM of pineapple in the greenhouse

We successfully measured CAM photosynthesis in pineapples using a LI-6400XT portable photosynthesis system. The chamber was equipped with a gasket and hinges designed to allow gas exchange measurements in thick leaves (Figure 2).

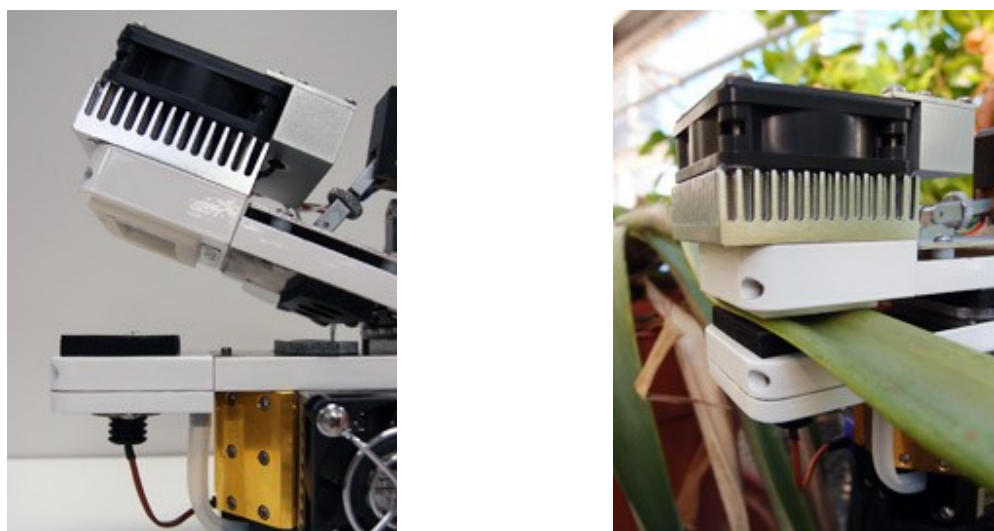


Figure 2. LI-6400 portable photosynthesis system with gaskets and hinges for thick leaves installed on the D leaf of a greenhouse-grown, 'MD-2' pineapple. Photos from Li-Cor Scientific, Lincoln, NE, USA.

CAM was measured under sunny, hot days in the greenhouse (Figure 3). PAR reached  $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$  and leaf T approached  $50 \text{ }^\circ\text{C}$  during mid-day hours. Maximum A rates of  $6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (similar to the results of Zhu et al., 2005), highest  $g_s$  (up to  $35 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), and lower  $C_i$  values ( $\sim 100 \mu\text{mol m}^{-1}$ ) were observed during the night. Marler (2011) recorded A rates lower than  $0.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in the lower leaves of pot-grown, vegetative pineapple plants, that did not increase during flowering and fruiting. In contrast, A of crowns and slips was considerably higher ( $>4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), which suggested that CAM activity of fruit crowns and slips may be essential to supply the assimilate requirements of reproductive, fruiting pineapple plants. We are extending these measurements to field-grown plants using state-of-the-art instrumentation, to assess photosynthetic performance and water use in the field, and the consequences of current agronomic practices on CAM in pineapples.

Agronomic practices should result in low  $C_i$  values in leaves during the night, an indicator of high capacity of a pineapple plant to draw  $\text{CO}_2$  from the air. In the greenhouse, A declined continuously during the course of three days of measurements probably due to rapid water depletion from the pots, located in a hot greenhouse under high PAR and evaporative demand, or to effects of continuous measurements during three consecutive days. Despite the occurrence of CAM and stomatal closure during the light period, E was higher during the day-time (phase IV), probably as a consequence of intense cuticular transpiration and incomplete stomatal closure (Figure 3).

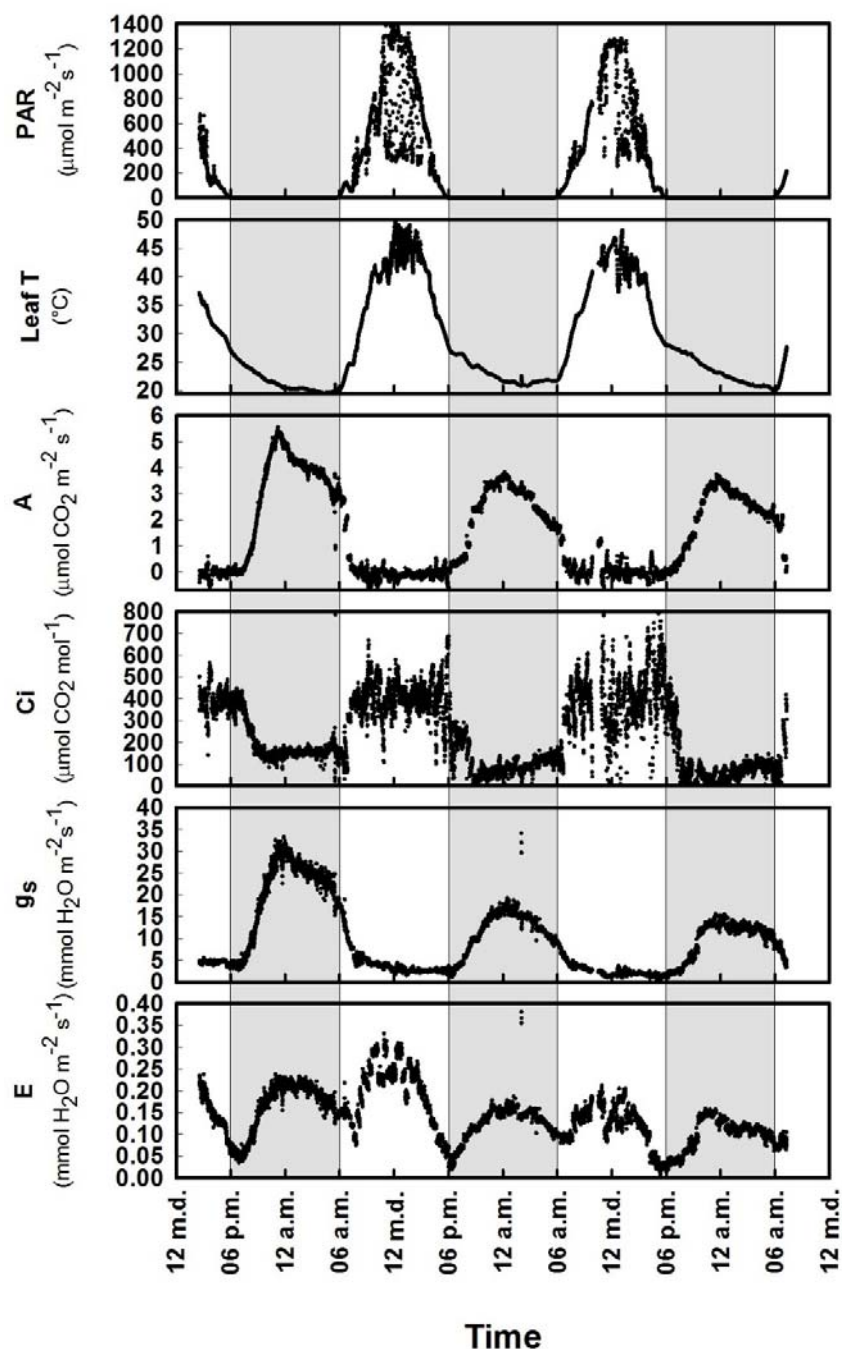


Figure 3. Diurnal courses of photosynthesis (A) and related parameters during three consecutive days in pineapple ‘MD-2’ plants growing in containers in the greenhouse, obtained in March 4-7, 2014. Photosynthetically-active radiation (PAR), leaf temperature (T), stomatal conductance ( $g_s$ ), transpiration rate (E), and internal  $CO_2$  concentration ( $C_i$ ) are also shown. Measurements were obtained every 10 sec. with the use of a LI-6400XT portable photosynthesis system. Vertical bars indicate the night-time periods.

Detailed examinations of individual CAM diurnal cycles would distinguish four phases (Osmond 1978): phase I occurs at night with open stomata and maximum  $CO_2$  fixation occurs; phase II is brief and begins with the light period, when stomatal closure causes a decline in  $CO_2$  fixation (some species, including pineapple (Zhu et al., 1999) show a small burst in  $CO_2$  fixation at the start of the day); phase III spans from early morning through late afternoon, the stomata remain closed, no  $CO_2$  fixation occurs, but intense internal de-acidification takes place;

phase IV starts in late afternoon with stomatal opening and high CO<sub>2</sub> influx. Depending on the environmental conditions (i.e. water availability), some CAM plants may engage in periods of CO<sub>2</sub> fixation during the day (longer phase IV), which may increase productivity and yield.

It is important to address the possible exploitation of night-time physiological activity of pineapples for agronomic applications, such as improvements in the effectiveness of foliar fertilization and the efficiency of water use, especially under irrigation regimes and current water scarcity. It is important to consider other properties of pineapples such as the presence of abundant water absorbing trichomes (Sakai and Sanford, 1980), which completely cover the adaxial surface of the leaf as well as the stomata on the abaxial surface (Bartholomew and Kadzimin, 1977; Krauss 1948, 1949; Benzing et al. 1976). However, water status indicators for field-grown pineapples are currently lacking.

### Leaf sap pH is a surrogate of CAM in the field

We wanted to know whether daily fluctuations of leaf sap pH can be used as an indicator of CAM performance in the field (Friend, 1990). We observed strong variations in pH in 24-h, day-night cycles (3.5 to 6.5, Figure 4), and indications from our studies and an earlier study of Aubert (1971) are that the intensity of this variation corresponds to PAR and temperature conditions of the previous phase IV. Fluctuations of pH were more intense in the photosynthetic parenchyma, although important pH changes were recorded in the water-storage tissue as well after sunny days.

In contrast to the results of Marler (2011), the D leaf of pineapple exhibited CAM rhythms just as intense as those recorded in the crown. We are extending these measurements to the pith tissue located in the core of the pineapple stem, an organ that may be important in water and acid storage, and constitutes the main site of bromelain biosynthesis, a potent anti-fungal metabolite.

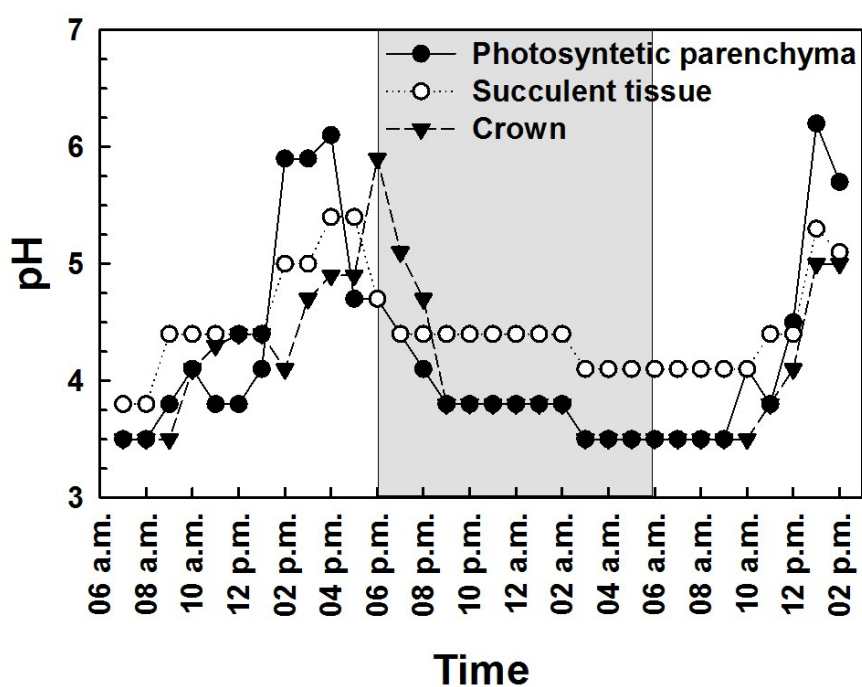


Figure 4. Daily changes in sap pH of the D leaf of 'MD-2' pineapple plants growing in the field (Costa Rica, North-Caribbean lowlands, March 21-22, 2014). Leaf sap pH was measured using SEOH pH Duotest analytical indicator paper inserted in the photosynthetic parenchyma and the water-storing tissue of the D leaf and fruit crowns.

The degree of de-acidification during the day (phases III and IV) and of stomatal aperture during the following night period (phase I) depended greatly on day-time PAR levels (data not shown). The effects of low light during overcast days were apparent as a reduced de-acidification of the leaf sap. As a consequence of a

relatively low leaf pH at the end of the light period, stomatal opening during the following night was reduced and night-time CO<sub>2</sub> fixation declined correspondingly. Recurrent low stomatal aperture and reduced nocturnal CO<sub>2</sub> fixation invariably would result in reduced biomass production and growth.

Daytime irradiance influences CAM in pineapples in several ways: the time of influx of CO<sub>2</sub> (phase IV) begins earlier, stomata open earlier in the afternoon (phase III shorter; phase IV longer), and high irradiance in phase IV results in maximum rates of CO<sub>2</sub> influx. Greater irradiance speeds the rate of ingress into phase I of high CO<sub>2</sub> fixation at night (Bartholomew, 2012).

Simple pH measurements like those described above allow the tracking of the de-acidification phase of CAM during the day. Portable gas exchange systems such as the LI-6400XT provide no information on de-acidification rate or C<sub>i</sub> increase during the day, because the stomata remain closed and gas exchange rates are nil. Like this carry-over effect of low light, the consequences of other stresses due to water deficits or excesses and high-temperature on field-grown pineapples have not been studied.

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## News from Cuba

### Effect of Previcur<sup>®</sup> Energy Fungicide on MD-2 Pineapple (*Ananas comosus* var. *comosus*) Plantlets During the Acclimatization Phase

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#### INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) is a tropical fruit of great commercial value with production being second only to bananas. 'MD-2', the most important fresh fruit cultivar, has dominated the fresh fruit market in recent years (Loeillet et al., 2011). The commercial importance of 'MD-2' has resulted in the need for plantlets to rapidly increase the planted area. Micro-propagation is much faster than traditional methods of vegetative propagation and its use has spread to various horticultural, agricultural and forestry crops (Jeon et al., 1995).

In the case of propagation protocol of pineapple plantlets, mortality in the acclimatization stage is greatly associated with fungal diseases. When mortality can not be reduced by environmental or substrate management, it is necessary to use chemicals to control the disease (Hernandez-Mansilla et al., 2010). Among the available options are the use of inducers of acquired resistance. The goal of this research was to study the effects of Previcur<sup>®</sup> Energy (Bayer CropScience) in controlling *Phytophthora* sp.

#### MATERIALS AND METHODS

All experiments were carried out at the Centro de Bioplantitas, Cuba (41°53'N, 78°41'W, 45 m a.s.l.). Uniform MD-2 pineapple plants (*Ananas comosus* (L.) Merr.) were micro-propagated as described by Escalona et al. (1999). The substrate used was a mixture of zeolite and filter cake (1:1) (v / v). Black polybags (8 cm diameter) were used. Small holes were made at the bottom of polybags using a paper punch to allow aeration and drainage of excess water. Irrigation was performed on a daily micro-aspersion of 5 minutes duration in morning hours. Light intensity (FFF) in the green house was maintained between  $458 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ , measured at midday and the photoperiod regime corresponded to natural cycles.

Homogeneous MD-2 pineapple vitroplants (plantlets) (120) with on average 8.8 leaves, a length of 8.9 cm and a fresh weight of 1.3 g were selected. The plantlets were divided into five replicates of 20 plantlets which were distributed randomly among the treatments. The plantlets were immersed for 3 minutes in solutions containing Previcur<sup>®</sup> Energy concentrations of 1.0, 2.0 and 3.0 mL/L. The morphological variables evaluated after 45 days in the acclimatization phase were:

- 1 - Survival (%)
- 2 - Number of leaves
- 3 - Number of roots

The statistical analysis of the results was developed with "STATGRAPHICS Plus". The normal distribution (Kolmogorov-Smirnov,  $P < 0.05$ ) and homogeneity of variances (Levene,  $P < 0.05$ ) Anova analysis of simple classification and Tukey test ( $P < 0.05$ ) were performed after checked.

#### RESULTS AND DISCUSSION

Number of leaves and roots were significantly higher at 1.0 mL/L (Figure 1) while leaf and root numbers at the higher concentrations were not significantly different from the control. Apparently, there was no negative effect of Previcur<sup>®</sup> Energy on the emission of roots and leaves between the control and the higher doses.

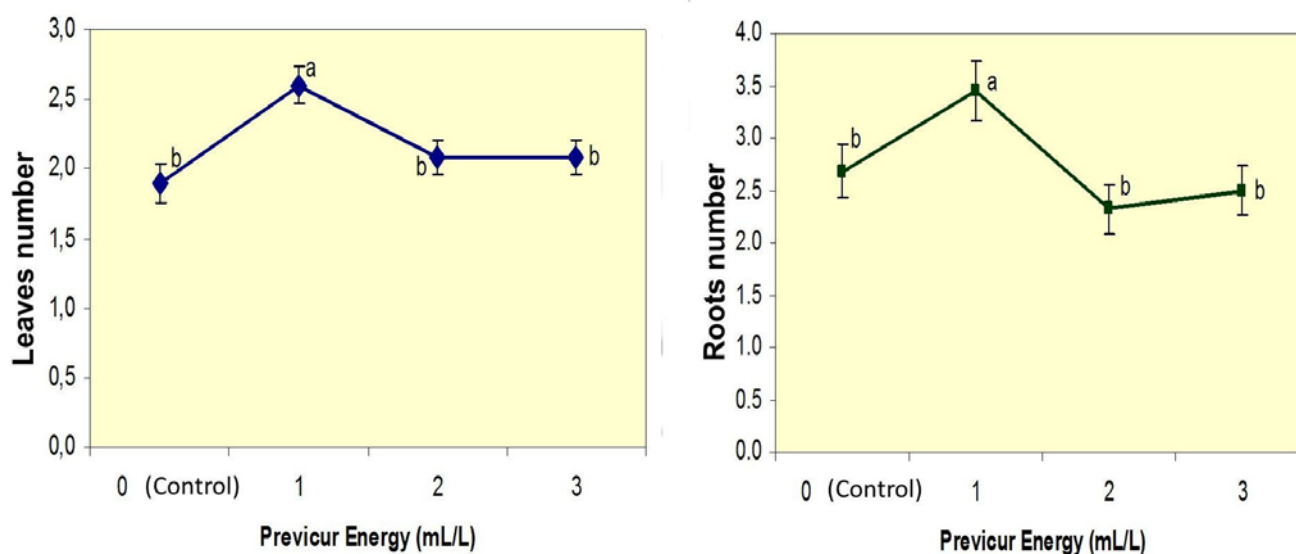


Figure 1. Effect of immersion of plantlets in different concentrations of Previcur<sup>®</sup> Energy on the numbers of leaves and roots produced by MD-2 pineapple vitroplants after 45 days of acclimatization in trays placed on house-cemented cover crop. Means with the same letters are not statistically different (ANOVA and Tukey, for  $p \leq 0.05$ ).

Roots formed *in vitro* are occasionally thick with abnormal root hairs and vascular systems when compared with those that developed in sand substrate. The roots that do not die during the transplant process produce normal new lateral and adventitious roots during acclimatization. That is why the root/shoot ratio is always higher in plants rooted *ex vitro* than those rooted *in vitro* (Amin et al., 2005).

Work to develop techniques to increase plantlet survival during acclimatization has been going on since the 1990s (Kozai et al., 2000; Saucedo, 2008). These efforts looked mainly at ways to reduce water loss from plantlets in growing houses, either by modifying the external environment to which they are transferred, or by direct treatment of plantlets before or after leaving the *in vitro* culture (Preece and Sutter, 1991).

If protective measures are not taken it is recognized that plantlet losses of 80 to 90% to *Phytophthora cinnamomi* or *P. parasitica* can occur shortly after planting in wet soils in climates with frequent rainfall and high temperatures (Taniguchi, 2007). High humidity is maintained during the first weeks of the plantlet acclimatization phase to prevent dehydration. Such conditions favor *Phytophthora* rots and Previcur<sup>®</sup> Energy helped to reduce the presence of this disease (Figure 2). An evaluation of the pathogenicity of *P. cinanmmomi*, *P. nicotiana* and *P. palmivora* to MD-2 pineapple plantlets, the highest mortality (100%) was caused by *P. nicotiana* followed by *P. cinanmmomi* (42%). These results demonstrate the high susceptibility of this cultivar to this pathogen (Taniguchi, 2007).

In conclusion, the lowest concentration of Previcur<sup>®</sup> Energy provided the highest level of protection of MD-2 pineapple plantlets to *Phytophthora*. Previcur<sup>®</sup> Energy can be used to protect MD-2 pineapple plantlets from *Phytophthora* infection during the acclimatization phase.

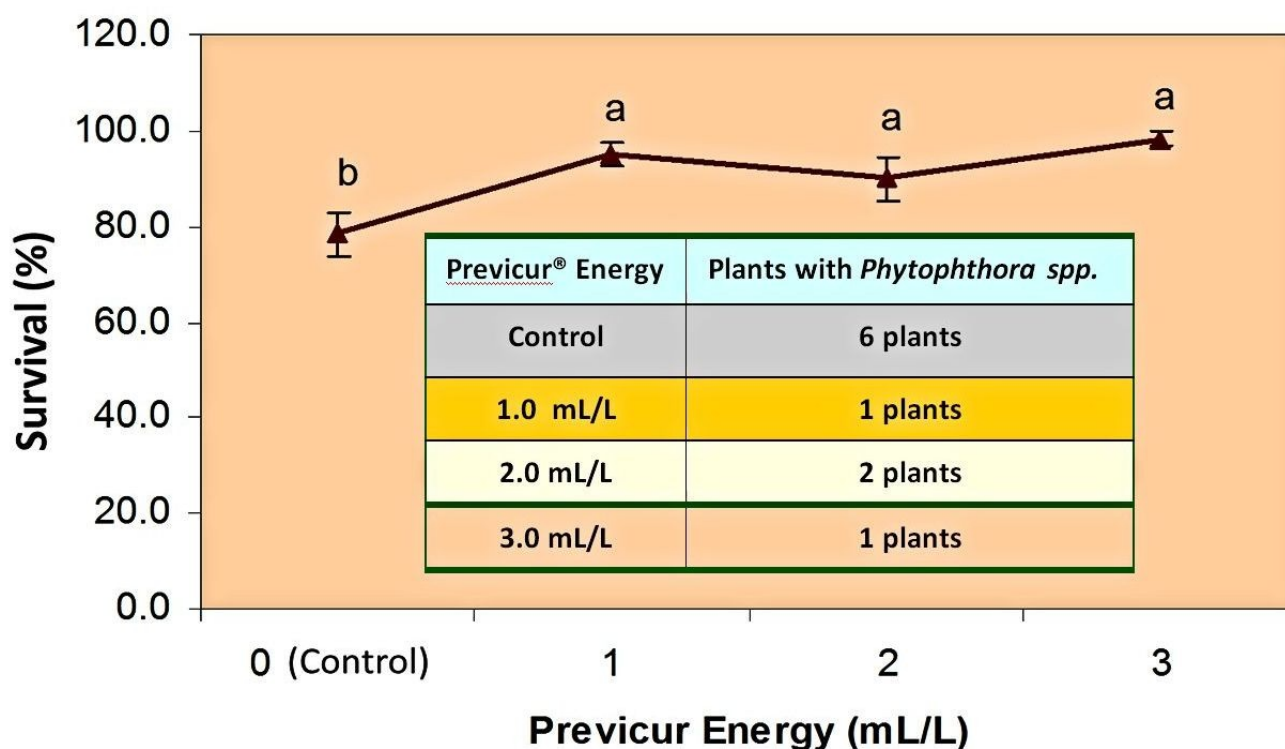


Figure 2. Effect of immersion of MD-2 pineapple plantlets in three concentrations of Previcur® Energy on survival and the presence of *Phytophthora* after 45 days of acclimatization in trays in a covered house. Means with same letters are not statistically different (Tukey for  $p \leq 0.05$ ) data were transformed according to  $y' = \sqrt{\frac{y}{100}}$  0.5.

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#### CAM Metabolic Changes of 'MD-2' Pineapple Grown Under High and Low Light

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## ABSTRACT

Some physiological and biochemical changes in ‘MD-2’ pineapple plantlets (*Ananas comosus* (L.) Merr.) grown under high photosynthetic photon flux density (EC) and greenhouse conditions (GC) (low photosynthetic photon flux density) were studied. Measurements were determined in the leaves at the same evaluation time and environmental conditions. In EC, maximum CO<sub>2</sub> exchange occurred at 0300 h (4.95 μmol m<sup>-2</sup>s<sup>-1</sup>), while the highest value in the GC was at 0900 h (3.8 μmol m<sup>-2</sup>s<sup>-1</sup>). Leaf chlorophyll content was reduced by long-term EC and there was a significant decrease in the chlorophyll *a/b* ratio (0.07 μg g<sup>-1</sup> FW). A significant increase of pH in leaf crude extract was found between 0900 h and 1500 h in plants in EC (pH 4 to 6,) while the pH of leaves from GC plants increased gradually between 0900 h and 2100 h. The activities of superoxide dismutase (SOD, EC 1.15.1.1) were significantly increased between 0900 h and 2100 h in both treatments but the increase was more pronounced in EC than in GC; SOD levels in plants grown under EC in all cases were twice those of GC plants. Crassulacean acid metabolism (CAM) in pineapple plants during days after acclimation was stronger when plants were grown under high photosynthetic photon flux density. Leaf acidity, water use efficiency, gaseous exchange profile, chlorophylls content and SOD activities are show changes in metabolic states favored to the CAM behavior in both treatments, but stronger in plants under environmental conditions that at the same time are more typical of field conditions.

**Index Terms:** CAM metabolism, *ex vitro* acclimatization, pH, photosynthesis, SOD activity.

## INTRODUCTION

*In vitro*-cultured plants are reported to be very sensitive to abrupt environmental changes, especially when they are removed from culture containers and placed under *ex vitro* conditions and later in nursery-field transition from vessels to field. Pineapple (*Ananas comosus* (L.) Merr.) plants have a somewhat plastic morphology and physiology that can change according to the environmental conditions under which the plants are grown (Villalobos et al. 2012; Aragón et al. 2012, 2013). It possess a mechanism whereby a switch from C<sub>3</sub> photosynthetic CO<sub>2</sub> fixation in small plantlets to nocturnal organic acid accumulation in darkness in mature plants allows a great decrease in water loss during drought due to stomata closure in the light period when atmospheric humidity and leaf temperature favors dehydration (Dodd et al. 2003, Herrera, 2009).

Pineapple plants in field conditions are well characterized (Dopazo et al. 2009). It is well established that the photosynthetic performance of plants acclimatized to high irradiance is higher than that of plants acclimated to low irradiance (Keller and Lüttge, 2005). Light acclimation consists in the long-term of an adjustment of the photosynthetic apparatus to the prevailing irradiance condition. In a shorter time perspective the plants have mechanisms by which it adjusts light harvesting to energy utilization (Skillman, 2008).

Due to existence of physiological and biochemical changes undergone by ‘MD-2’ pineapple plants during the hardening phase of the micro-propagation protocols, the aim of the present work was to examine some physiological and biochemical variables during 24h in plants at six months age grown under low and high photosynthetic photon flux density. We wanted to determine whether plants acclimatized under greenhouse or field conditions were most adapted to field conditions.

## MATERIALS AND METHODS

### Plant material and growth conditions

Pineapple plants (*Ananas comosus* (L.) Merr.) ‘MD-2’ were micropropagated (Escalona et al. 1999). Micropropagated plantlets (plants) were dipped in 3.0 mL L<sup>-1</sup> Previcur<sup>®</sup> Energy (Bayer CropScience) for 5 min and planted in plastic vessels (volume 256.26 cm<sup>3</sup>) filled with a substrate composed of 1:1 (v:v) red ferralytic soil and filter cake (derived from sugarcane bagasse) (Villalobos et al. 2012). Plants were acclimatized for six months in a greenhouse under 75 ± 3% relative humidity (RH), 25.5 ± 2°C temperature and an average photosynthetic photon flux density (PPFD) of 300 ± 25 μmol m<sup>-2</sup>s<sup>-1</sup> and natural photoperiods. After this time, uniform plants of 34-36 g fresh weight, with 11-12 leaves and 12-13 roots were selected for further study. The plants were misted for 30 min at 9:00 am every day and a foliar fertilizer mix containing 16.0 g of crystalline N-P-K+1.0 g of Multimicro Combi (Haifa Chemicals Ltd., Haifa Bay 26120, Israel) in 16 L of water was applied every ten days. After six months of additional growth, the plants were divided in two groups of 120 plants and grown for 28 d at two light levels (Table 1). Irrigations in both groups consisted of saturating the substrate every two days. A foliar fertilizer mix was applied early in the morning every ten days.

**Table 1 Experimental conditions measured under greenhouse and natural environment.**

Conditions	Light (Day) (PPFD= μmol m <sup>-2</sup> s <sup>-1</sup> )	Relative Humidity (%)		Temperature (°C)	
		Day	Night	Day	Night
Greenhouse conditions (GC)	250 ± 30	69 ± 3	78 ± 3	33 ± 2	22 ± 2
Environmental conditions (EC)	800 ± 30	71 ± 3	80 ± 3	32 ± 2	23 ± 2

PPFD= Photosynthetic photon flux density (PAR).

After 28d all physiological and biochemical measurements were made on plants every 6 hours during a whole day (24h) beginning at 9:00 am. ‘D’-leaves (defined as a leaf forming a 45° angle with the vertical axis) of six plants were collected from each treatment (GC; EC) and taken for analyses. Plant material was stored in liquid nitrogen for later biochemical determinations.

### Photosynthetic rate, total transpiration rate and CO<sub>2</sub> concentration of sub-stomatal cavity measurements

Photosynthesis rate (A, μmol m<sup>-2</sup>s<sup>-1</sup>) and total transpiration rate (T, mmol m<sup>-2</sup> s<sup>-1</sup>) were measured at both environmental conditions with an infrared gas analyzer (PP Systems CIRAS-2 Portable Photosynthesis System with a 1.7 cm<sup>2</sup> cuvette (PLC6 (U))). PPFD was fixed at 600 μmol m<sup>-2</sup>s<sup>-1</sup> for each determination and thirty determinations were done on 5 leaves for each time and treatment.

### Analytical methods

**1. Chlorophyll content.** Chlorophyll *a* and *b* were determined in leaves by grinding frozen samples to a fine powder using liquid nitrogen with a pestle and mortar; 0.25 g were taken and diluted into 1500 μl acetone (80%) and centrifuged at 12000g during 5 min at 4°C. The supernatant was collected for direct measurement. Chlorophyll concentration was calculated using the equations described by Porra, (2002). Samples were taken at the beginning of the day and chlorophyll contents were expressed as μg g<sup>-1</sup> FW (fresh weigh).

**2. pH determination in crude leaf extracts.** Leaves were crushed until 0.5 mL of crude extract was obtained. The extract was clarified though the addition of one mg of activated charcoal to eliminate pigments. The crude extract pH was immediately quantified with pH indicator strips (Merck KGaA 64271) according to Aragón et al. (2012).

**3. Succulence index.** The values of total chlorophyll content, fresh weight (FW) and dry weight (DW) were used for succulence index determination by the equation: SI = (FW-DW)/[Chl(*a*+*b*)], as described by Kluge and Ting, (1978).

**4. Superoxide dismutase (SOD).** Superoxide dismutase and protein extraction was released according to Wang and Jiang, (2007). Total soluble protein was determined according to Bradford, (1976). Superoxide dismutase activities (SOD) (EC 1.15.1.1) were assayed according to McCord and Fridovich, (1969). The SOD were expressed as specific enzymatic activity defined with respect to protein content (U mg<sup>-1</sup> prot.). All biochemical measurements were performed using a Pharmacia Bio Spectrophotometer.

### Statistical analysis

All experiments were performed three times at each of three biological samples. All statistical analyses were carried out using SPSS version 12 (Pérez, 2005). All situations were analysed using parametric one-way ANOVA followed by Tukey's Multiple Range Test or Student's *t*-test. All tests at 5% significance level.

## RESULTS AND DISCUSSION

### Gas exchange

CO<sub>2</sub> exchange rate in leaves of plants growing under EC reached a maximum value at 0300h (4.95 μmol m<sup>-2</sup>s<sup>-1</sup>), while the highest value in GC was at 0900h (3.8 μmol m<sup>-2</sup>s<sup>-1</sup>)(Fig. 1B). Between 2100h and 0300h (night) GC plants assimilated approximately 23% less CO<sub>2</sub> than did EC plants.

CO<sub>2</sub> assimilation of plants in both treatments occurred predominantly at night, although by 1500h the CO<sub>2</sub> assimilation was substantially negative in both treatments (-0,83 μmol m<sup>-2</sup>s<sup>-1</sup> in EC and -2,27 μmol m<sup>-2</sup>s<sup>-1</sup> in GC). Although net CO<sub>2</sub> assimilation in both treatments increased overnight, EC plants assimilated much more CO<sub>2</sub> than did GC plants. Conversely, during day GC plants assimilated more CO<sub>2</sub> at 0900h than did EC plants. Both groups show the typical decrease in CO<sub>2</sub> assimilation during the day associated with stomatal closure because intercellular-space CO<sub>2</sub> concentrations increase due to decarboxylation of malate accumulated during the previous night (Winter et al., 2008). The differences between GC and EC plants at 0900h are likely due to the greater levels of malic acid in vacuoles of EC plants.

The CO<sub>2</sub> assimilation in CAM plants, including pineapple, occurs via the photosynthetic carbon reduction cycle. It is assumed that photosynthesis will increase with increasing irradiance, and the results of Shiroma (1977) and Nose et al. (1986) support this assumption. Nose et al. (1986) found that light saturation of pineapple plants occurred at a photosynthetic photon flux density (PPFD) of about 500 μmol m<sup>-2</sup>s<sup>-1</sup>.

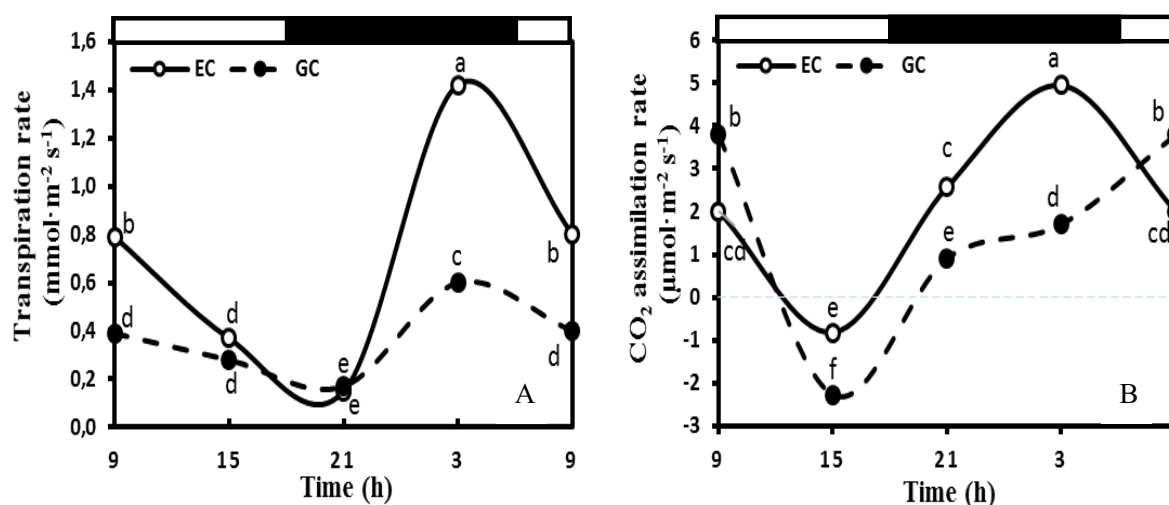


Figure 1. Total transpiration rates (mmol m<sup>-2</sup>s<sup>-1</sup>) (A) and CO<sub>2</sub> assimilation rates (μmol m<sup>-2</sup>s<sup>-1</sup>) (B) and in pineapple plants (*Ananas comosus* (L.) Merr.) 'MD-2' grown under two different light intensities (EC and GC). Values recorded at 0000h and 2400h were similar. Means followed by different letters are significantly different using ANOVA, Tukey's test, *p* < 0.05. Means was compared between all times and treatment. The black bar indicates hours of night. Data represent means of an n=30 samples.

In greenhouse and field conditions pineapple plants should be functioning in the CAM mode (Borland, 1989, Nievola et al. 2005, Antony et al. 2008, Aragón et al. 2013), so an increase of net CO<sub>2</sub> fixation in both treatments was expected. Night CO<sub>2</sub> fixation is initiated by PEPCase in a light-independent fashion, whereas daytime assimilation is light dependent and effected by RuBisCO. The results displayed in Fig. 1 are consistent with a well-established CAM mode, albeit EC plants showed it to a greater degree.

Transpiration rate in leaves of EC plants was significantly higher than the rate for GC plants at 0300 h (1.42 vs. 0.6 mmol m<sup>-2</sup>s<sup>-1</sup>, respectively). At 0900 h transpiration rates in the two treatments declined significantly, with a greater decline in EC plants. Stomatal opening of CAM species normally occurs at night when the highest rates of CO<sub>2</sub> assimilation occur (Borland, 1989, Antony et al. 2008; Nievola et al. 2005, Aragón et al., 2012).

On the other hand the CO<sub>2</sub> concentration of the sub-stomatal cavity always was highest in GC plants (Figure 2A). The day-time values were higher than those at night, although both treatments show a similar tendency. The highest values occurred at 15 h (816 μmol·mol<sup>-1</sup> to GC and 554 μmol·mol<sup>-1</sup> to EC) in both treatments.

In CAM plants the CO<sub>2</sub> is released at a high internal partial pressure (pCO<sub>2</sub>) which is sufficient to result in stomatal closure, thus conserving water. The high pCO<sub>2</sub> generated via decarboxylation of malate accumulated during night also suppresses photorespiration (Borland and Taybi, 2004). These values can also be explained with the results of pH measurements (Figure 4A) which show possible decarboxylation of malic acid accumulated during night and show a low consumption of atmospheric CO<sub>2</sub> during the day.

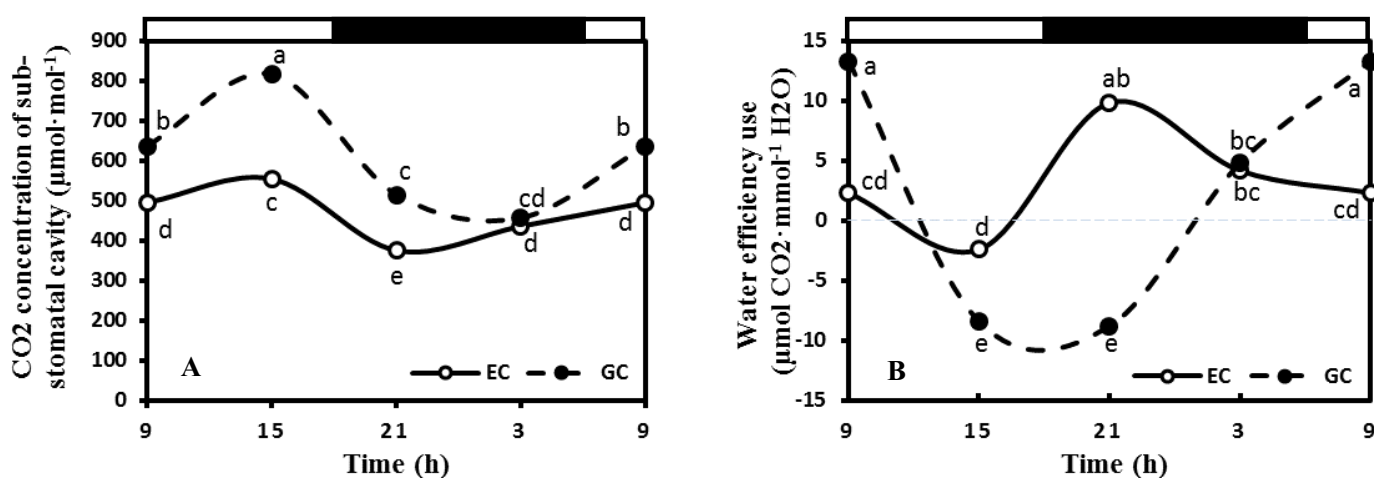


Figure 2. CO<sub>2</sub> concentration of sub-stomatal cavity (μmol·mol<sup>-1</sup>) (A) and water efficiency use (μmol CO<sub>2</sub>·mmol<sup>-1</sup> H<sub>2</sub>O) (B) in pineapple plants (*Ananas comosus* (L.) Merr.) 'MD-2' grown under two different light intensities (GC and EC). Means followed by different letters are significantly different using ANOVA, Tukey's test, p < 0.05. The black bar indicates hours of night. Means was compared between all times and treatment. Data represent means of n=30 samples.

### Response in chlorophylls content

Leaves of pineapple plants growing in GC contained significantly more chlorophyll (*a*, *b*, *a+b* and *a/b*), than leaves of plants growing in EC (figure 3). Leaf chlorophyll content was increased by long-term high light exposure and there was a significant decrease in the chlorophyll *a/b* ratio (0.07 μg g<sup>-1</sup> FW). This higher chlorophyll content in leaves under GC is indicative of adaptation to shade conditions.

Higher total chlorophyll without a concomitant decrease in the chlorophyll *a/b* ratio during acclimation/adaptation of photosynthetic tissue to lower light levels will occur if there is an increase in the number, but not size, of the "photosynthetic units" (PSU) in the tissue. An increase in PSU size in response to lower light levels typically reflect, in particular, an increase in the light-harvesting portions of the photosynthetic pigment/protein complex in both photosystems (Jia et al. 2012).

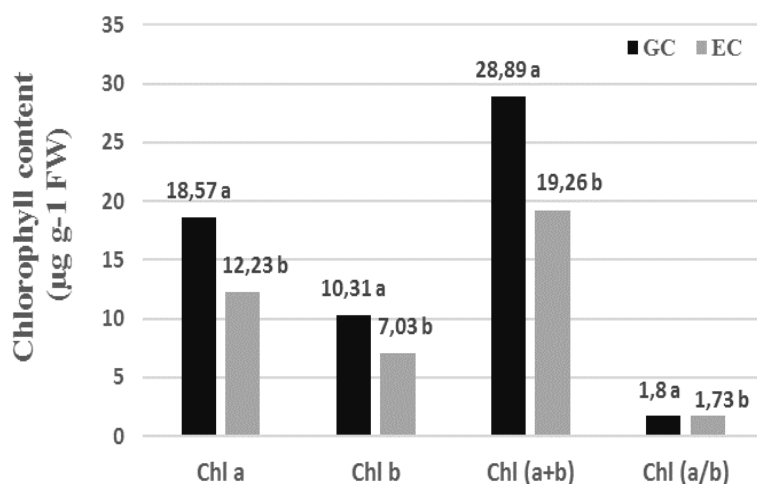


Figure 3. Change in chlorophyll a, chlorophyll b, total chlorophyll content (a+b) and chlorophyll a/b ratio in pineapple plants grown under two different light intensities (under greenhouse and natural environment). Means followed by different letters in rows are significantly different using Student's t-test  $p < 0.05$ . Means was compared between treatments. Data represent means of 9 samples.

### pH and succulence index

In both treatments there is a clear trend of nocturnal acidification/daytime deacidification, typical of CAM. However, the degree of acidification and the time frame in which it changes are clearly different among treatments. The pH was higher in GC treatment compared to the EC at all times during the night period. Significant differences in leaf pH values between EC and GC leaves was found at 15h (EC > GC) and 21h (GC > EC) while the pH of EC leaves at 15 h equaled that GC at 21h (figure 4A).

The succulence index (SI), another parameter related to CAM function, was significantly higher in EC plants during the whole period analyzed and increased at night in both treatments (Fig. 4B). Again, a clear difference is visible between treatments that may be the result of different degrees of CAM.

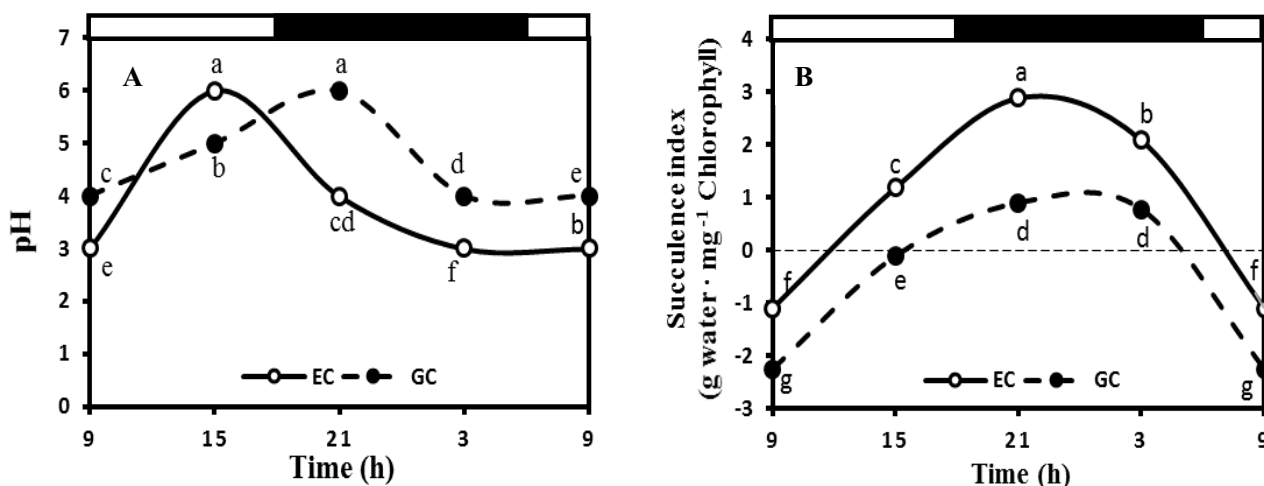


Figure 4. pH values in leaves crude extract (A). Succulence index (SI) in leaves of pineapple plants (*Ananas comosus* (L.) Merr.) 'MD-2' grown under two different light intensities (UG and NE). Means followed by different letters are significantly different using ANOVA, Tukey's test,  $p < 0.05$ . Means was compared between all times and treatment. The black bar indicates hours of night. Data represent means  $n=9$ .

### SOD enzymatic activities

SOD activity was significantly higher in EC plants than in GC plants and the diurnal variation also was greater in EC plants (Fig. 5). The specific enzymatic activities of SOD also was much greater in GC plants than in EC plants. High light intensity probably caused ROS increase and it was reduced by ROS trapping enzymatic activities including SOD as was observed in Figure 5. During day of CAM the stomata are closed, and this leads to a parallel increase in both the intercellular partial pressure of O<sub>2</sub> (piO<sub>2</sub>) and CO<sub>2</sub> (piCO<sub>2</sub>). An increased O<sub>2</sub> level in these conditions can promote overproduction of H<sub>2</sub>O<sub>2</sub>/ROS (Lüttge, 2004).

We argue that these results will expand our knowledge on the induction and description of the CAM pathway in pineapple plants during hardening. CAM metabolism in pineapple plants was stronger under natural environment than under greenhouse conditions demonstrated mainly by results of the succulence index, pH of the sap and SOD enzymatic activities.

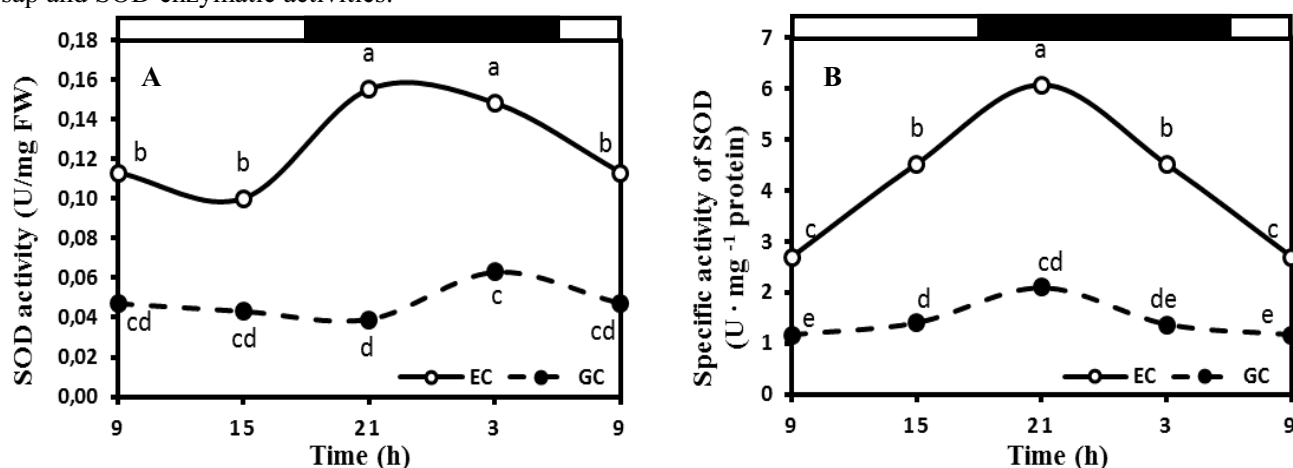


Figure 5. Superoxide dismutase (EC 1.15.1.1) enzymatic activities in leaves of pineapple plants (*Ananas comosus* (L.) Merr.) 'MD-2' grown under two different light intensities (GC and EC). (A) SOD activities per g of fresh weight, and (B) Specific activities. Means followed by different letters are significantly different using ANOVA, Tukey's test,  $p < 0.05$ . Means was compared between all times and treatment. The black bar indicates hours of night. Data represent means of an  $n=9$  sample.

### ACKNOWLEDGEMENTS

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## News from France

### **Pineapple Taxonomy: Species, Botanical Varieties and Cultivars, and Their Importance in Understanding and Managing Pineapple Diversity**

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#### **Objectives and problems of formal plant taxonomy**

A widely accepted plant nomenclature, based on precise identifications and descriptions, is essential in defining properly the objects of our scientific and economic activities and making it possible to understand each other and compare results. As it also deals with the classification of organisms, taxonomy includes and synthesizes much information in a plant name. Indeed, it is intimately related to plant systematics, i.e. the study of botanical diversity and its origins, which focuses on understanding evolutionary relationships among plants. Thus, the practice of taxonomy is “a complex summing up of knowledge, i.e. it starts with a thorough analysis to base the synthesis that will end up in a “simple” name: the name of the species, of the genus, or of whatever ... A taxonomist never merely applies a name, but rather he draws conclusions for a name” (Bicudo, 2004).

Further, the knowledge to be synthesized in a classification has increased enormously since Linnaeus' time, in quantitative terms as well as in the range of disciplines involved. Taxonomists cannot be satisfied with an intuitive morphological approach, as they now have to consider the contributions of numerical taxonomy, reproductive biology, evolutionary biology, phylogenetics, Mendelian genetics, population genetics, molecular genetics, and ecology. This is a source of difficulty, as these contributions are very unequally distributed among plant families and the number of taxonomists has not increased, even less followed the number of described species.

Taxonomy is governed by strict rules, gathered in a slowly evolving international code of nomenclature. For example, the principle of priority, dictating that the oldest validly published name of a taxon prevails over its more recent synonyms, ensures stability to the nomenclature. On the other hand, the oldest name is also inevitably the one bearing only the poor information that was available when the taxon was described for the first time. Thence the principle of priority may lead to some form of stability for mistakes too, and some taxonomists have questioned its general application. For example, Ochsmann (2004a) cites Burt (1970):“...I referred to the muck-heap of two centuries of unindexed and inadequately described names. The best thing to do with a muck-heap is to leave it undisturbed so that it quietly rots down.”

Plant taxonomy has long had difficulties with cultivated plants, as attested by the existence of an international code of nomenclature for cultivated plants. The two systems diverged when the idea of phylogenetic reconstruction instead of formal classification gained importance in general taxonomy. The phylogenetic background was not important in cultivated plant taxonomies and the categories specific for cultivated plants aimed at providing a formal classification for practical use. Much confusion came from the use of categories with different interpretations, and particularly the use of the term “variety” with quite different purposes and meanings for global vs. cultivated plant classifications (Ochsmann, 2004b).

#### **Historical problems in pineapple taxonomy**

Pineapple classification has particularly suffered from the above-mentioned problems and limitations. Despite the relative simplicity of the current picture of pineapple diversity, its evolution has been chaotic, with an inflation of synonyms, initially based on the knowledge of a handful of cultivars of the edible pineapple and, later, reinterpretations of poorly described forms of lesser economic importance (Leal et al., 1998). Furthermore, the clonal nature of most observed materials exaggerated the importance of seemingly stable morphological diversity. For example, Smith (1962) raised his *A. ananassoides* var. *nanus* L.B. Smith to the specific rank (*A. nanus* (L.B. Smith) L.B. Smith), based on the morphological stability of the cultivated clone he used as a type.

Such problems have arisen from the beginnings of Linnaean taxonomy, as pineapple classification was greatly influenced by European gardeners that were highly sensitive to the differences between clonal “garden forms” and had absolutely no knowledge about other forms, either wild or cultivated for fibers. This is why they

gave so much importance to such traits as leaf spines or leaf variegation. Indeed the latter is of importance only for the ornamental horticulturist, and there is no reason to regret that the new classification does not take into account this trait, better treated at the cultivar level, as proposed by Butcher and Gouda (2014).

Drs Butcher and Gouda usefully emphasized that “garden forms” (in fact cultivars) should not be named in Latin. Indeed, this practice also contributed to the confusion in pineapple taxonomy. We have to remember that *Ananas* was first proposed as the pineapple generic name in the 1754 edition of Miller’s *Gardeners Dictionary*. Its description was immediately followed by a series of six Latin polynomials describing particular cultivars of the edible pineapple. In the 1768 edition, an English translation and a short Latin name was added for each of them. In a way, this list may have been the first “Bromeliad Cultivar Register”, including cultivars *Ovatus*, *Pyramidalis*, *Glabra*, *Lucidus*, *Serotinus*, and *Viridis*. It was not exhaustive, as Miller immediately stated that “there are several other varieties of this fruit, some of which may have been obtained from seeds.” The problem came from the fact that many distinguished northern taxonomists only gave importance to the Latin name, so they raised them automatically to the species level, without any consideration to any concept of biological species. Regrettably, the World Checklist of Selected Plant Families (WCSPF) now includes the following names: *A. glaber* Miller, *A. lucidus* Miller, *A. ovatus* Miller, *A. pyramidalis*, *A. serotinus* Miller, and *A. viridis* Miller. All of them are considered synonyms for *A. comosus* var. *comosus*, the edible pineapple, except *A. lucidus* which was finally given by Smith and Downs (1979) to curagua, the pineapple cultivated for fiber (Leal and Amaya, 1991). This is particularly strange because curagua had been described by Smith himself in 1939, as a new species: *A. erectifolius* L.B. Smith. How, 40 years later, did Smith come to give curagua the Latin name of a cultivar of a different species (unlike Freddy Leal and myself, Smith always considered the common pineapple and curagua different species)? To me, this remains a mystery.

An even stranger example is that of *Ananassa monstrosa*, a name that Carrière (1870) associated with crownless forms of the common pineapple. Although a simple curiosity, this was accepted by Smith and Downs (1979) under the name *A. monstrosus* (Carrière) L.B. Smith, a name still cited as a valid synonym in the WCSPF. Indeed, the WCSPF has not taken into account the fact that Freddy Leal invalidated it in an issue of the *Journal of the Bromeliad Society* of 1990, stating that it was a nude name (*nomen nudum*). He also showed that this was neither an omission nor a detail. In fact, Carrière openly mocked botanists. I cannot resist translating a few sentences from the original French text: “by its general aspect and vegetation, *Ananassa monstrosa* presents nothing particular ... except the traditional crown that tends to disappear. Is that a sign of time? In any case, this anomaly will oblige botanists to modify the specific traits of *Ananassa sativa*, just as legists have ... to abrogate or modify some (law) articles, or add some. Will they consent to that? We are not sure, as scholars, some of them at least, are still in this period of innocence that could be seen under the reign of the old Saturn; doubt has not penetrated yet in their candid conscience”...”We do not ignore that the Pineapple, considered as a type and provided with a crown, sometimes produces, by a kind of low dimorphism, crownless individuals (democratized pineapples in a way), which is, according to us a reason more to consider this fact as fully natural.”

The preceding stories show that important errors in name interpretation have persisted in pineapple taxonomy for more than two centuries, even after they were corrected by Mez (1892, 1934), a prominent bromeliad authority. In essence, Mez’s taxonomical treatment, recognizing both *A. comosus* (L.) Merrill and *A. macrodontes* Morren, and hesitating on the specific (in 1934) or varietal (in 1892) rank of *A. comosus* var. *bracteatus* (Lindl.) Coppens & Leal, was very similar to ours (Coppens d’Eeckenbrugge and Leal, 2003). The difference mostly came from the fact that we could use a wealth of data accumulated on pineapple reproductive biology, morphology, distribution and genetics (Coppens d’Eeckenbrugge et al., 1997; Duval et al., 2001, 2003), to opt for the varietal rank for *A. comosus* var. *bracteatus* as well as for two more recently described taxa, *A. comosus* var. *parguazensis* (Camargo & L.B. Smith) Coppens & Leal and *A. comosus* var. *erectifolius* (L.B. Smith) Coppens & Leal. The same historical causes seemed to lead to the same effects, as our classification too has been relatively ignored by formal botanists. On the other hand, we are satisfied that it has been recognized by the USDA Germplasm Resources Information Network, and largely accepted by ‘Pineapple People’, and particularly pineapple breeders, who manage pineapple collections and manipulate pineapple reproduction (including interspecific barriers or lack of it) in their everyday work. Breeders are inclined to give a higher importance to systematics, and thence to the biological species concept, than to the formal aspects of taxonomy.

Obviously, our will to produce a taxonomy that reflects biological relationships among pineapple taxa (and beyond them, with other bromeliads; see Duval et al., 2003) did not exempt us from caring about the formal aspects. This is why we first proposed a historical revision of pineapple taxonomy in 1998 (Leal et al., 1998),

where formal problems were underlined. That paper was the basis for our classification, and this is where we justified the synonymies we recognized and those we refuted in Coppens d'Eeckenbrugge and Leal (2003). We still believe these reasons are valid, except for the taxon *A. comosus* var. *ananassoides* (Baker) Coppens & Leal, which should have been *A. comosus* var. *microstachys* (Mez) L.B. Smith as was proposed by Smith (1934). This combination is fully consistent with our classification. I have to admit that we ignored the rule of precedence at the varietal level. This has been mentioned in the chapter of a collective book, written with Brazilian breeders, which will be published soon.

Although not an expert in formal taxonomy, I am quite surprised by the proposal of Drs Butcher and Gouda (2014) at the end of their paper. Indeed, I do not know of any rule giving some kind of priority to wild forms over cultigens. Plant domestication preceded Linnaean taxonomy by thousands of years, so that western botanists, as a group, had named the cultivated plants that fed them well before they were even acquainted with the existence of their wild relatives (in the cases where these wild relatives still exist). Reasoning by analogy, I cannot imagine that *Zea mays* subsp. *mays* (maize) might disappear from our fields, to be replaced only by duly recognized registered cultivars, so that biologists could focus their efforts on the three wild teosintes they have recognized so late in history (in the late XXth century).

Another point is that Drs Butcher and Gouda seem to confuse two levels of variation in cultivated plants: cultivars and cultigens. "Cultivar" is strictly a synonym of "cultivated variety", designating "an assemblage of plants that has been selected for a particular attribute or combination of attributes and that is clearly distinct, uniform, and stable in these characteristics and that when propagated by appropriate means retains those characteristics." Thus, "cultivars" are opposed to cultivated populations that are too unstable and/or heterogenous to be clearly distinct and that do not breed true to type. Plant taxa (species, subspecies or botanical varieties) that owe their mere existence to man are better designated as cultigens ("Plant or group known only in cultivation; presumably originating under domestication"; Bailey, 1924). *Zea mays* subsp. *mays*, *Malus domestica* Borkh. (the domesticated apple), *A. comosus* var. *comosus*, *A. comosus* var. *erectifolius*, and *A. comosus* var. *bracteatus* are all cultigens, each one presenting an unlimited number of cultivars (each selectable mutation or recombination may provide a new cultivar). Even the rather uniform *A. comosus* var. *bracteatus* has at least four cultivars, created by natural mutations that were selected by man (several variegated forms and at least one with smooth-leaves). Sound cultivation practices, starting with the choice of cultivation materials, require the recognition of the different levels of variation in cultivated plants. Furthermore, if we want to preserve a constructive dialog between formal taxonomists and horticulturists, we need a common vocabulary. Thus, it is useful to revise basic concepts and their application to pineapple.

### **Applying concepts of species, botanical varieties, and cultivars to pineapples**

Species are taxa whose members interbreed freely in the absence of spatial barriers. Fertile representatives of *Ananas* are either diploids (most common case in *A. comosus*) or tetraploids (general case in *A. macrodontes*). The main interspecific barrier appears to be the reduced fertility of crosses between diploid and tetraploid forms in the genus (Collins, 1960). Among normal diploid forms of *A. comosus*, the only general barrier to fertilization is the presence of a self-incompatibility system that inhibits self-fertilization and prevents consanguinity.

A botanical variety "consists of more or less recognizable entities within species that are not genetically isolated from each other" (USDA, 2010), a definition that applies perfectly well to varieties under *A. comosus*. Thus, representatives of *A. comosus* var. *microstachys* (incorrectly named *ananassoides*) are readily distinguished from the edible pineapple (*A. comosus* var. *comosus*) by their combination of narrow leaves with antrorse spines, long and narrow peduncles and small fruits. *A. comosus* var. *parguazensis*, is mostly characterized by its wider leaves with antrorse and retrorse spines and a basal constriction; however intermediate types are not rare, probably because of natural hybridization with neighboring representatives of *A. comosus* var. *microstachys*. *A. comosus* var. *erectifolius* is similar to *A. comosus* var. *microstachys*, except for its smooth and erect leaves with high fiber content. The molecular study of Duval et al. (2003) confirmed their strong genetic relationship. *A. comosus* var. *bracteatus*, with its wide spiny leaves, shorter and wider peduncle, and larger fruit with conspicuous bracts, is a special case because it descends from an initial hybridization event between *A. comosus* and *A. macrodontes*. However, it is a diploid form presenting all the diagnostic traits of *A. comosus* (vegetative propagation by fruit crown and suckers), and it is sexually compatible with other representatives of the species.

The coexistence of cultivated and wild botanic varieties is not unique to pineapple. For example, *Passiflora tripartita* (Juss.) Poir. (banana passion fruit) includes one cultivated botanical variety (*P. tripartita* var. *mollissima* (Kunth) Holm-Niels. & P.M.Jørg.) and two wild ones (*P. tripartita* var. *tripartita* and *P. tripartita* var. *azuayensis* Holm-Niels. & P.M.Jørg.) (Yockteng et al. 2011).

“Cultivar names may be given to the following types of propagated materials: clones, graft-chimeras, seed (as long as the propagated material retains the unique characteristics of the parents), line, multi-line, F1 hybrids, and genetically modified plants” (USDA, 2010). In the pineapple case, homogeneity is ensured by vegetative propagation, so all cultivars are clones. When horticulturists refer to pineapple “hybrids”, they only refer to the fact that the first representative of the clone was obtained through sexual hybridization between two other cultivars or experimental materials (intermediate products of pineapple breeding). This is not only true for modern breeding. Indeed, only sexual recombination can explain the very high diversity observed among pre-Columbian pineapple cultivars and the existence of homozygotes for rare mutations, such as the piping trait in cultivar Primavera (Cabral et al., 1997). Somatic mutation is a secondary but important source of variation, as it is particularly frequent in pineapple (Collins, 1960). Somatic mutations may appear anywhere on a plant, which becomes a chimera. Suckers originating from a completely mutated sector are generally stable mutants. If the mutation affects a qualitative trait, the mutant is both clearly distinct and stable, so it can be recognized as a new cultivar. The most common case is ‘Baronne de Rothschild’, which is a spiny mutant of ‘Smooth Cayenne’. At the same time, this is a strange case of a name given to a cultivar bearing an unfavorable mutation. Variegated mutants provide another example of readily identified new cultivars, if they are stable. Indeed, the suppression of chlorophyll may be unstable, if green and white sectors can produce green and white suckers respectively.

Except for ornamental mutations, such as variegated leaves or bright red fruits, most qualitative mutations are unfavorable or anecdotal. Mutations for quantitative traits may progressively differentiate (sub)clones from a same cultivar. Although the resulting variations are not clear enough to justify their registration as new cultivars, these selections are important in terms of farm yield and income, so they can justify the use of a distinct name or code along that of the original cultivar (Coppens d’Eeckenbrugge and Leal, 2001).

Cultivar names must not be confused with commercial names, such as “Gold Extra Sweet” or “Tropical Gold,” used by important companies in order to differentiate their production/sale from that of their competitors.

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## News from Ghana

### **An Integrated Approach to Disease Control and Soil Fertility Management for ‘MD-2’ Pineapple: A Preliminary Trial in Ghana**

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#### **INTRODUCTION**

European supermarkets prefer ‘MD-2’ (MD2) pineapples, a variety that is highly susceptible to rots caused by *Phytophthora*. *Phytophthora* sp. thrives in wet stagnant conditions caused by high rainfall and impeded drainage. The commercial production system for MD2 in the wetter regions of Central America is based on forming soil into high ridges and building primary, secondary and tertiary drains through which surface water is removed (a ‘ridge-and-drain’ system). Pineapple suckers are then planted on the ridges, sometimes through black plastic mulch. This system works well but requires heavy equipment, is expensive to implement and even when well managed there is a risk of soil erosion. There is evidence that this system may be unnecessary in Ghana where rainfall is lower and where there is good sub-soil drainage and where compaction caused by heavy tractors has not occurred. Better sub-soil drainage can be enhanced by shallow ripping to loosen a line of soil down through the root-zone.

We report here the results of an un-replicated observation trial using reduced-tillage equipment and non-conventional products to evaluate an alternative production system based on suckers planted through plastic foil (mulch) into the rip-lines of flat beds.

#### **MATERIAL AND METHODS**

##### ***Phytophthora* disease management**

Four non-conventional products that can reduce fungal disease (e.g. *Phytophthora*) were used in this trial. Plants were treated with two different strains of *Trichoderma* fungus in high quality formulations; one that colonize the root zone the second that has been selected for use as a foliar spray to control fungal pathogens (Eco-T and Eco-77 respectively; [www.plant-health.co.za](http://www.plant-health.co.za)), Trichodermas are known to increase resistance to biotic and abiotic stress and to improve growth and yield, so these may also contribute to higher yield. One product, Enrich ([www.bioteq-ouest.com](http://www.bioteq-ouest.com)) also contains a mix of bacteria that also colonize plants and the root zone. A fourth product, potassium silicate fertilizer, is reported to block *Phytophthora* in avocado seedlings (Agrisil; [www.plant-health.co.za](http://www.plant-health.co.za)).

##### **Field preparation**

Solidaridad West Africa has been promoting sustainable production of soy and cotton in Ghana, and as part of this work has been testing an Ndume ([www.ndumekenya.com](http://www.ndumekenya.com)) ripper for reduced tillage farming. In this trial, the ripper was used to produce two 28 cm deep rip lines in flat beds in a single pass. The beds were shaped to be convex, such that rainwater would pool in the beds and not run off. The beds were covered in plastic mulch which was perforated in the middle to allow water to infiltrate into the soil. MD2 suckers were then planted into the rip lines through the plastic. The trial area was 4 x double rowed beds each 2 meters part and 30 meters long. Ripping loosened the soil, created zones of good drainage and enhanced root penetration and water capture. The trial was planted in January 2013 on a pineapple farm in Ghana. The non-conventional products were used in addition to chemical controls to suppress *Phytophthora* (Alliette 80%, Chlor-pyrifos 48%, Ridomil Gold MZ 68 % are all used on the farm as insurance sprays). Eco-T was applied as a dressing to suckers at planting; Eco-77

and Agrisil were applied in several foliar sprays; Enrich was applied twice as a foliar spray). The timeline for the development of an alternative production system is shown in Table 1.

### TRIAL RESULTS

The trial was harvested in March 2014. *Phytophthora* control and fruit size was as good or better than is typical of the host farm (Table 2). This result suggests that scaling-up the trial programme is justified. Larger trials will assess performance in conditions relevant to a commercial production system. Larger trials should also consider the need to adapt equipment to the new system. The non-conventional products also show potential and further testing is needed in research trials in a commercial farm setting.

Table 1. Timeline for developing an alternative production system

Timing	Development phase
2009-2013	<b>Background research:</b> (1) on soil compaction in ridged systems in potato - the only other major crop where ridges are used; (2) contact with equipment developers; (3) review of literature on integrated management options for phytophthora; etc.)
2010-2012	<b>Mini-trial</b> comparing ridges with flat-beds (on a research station)
2012	<b>Survey of the host farm:</b> using penetrometers (to measure soil compaction) and water infiltration tests (to measure the rate of drainage into subsoil).
Feb-2013 to Mar-2014	<b>Planting observation trial –Gold Coast Fruit Limited, Ghana</b>
In planning.	Larger, commercial scale tests including adaptation of ancillary equipment
In planning	Replicated research trials with non-conventional products to manage <i>Phytophthora</i>
In planning	Scaling up into regular commercial production.

Table 2. Results of a preliminary trial results (note that the ‘reference plot’ was planted with the same planting material in the same week).

	Trial plot	Reference plot	Farm average March 2014
Suckers per ha	50000	72000	72000
Yield (t/ha)	78.4	94.2	86
Average weight (kg) of harvested fruit	1.82	no data	no data
Average weight per sucker planted (kg)	1.65*	1.31	1.19
Mortality from <i>Phytophthora</i>	0.10%	4.50%**	no data

\*Some fruits were stolen from the trial plot and very small fruit was not harvested.

\*\* A survey of a small section of the reference plot; the level of infection is typical of the farm as a whole.

### Soil quality

Composite soil samples were taken within the trial plot at harvest from the top 20 cm from: 1) the soil between beds, 2) under the plastic mulch between rip-lines and 3) under the plastic mulch within the rip-lines. The percentage of silt under the plastic mulch was higher than between the beds and there was more sand between the beds (presumably the fine particles had washed into the beds and percolated through the perforations in the mulch). Soil organic matter was 33% higher in the rip-line than the other zones (Table 3). A number of chemical properties were different in the rip-lines (see table), presumably due to complex causes (e.g. retention of nutrients from fertilizer nutrients from roots and root exudates, etc.). Further trials are needed to judge the relevance of these differences.

Table 3. Variation in mineral texture and mineral nutrients in the trial plot.

	Between beds	Under mulch, between rip-lines	Under mulch, within rip-lines
Sand (%)	12.28	4.5	5.67
Clay (%)	28.74	26.3	26.12
Silt (%)	58.98	69.2	68.21
Organic Matter (%)	1.1	1.0	1.4
Potassium (ppm)	86	63	77
Phosphorus (ppm)	13	12	37
Magnesium ( ppm)	95	104	55
Calcium (ppm)	678	880	434
Sulphur (ppm)	6	6	25
Zinc (ppm)	8.3	5.7	12.9
Iron (ppm)	238	264	395
Fe/Mn ratio*	0.65	0.686	1.15

\*The farm has a problem of manganese toxicity, which antagonizes iron, requiring iron sulphate application.

### **Biomass and nutrient uptake in crop residues at harvest, harvest data and a comparison of performance in flat-bed with the Costa Rican ridge and drain system**

Random numbers were used to select six plants from the inner rows. Fresh and dry weights were calculated. A sample of the homogenized dry plant was sent for analysis to assess the nutrients remaining in the crop residues. These high levels of organic matter in the standing crop and the large amounts of nutrients (Table 4) confirm the value of the residues and the logic of trying to optimize the system in subsequent trials, in order to enhance nutrient recycling and improve top-soil quality from one crop cycle to the next.

The percentage of fruits by size class in the trial are shown in Table 5. Theoretical boxes for export in each size class was calculated based on a plant population of 50,000 plant/ha. A comparison of the expected benefits of the flat bed system used in the trial over the deep ridge system is presented in Table 6.

Table 4. Average (n= 6) fresh and dry weight of pineapple crop residues and mineral nutrients contained in a composite tissue sample after fruit harvest.

<b>Analysis of crop residues at harvest (per hectare)</b>	
Fresh weight of plants	199 tonnes
Dry weight of plants	29 tonnes
Potassium	441 kg
Nitrogen	371 kg
Calcium	284 kg
Magnesium	87 kg
Sulphur	46 kg
Phosphorus	26 kg
Manganese	11 kg
Iron	8.5 kg
Boron	1.5 kg
Zinc	1.4 kg

Table 5. Percentage of marketable fruits by size class and theoretical boxes/ha for export based on a plant population of 50,000 plants/ha.

<b>TRIAL RESULTS</b>					
<b>(average fruit weight = 1.82 kg)</b>					
Size class (fruits per 12 kg box)	min' fruit weight for packing (kg)	max' fruit weight for packing (kg)	% fruit in class (frequency)	% fruit in class(by weight)	Number boxes for export
	<0.9 (too small)	-	3.90%	1.30%	-
12	0.9	1.1	5.30%	3.00%	192
10	1.101	1.3	5.90%	4.00%	257
9	1.301	1.5	12.20%	9.90%	590
8	1.501	1.7	17.10%	15.60%	930
7	1.701	1.9	13.50%	13.70%	839
6	1.901	2.3	29.70%	34.80%	2153
5	2.301	3	11.80%	16.70%	1027
	-	>3 (too big)	0.70%	1.00%	-
Potential export boxes per hectare					5988

Table 6. Comparison of the expected performance of the alternative system with the conventional system currently used.

<b>Attribute</b>	<b>Alternative flat-bed system</b>	<b>Ridge-and-drain system</b>
Soil compaction	One pass by light ripper once per cycle (30 months) is all the land preparation that is needed. So there is little tractor traffic.	Land preparation requires moving thousands of tonnes of soil with heavy tractors dragging deep rippers, ploughs, bed formers, ditchers. This can all contribute to sub-soil compaction.
Flat beds or high ridges	Planting on flat beds with no ridges.	Planting on high ridges.
Permanence	This system would permit the establishment of permanent planting beds and a transition to modern 'CTF' (see: <a href="http://controlledtrafficking.com">controlledtrafficking.com</a> ) and precision farming methods. CTF is based on permanent beds with machinery wheels running only on inter-bed tracks.	Beds need to be broken up and re-formed every cycle.
Integrity of topsoil and topsoil quality	Top-soil is kept in place and top-soil quality in the planting zone improved during the trial (analysis showed increased silt portion and organic matter in the rip-lines compared to areas outside the rip-lines).	Top-soil is mixed with subsoil during land preparation. This leads to fine particles and organic matter being lost. Stones may be dragged up from the sub-soil during land preparation.
Timing of soil preparation	Ideally ripping should be in the dry season when soil shatters easily. Ripped land can be left and utilized when required.	Preparation of beds must be before planting, which is impossible during periods of heavy rain.
Utilization of crop residues	In a reduced tillage or minimum tillage system these should be left on the soil surface along the rip-lines so that as much organic matter and nutrients are available in the root zone of the plants in the subsequent cycle. For a commercial pineapple system this may mean adapting equipment to better chop residues (e.g. with a	Normally residues are chopped and incorporated with a horizontal chopper then mixed with soil during the plowing and bed forming operations. Sometimes residues are burnt to remove plastic mulch from previous cycle. It should be expected that fine chopping and leaving residues on the surface in a

	vertical axis chopper) and adapting equipment used in other types of horticulture to lift remaining plastic mulch from the previous cycle (biodegradable mulch is probably too expensive).	reduced tillage system would allow more efficient recycling, however, this supposition needs testing in further trials.
Fuel consumption	Low (not yet calculated – in soy/maize farming fuel consumption halves when plowing is replaced by ripping)	Very high
Cost of land preparation	Low (not yet calculated)	Very high
Fertilizer efficiency	All commercial pineapple has a high fertilizer requirement, but this alternative system should need less fertilizer due to higher soil organic matter and top-soil quality and better recycling of nutrients and organic matter in crop residues for subsequent crops.	Not good (though this is a characteristic of all pineapple systems).
Control of phytophthora	Plant losses to phytophthora have been low or zero in trials with prototypes of this system.	Even with high levels of chemical control <i>Phytophthora</i> is still present
Use of chemical herbicides	Flat beds enable use of ULV (ultra-low-volume) sprayers for herbicide which do not require water (less arduous, 50% less herbicide) This needs further work and tests.	Moderate
Use of chemical fungicides and transition to integrated control of <i>phytophthora</i>	The system appears to reduce the underlying risk of <i>phytophthora</i> ; this is a basis for developing an integrated system of control that includes several ‘soft’ products in a package. With fine-tuning we expect that chemical fungicides can be much reduced or possibly eliminated	Highly dependent on chemical fungicides. This is a feature of MD2 management in Costa Rica and Hawaii.
Relevance to out-growers and smaller farmers.	Easy to manage much and so easier for smaller farmers to copy the system of large farms. Simpler equipment required. More suited to uneven land and small plots.	Difficult to manage. Very hard for out-growers to adopt system without a lot of support for land preparation.
Erosion risk	Very low / non-existent (in this trial all water soaked into the soil within the plot – so no runoff – the plastic was perforated in the middle to allow water to enter the bed).	Moderate risk even with the best management. High risk if management is not good.
Drought resilience and water harvesting	In Ghana dry season drought is a production risk. Encouraging water to drain into the beds should increase water availability in dry months, especially from the isolated storms that occur from time to time in dry months in Ghana.	Storm water in the dry season runs off plastic mulch into the drainage system.



Penetrometer and water infiltration tests were part of a survey aimed at understanding soil compaction and sub-soil drainage patterns.



Free draining gravel subsoil, typical of the farm.



Erosion in commercial pineapple fields using a poorly managed ridge-and-drain system.



Ndume ripper used to create double rip lines for the pilot trial.



Pineapple suckers planted through the plastic foil into flat-beds.



The pilot-trial plot after flower induction.



Soil profile across the ripline: 30 cm rule alongside pineapple roots emerging from the soil.



Large fruit in the pilot trail.



Healthy root ball of a plant pulled from the pilot trail on harvest day.

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## Services

The listings below are provided as a convenience to readers of Pineapple News and should in no way be construed as an endorsement of those offering to provide commercial or professional services. Those wishing to provide commercial or professional services to pineapple producers are invited to contact the editor for possible inclusion in the listings below.

### Commercial Services

- **Centro de Bioplantas.** Dr. Justo L. Gonzalez Olmedo, Director of Foreign Affairs Office, Centro De Bioplantas. Universidad De Ciego De Avila, Carretera a Moron Km 9. Cp69450. Cuba. Centro De Bioplantas offers certificates of authenticity for pineapple material propagated in their tissue culture facility. Web site: <http://www.Bioplantas.cu>.
- **Maintain CF 125** continues to be available for use in pineapple plant propagation anywhere in the world. Supplies can be obtained from N. Bhushan Mandava, Repar Corporation, 8070 Georgia Ave., Suite 209, Silver Spring, MD 20910. Tel: (301) 562 – 7330; Fax: (202) 223 – 0141; On the web at [www.reparcorp.com](http://www.reparcorp.com); E-Mail: [mandava@compuserve.com](mailto:mandava@compuserve.com).
- **Thai Orchids Lab,** Dr. Paiboolya Gavinlertvatana. Horticulture/ agriculture/ forestry tissue culture laboratory with exports to Australia, U.S.A., Africa, and Asia. CO2 & MD2 pineapple available (open to acquiring additional varieties) or confidential exclusive contract propagation. Phone: +1.617.910.0563 Website: <http://www.tolusa.com/>.
- **Vitropic,** Zone d'Activités Economiques des Avants, 34270 Saint Mathieu de Trévières 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

- **Dr. Surya P Bhattarai.** CQUniversity Australia, Rockhampton, QLD 4702, Australia; Tel: +61 7 4923 2140 (w), +61 438191391 (m) ; E-mail: [s.bhattarai@cqu.edu.au](mailto:s.bhattarai@cqu.edu.au). Experience: More than 25 years of experience in agronomy/physiology in a large range of horticultural crops including pineapple. Recent focus on crop water management and irrigation of pineapple in Australia. Will work on collaborative research projects (global, regional or local) on pineapple in the area of crop agronomy, postharvest and value chain.
- **Szu-Ju Chen.** 2-6 Dehe Rd., Dehe Village, Changjhih Township, Pingtung County 90846, Taiwan. E-mail: [suju@mail.kdais.gov.tw](mailto:suju@mail.kdais.gov.tw). Assistant Researcher, Kaohsiung District Agricultural Research and Extension Station. Area of specialization: Improvement of cultural practices and postharvest management of pineapple and other tropical fruits.
- **Dr. Mark Paul Culik.** INCAPER, Rua Alfonso Sarlo 160, CEP 29052-010, Vitoria, ES, Brazil; Tel: 27-3636-9817; E-mail: [markculik3@yahoo.com](mailto:markculik3@yahoo.com). Experience: PhD in Plant and Soil Sciences 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 mites to fruit flies, and current work on scale insects, including pineapple mealybugs. Areas of specialization: Entomology, Insect and Pest Identification, Integrated Pest Management.
- **Dr. Herve Fleisch.** Interested in consulting on most agronomic and managerial aspects of production operations. See on-line profile at <http://www.linkedin.com/pub/herve-fleisch/28/536/21a> and web page at [www.cuenca-caribe-consultores.com](http://www.cuenca-caribe-consultores.com).
- **Eng. Agr. Rafael Garita C.** E-mail: [rafagarita8@yahoo.com](mailto:rafagarita8@yahoo.com). Twenty years of experience with 'MD-2' pineapple at Del Monte Fresh Produce (Pindeco) in Costa Rica under the guidance of Mr. George Yamane who led development of 'MD-2' at Del Monte. Worked on pineapple projects in the Philippines (Cagayan de Oro) and was involved in preliminary studies in Indonesia: Sulawesi Islands Molukas (Halmahera). Also consultant in Costa Rica, Panama, Puerto Rico, the Dominican Republic, Colombia, Ecuador, El Salvador, Guatemala and others.
- **Ching-San Kuan.** No.2, Minquan Rd., Chiayi 60044, Taiwan. E-mail: [Kuan@dns.caes.gov.tw](mailto:Kuan@dns.caes.gov.tw). Associate Researcher, Taiwan Agricultural Research Institute, Chiayi Agricultural Experiment Station. Area of specialization: Pineapple breeding and cultural practice improvement.
- **Juan Luis Morales Ch.** E-mail: [jlmoralesch@gmail.com](mailto:jlmoralesch@gmail.com) Phone:506-83988772. I was responsible for research, technical services and quality control of 'MD-2' pineapple in Pinedeco-Del Monte, for over 20 years and have experience in many parts of the world. I can advise pineapple growers on agronomy practices, crop protection and quality control.

- **Ing. Jhonny Vásquez Jiménez**, MSc. San Carlos, Costa Rica. E-mail: [jvasquez@proagrocr.com](mailto:jvasquez@proagrocr.com), Phone: (506) 89103878, (506) 24756795. Advice on the agricultural management of pineapple crop. Analysis and improvement of pineapple crop systems for producer companies (environment and productive potential, nutrition, control pathology, crop management). For Agrochemical Companies, designing and conducting researches for new production technologies in the area of nutrition, plant pathology, weeds and other disorders.
- **Mr. José R. Vásquez**, MBA with emphasis in Agribusiness (E-mail: [jrva46@excite.com](mailto:jrva46@excite.com), [jrva46@gmail.com](mailto:jrva46@gmail.com)). Phone: 504 2668 1191; 504 94899901. Experience: Environmental and Sustainable Agriculture. Pineapple and melon production, from seed propagation, planting, field maintenance, forcing, harvesting, post-harvest management and commercialization.
- **Ren-Huang Wang**. 2-6 Dehe Rd., Dehe Village, Changjhih Township, Pingtung County 90846, Taiwan. E-mail: [rhwang@mail.kdais.gov.tw](mailto:rhwang@mail.kdais.gov.tw). Assistant Researcher, Kaohsiung District Agricultural Research and Extension Station. Area of specialization: Improvement of cultural practices and postharvest management of papaya, pineapple and other tropical fruits.

### New Books, Book Reviews and Web Sites

#### Garita Coto, Rafael Ángel. 2014. *La Piña*

Editorial Tecnológica de Costa Rica. 568 pages. In Spanish only. ISBN 978-9977-66-301-2. Available in paperback from Amazon.com for \$78.00. A description in Spanish is provided by Amazon.com.

**Carr, M.K.V., 2014. Pinapple.** Pages 265-278, p. 350, *Advances in Irrigation Agronomy: Fruit Crops*. Cambridge University Press. Crops covered include avocado, cashew, citrus spp., date palm, lychee, macadamia, mango, olive, papaya, passion fruit and pineapple. Available from Amazon.com for \$115.00. A description and very generic review are provided at <http://www.cambridge.org/us/academic/subjects/life-sciences/plant-science/advances-irrigation-agronomy-fruit-crops>.

#### Web Sites of Possible Interest

- Guia Para La Identificación Y Manejo Integrado De Plagas En Piña (Guide to the identification and Integrated Pest Management in pineapple) <http://cep.unep.org/repcar/proyectos-demostrativos/costa-rica-1/publicaciones-proagroin/Guia20Manejo20de20plagas20en20pina.pdf>
- National Pesticide Information Center: <http://npic.orst.edu/npicfact.htm>
- Reduciendo el Escurrimiento de Plaguicidas al Mar Caribe (Reducing Pesticide Runoff to the Caribbean Sea) <http://cep.unep.org/repcar/proyectos-demostrativos/costa-rica-1/publicaciones-proagroin/>
- <http://www.daff.qld.gov.au/plants/fruit-and-vegetables/fruit-and-nuts/pineapples>

### 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 for the period since the last issue up to about March 31, 2014. Some papers may seem relatively unrelated to pineapple but the list follows the principle of inclusion to provide the widest possible content. Abstracts of most of the papers listed below can be found on the internet by pasting the title into the search window. All abstracts of papers published in *Acta Horticulturae* are available from [actahort.org](http://actahort.org). For a larger view, adjust the magnification in Adobe Reader.

Abadias, M., Alegre, I., Oliveira, M., Altisent, R., and Viñas, I., 2012. Growth potential of *Escherichia coli* O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. *Food Control* 27:37-44.

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Adornado, H.A. and Yoshida, M., 2008. Crop suitability and soil fertility mapping using geographic information system (GIS). *Agricultural Information Research* 17:60-68.

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## Contributions to Pineapple News

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

- Timely news about research on issues related to culture, processing, storage, and marketing of pineapple.
- New, interesting, or unique problems encountered by growers.
- Country or status reports on the local pineapple industry.
- For articles not included in the above, please contact the editor.

The language of Pineapple News is English. Editing assistance will be provided on request and internet language translation, e.g., google translate at <http://translate.google.com>, or <http://www.spanishdict.com/translation> for Spanish, provide quite accurate translations.

Article length: Papers should be approximately 4 double-spaced pages in 11 point font or equivalent, not including tables, figures and photos. However, longer papers can be found in past issues of Pineapple News. Please contact the editor when considering submitting articles longer than 4 pages of single-spaced text.

Article number for one author: There is no limit to the number of articles that can be submitted. However, acceptance and publication is at the discretion of the editor.

Tables and graphs: Submit tables in Word format or as spreadsheet files. When submitting graphs, provide the original file or submit as a graphics file (jpg, png or other format).

Photographs: Submit photographs that can be scanned or provide digital files in jpeg or other format recognized by MS Word. The minimum resolution should be 300 dpi.

Author guide: Use the guide at <http://www.ishs.org/wri/pap1.htm> when preparing contributions to the newsletter.

Send contributions and inquiries to: D.P. Bartholomew, Dept. of TPSS, Univ. of Hawaii, 3190 Maile Way, Honolulu, HI 96822 U.S.A. (Phone (808) 956-7568; Fax (808) 956-6539; E-mail: [duaneb@hawaii.edu](mailto:duaneb@hawaii.edu)).

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