Pineapple News

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

Volume II No. 2 March 1996

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From The Editor

My apologies to those who hoped, as I did, for a more frequent newsletter. I planned for two issues yearly but other commitments have delayed this issue. And hanks to all who have helped to support Pineapple News with their monetary contributions. Thanks especially to those who contributed more than $10, some more than $100, to help pay printing and mailing expenses, which are the greatest costs in producing an international newsletter.

Finally, thanks to those who contributed abstracts, brief papers, and other information about pineapple. Without your articles and notes the newsletter would be very slim. In that regard, one reader asked for a review of the factors affecting fruit size. I have briefly summarized the factors that immediately came to mind, but I didn't take the time to review the subject comprehensively. The summary of work done in Queensland, Australia (see abstract below) should provide additional guidance in that regard. I would appreciate hearing from readers who might have additional information on the subject. There also is recurring interest in inhibiting natural flowering of pineapple. I have included some comments on that subject also, but it may only be solved by genetic engineering. Lastly, I have included a newsletter on precision farming, which I thought put those who manage large land areas in touch with the future.

3rd International Pineapple Symposium: It has been confirmed by Dr. Suranant Submadrabmandhu, Department of Horticulture, Kasetsart University, Bangkok, Thailand, that the Horticultural Society of Thailand will sponsor the 3rd symposium sometime in 1998. I expect more information will be forthcoming as the time for the meeting nears.

Professional Directory: Occasionally, I get requests from people or organizations looking for professionals or consultants with expertise on pineapple. I could respond better to those requests if readers who are interested in consulting would provide me with a professional profile. My plan is to make the directory an occasional section of the newsletter so over several issues a fairly comprehensive listing would be compiled. Those seeking consultants could refer to the listing to find persons with the desired expertise in production, processing, management, research, etc.

Pineapple News on the World Wide Web: Pineapple News is now available on the World Wide Web (WWW) at: http://agrss.sherman.hawaii.edu/pineapple/pineappl.htm. This format requires a web browser such as Mosaic or Netscape running under Microsoft Windows or a comparable system. I also plan to get the text on the Univ. of Hawaii gopher because I know it will be accessible to more readers in that format, but first I need to learn more about this older technology.

Expect each issue of Pineapple News to be available on the WWW a month or two after it is printed. One of the most interesting inquiries that came as a result of the Web page was a request for names and phone and fax numbers of companies that produced pineapple juice concentrate. I supplied what I had but certainly did not expect to be involved in marketing as a result of a Web page about pineapple.

Membership in ISHS: For membership in the International Society for Horticultural Science, write to:
International Society for Horticultural Science, Englaan 1, 6703 ET Wageningen, Netherlands

Reader Feedback Requested: It has been my policy to print anything submitted by readers in *Pineapple News*. In this issue are three papers that were submitted for the newsletter. I would be interested to know what readers would like to see in the newsletter and so have included a short questionnaire at the end of the newsletter to obtain reader feedback. Until the policy is better defined, please continue to send abstracts, brief papers, research reports, summaries, inquiries, etc. related to pineapple to me for inclusion in the newsletter. Because of cost, I cannot print photographs at this time. Direct all correspondence related to Pineapple News to:
D.P. Bartholomew, Dept. Of Agronomy and Soil Science, Univ. Of Hawaii, 1910 East-West Rd., Honolulu, HI 96822 U.S.A. (Phone (808) 956-8708; Fax (808) 956-6539; E-mail: duaneb@hawaii.edu. ♢

Factors Influencing Fruit Size of Pineapple

The important factors affecting pineapple fruit size at harvest are the pineapple group (Cayenne, Spanish, etc.; Smooth Cayenne has much larger fruit on a plant of a given size than does Spanish), plant size (larger plants produce larger fruit, but there appears to be an upper limit that usually isn't reached because the market for very large fruit is limited), plant population density (average fruit weight declines predictably with increasing density), season (there is very little data on this subject and what there is isn't always consistent), and climate (a poorly researched subject because few growers and no researchers have an opportunity to explore productivity in different climates. It is my impression that plants fruiting in the warm tropics (Malaysia, Thailand) must be larger to produce a fruit of a given size than plants in cooler environments, but I know of no data to back up that impression. Type and size of planting material are often mentioned as important factors affecting fruit size, but I believe they are only important to the extent that they affect the time required to reach a given plant weight, which is not an insignificant consideration. Growers need to measure productivity per unit of time if resources are to be used efficiently. Plants grown from large planting material (suckers) reach a given weight in less time than do plants grown from smaller (crowns or slips) planting material. Environmental stresses and pests and diseases primarily impact fruit weight by reducing plant growth. ♢

Natural Flowering

One of the important unsolved problems in pineapple culture and management is naturally-induced flowering (natural induction or NI to keep it short), which occurs unpredictably almost everywhere Smooth Cayenne pineapple is grown. Is NI also a problem for growers of rough-leaf pineapple? NI is a problem because it occurs unexpectedly in fields managed to minimize such flowering. Minimizing NI is done by maintaining as near-optimum growing conditions as possible and planting so

plants are small, and thus less susceptible to NI, when NI is most likely to occur. NI has been an occasional problem in Hawaii since year-round production became a common practice. A major problem in investigating NI is its sporadic nature, which often results in a no-test situation. We had no success in three attempts at conducting research on NI in the field. Dr. Xiang-jia Min, who recently completed his doctorate here in Hawaii, inadvertently found that a high percentage of NI occurred in season when plants were grown in pots. Pot culture is labor intensive but it does permit real tests to be carried out. The results of those pot tests are presented in a final report of our research below. Our best success was obtained with the growth inhibitors paclobutrazol and uniconazole. Glenn Taniguchi reports below on tests with Tilt (propaconazole) and Elite (tebuconazole), two new chemicals that are effective in controlling *Chalara paradoxa*. Elite also strongly inhibits the growth of pineapple and its effects on flowering are being looked at.

Our data suggest that paclobutrazol and uniconazole inhibit leaf growth relatively more than that of the fruit so there may be a potential to inhibit NI and reduce plant size, thereby making harvesting easier. A major problem with paclobutrazol and uniconazole is that they are unlikely to be registered for use on pineapple. The situation for Elite may be more promising because the compound is an effective fungicide. If registration could be obtained on the basis of fungicidal capability, the compound might also be useful in inhibiting natural flowering, giving growers a new management tool. ♢

Spatially-Variable Agriculture

While "surfing the internet" recently, I came across a newsletter on precision farming put out by Dr. John K. Schueller of the University of Florida. Dr. Schueller provided me with an introductory paragraph about spatially-variable agriculture and was kind enough to give me permission to reproduce his newsletter on the subject; I have done some minor editing of the newsletter for the sake of brevity. While such technology may not be immediately applicable on pineapple farms and plantations, it is good to be aware of new developments in the highly competitive field of agriculture and some very progressive farmers might be interested in exploring the potential of these innovations on their farms.

Spatially-variable Agriculture

Dr. John K. Schueller, Affiliate Associate Prof., Agricultural & Biological Engineering, Assoc. Prof., Mech. Eng., Univ. of Florida. E-mail: schuejk@pine.circa.ufl.edu; Fax: (904) 392-1071; Leave message at (904) 392-0828).

Spatially-variable agriculture is becoming widespread in the grain production regions of the United States, especially in the Midwest. There is also substantial activity in regions of Canada, Germany, England, and Italy. The keys to the production systems are grain combine harvesters that automatically generate maps of the harvested crops. These maps are then used for management decisions. The most common management practice is to generate maps on personal computers of the desired variable rates of application of fertilizers and pesticides within the yield-mapped fields based upon the yield maps and similar maps of soil properties and fertility. These maps are then loaded into mobile applicators, which apply the fertilizers and pesticides accordingly. These techniques should also be applicable to tropical crops, such as sugar cane and pineapple. All that is needed is for engineers and agricultural scientists to develop the appropriate equipment and management techniques. As I indicated in a 1992 article in Fertilizer Research, the large inherent variability within tropical fields should make them particularly appropriate for these technologies which minimize economic input costs and the introduction of agricultural chemicals into the environment.

Newsletter on Spatially Variable Agriculture

It has been over a year since I sent around my little newsletter on some of the news in spatially-variable agriculture. So it is time to clean off my desk again. Remember that this isn't complete, balanced, or accurate--but it's free. This is being distributed on the mailing list PRECISE-AGRI @SOILS.UMN.EDU so that I can reduce the mailing costs and delays. If you do not subscribe to it, I suggest you do. Information can be found by sending the email message "help" (without quotes and in the body of the message, not the subject line) to "listproc@soils.umn.edu" and then subscribing to the list following the directions in the return message. I will send some paper copies to SOME people who are not on the mailing list, but there are no guarantees.

Once upon a time, this little newsletter could document all that was going on. It now is impossible. But there are many sources of information. Grant Mangold's ag/INNOVATOR (Grant=712-296-3615,Circulation=800-564-4005) is one of the best. K. Elliott Nowels's Dealer PROGRESS special issue on Precision Agriculture in January was also valuable for its viewpoint from that of the fertilizer and chemical retailers and its listing of 30 companies in the area.

Conferences: Many conferences now include some material on spatially-variable agriculture (svAg) or are even focused on it. These include: The American Society of Agricultural Engineers (612-429-0300) meeting in Chicago on June 19-22.

The Information Agriculture Conference in Champaign, Illinois on June 27-30 is being sponsored by the Potash & Phosphate Institute. For more information contact Mary Hughes (913-776-0273). The GIS in Production Agriculture workshop in Vail, Colorado sponsored by the Farmers Software Association (800-237-4182) is tentatively August 21-25.

The National Crop Conference for Large Acreage Farmers in Galena, Illinois on July 5-8 has updates on svAg. It is sponsored by Brock Associates (800-558-3431).

The North American Agricultural Equipment Conference in Chicago November 1-5 includes sessions organized by ASAE's Power and Machinery Division.

For those interested in European activities, the AgEng 96 meeting will be held in Madrid on September 23-26, 1996.

Regional farm shows often feature svAg. A good example was the 1994 Wisconsin Farm Progress Show where the AgTech

2000 tent brought together various equipment suppliers and fair goers could watch a yield map being created from a combine in a nearby field.

Marvin Stone (Oklahoma State) and I gave talks on tractor-implement communications and svAg respectively at the Marketing & Management Conference of the Equipment Manufacturers Institute in January. The inclusion of this material at that particular conference and the response we got indicated to me that the traditional agricultural equipment manufacturers are increasing their respect for these technologies.

Bill Stout (Texas A&M) and I are giving a keynote speech on agricultural equipment technological levels in North America and western Europe at the Club of Bologna meeting in November (coinciding with the big EIMA equipment show). The Club of Bologna deals with "strategies for the development of agricultural mechanisation" and includes such leaders as Profs. Legg (U.K.), Pellizzi (Italy), Renius (Germany), and G. Singh (India). Prof. Stout and I are looking for any input you may have on the topic.

Publications: The October 1994 issue (Vol. 11, No. 1) of Computers and Electronics in Agriculture (Compag) was a special issue on GPS in agriculture edited by Prof. Auernhammer. Dr. John Stafford is editing a nine-paper Compag issue on svAg due out in late 95 or early 96. At the 1994 Winter ASAE Meeting, I found the papers by Macy, Shearer, and Auernhammer to be the most interesting--although others have their own favorites. The papers at the AgEng'94/CIGR meeting in Milano, which I attended last August, generally were by the same researchers (Auernhammer, Stafford, de Baerdemaeker, Fekete, etc.) whose work is accessible in North America through Compag and ASAE papers.

The book based on Pierre Robert's first Minnesota conference Soil Specific Crop Management (ASA-CSSA-SSSA, 1993) is still useful. (I should clarify again that the section on "Engineering Technology for Site-Specific Crop Management" was written by Steve Searcy. I only chaired the oral working group.) The book on the second Minnesota Conference, Soil Specific Management for Agricultural Systems, is almost out from ASA-CSSA-SSSA.

Visits: I have not made as many visits as I would have liked recently. But I did get to see Prof. Auernhammer's activities in Freising (Munich). We're looking forward to the results of yield mapping with four different meters on a combine and the yield mapping of other crops, especially forage. One of his students, Thomas Muhr showed me the company Thomas has in Adelschlag. They have done a lot of automatic field mapping, especially in the former East Germany. Spectrum Technologies in Plainfield, Illinois is doing good business with hand-held meters for pH, chlorophyll, nitrate, and potassium. Harold Schramm, who many of you know from his leadership at International Harvester and ASAE, is leading the technical efforts. Craig Kvien (912-386-7274) and others at the National Environmentally Sound Production Agriculture Laboratory (NESPAL) in Tifton, Georgia (USA) are ramping up fast from a recent start in doing research on svAg in peanuts and cotton.

Products: I can't keep up with all the products anymore. That is the job of the farm press now I guess. Concord Inc. (701-280-1260) announced they have added more site-specific staff and sent out (at least to me) a nice folder of their various products in the field. The Deere Precision Farming Group (1800 158th Street, East Moline, IL 61244-9532) is closely watched due to the dominant position of Deere in the agricultural equipment marketplace. They have an agreement with John E. Chance & Associates to provide a DGPS receiver and correction signals. A combine yield mapping system will be available in limited quantities for this year's crop. They are committing themselves to a spray rate control system, a seed rate control system, a cotton yield monitor, a tractor precision farming package, and other items. GPS World (p. 62, April 1995) says that "Over the past year, other companies have entered GPS-based products, systems, and related software into the precision farming market, which appears poised for rapid growth. Among these are Rockwell Collins (Vision GPS/GIS system), Del Norte (Flying Flagman), NovAtel (AG-20), and SatLoc (TerraStar)." Deere hosted a VRT (Variable Rate Technology) Economic Consortium for some of us university professors in Chicago last July. The economics of this area are undeveloped at this time.

Case (merger of J.I. Case and International Harvester, now separate from Tenneco) is integrating site-specific farming into their design teams. Case will have combines with their own yield monitors in test fields this fall.

John Posselius is active in this area at New Holland (formerly Ford-New Holland). I didn't talk to my contacts at AGCO (formerly Massey-Ferguson, New Idea, Deutz-Allis, Heston, etc.) early enough to get a response before I had to send this out.

Ag-Chem continues its leadership in this area with the SoilTeq Soilection system. The big video screen and EPROMs are a thing of the past with the FALCON controller introduced last summer. They are putting the controller on four-bin dry spreaders, multi-tank liquid applicator, anhydrous sidedress applicators, spinner spreaders for lime, and planter controllers.

Tyler is marketing a three-bin dry applicator with variable rate control. The Minneapolis judge still has not decided whether it infringes upon the Ag-Chem patent or not. Both firms are continuing developments of more and better svAg equipment. Ag-Chem has excellent manufacturing facilities and Tyler is now in the construction phase of a large plant expansion and transfer of the former Mertz line to its Benson, Minnesota headquarters. For information on other manufacturers (many small manufacturers have innovative products), see ag/INNOVATOR or other farm press outlets. For news on GPS and GIS I recommend GPS World (interesting article on page 40 of March issue on increasing accuracy with auxiliary devices) and Geo Info Systems respectively.

In the May issue of Resource (formerly Agricultural Engineering), I editorialize strongly on page 40 for some sort of testing to determine svAg equipment performance. I did not mean to criticize products, just to motivate the development of standards and comparisons so that purchasers can make informed decisions. A refined version will appear in ag/INNOVATOR.

Some of the interesting research: The USDA soil tilth lab in Ames, Iowa is doing some good work on long-term yield

mapping, yield variability modeling, variability causes and management, electromagnetic induction sensing, and climate trend removal. Tom Colvin (515-294-5724, colvin@nstl.gov) is one of the leaders. Steve Searcy at Texas A&M is in the second year of variable herbicide application research. Their team has implemented look-ahead control (some of us call it feedforward control). They are working with the sensor from Wilkerson (U. Tennessee) for cotton yield mapping.

Pierre Robert (612-625-3125) is talking with industry about trying to establish a Precision Agriculture Center at the U. of Minnesota. They are continuing their research work. Besides the Gopher list I discussed at the start of this newsletter, they hope to set up a World Wide Web homepage for us which will also be able to have field images. The USDA/U. Missouri group in Columbia (Ken Sudduth, Steve Borgelt, etc.) received a USDA/NRI grant to look at methods of making recommendations based upon in-field data. They also continue long-term field data collection, looking at spatial correlations, and sensor (EM induction conductivity and corn population at combining) development. Some researchers are taking advantage of the natural geographic referencing and physical structure of irrigation systems for svAg. For example, E. John Sadler and Carl Camp are controlling nitrogen application in 7.5 degree/30 foot long sections of a center pivot. Some research and development is being conducted by small firms under U.S. government SBIR projects. For example, Field Control Systems, Inc., (White Bear Lake, Minnesota) had a weed mapping project using video for near-real-time field management.

There is a lot of British activity. For example: a precision farming specialist group within the Institution of Agricultural Engineers, a precision farming conference for farmers on June 14th, and significant research. Simon Blackmore (Silsoe College) is setting up a Centre for Precision Farming at Cranfield University. The work of John Stafford and others at Silsoe Research Institute includes two EC projects, one on patch spraying and one on using soil and crop data and yield maps in a N uptake model for spatially-variable fertilizer application. Their patch spraying methods are undergoing evaluation trials and may be commercialized. There seems to be a mini-resurgence in looking at machine vision for agricultural vehicle guidance, including efforts at Silsoe Research Institute and in Queensland, Australia. (There were efforts documented at AgEng'94 also in the area.)

I had the pleasure of being the Engineering referee on the Master thesis of Paul Tyndale-Biscoe, studying under Graham Moore in Civil and Environmental Engineering at the University of Melbourne. He did an excellent job building a soil sampler and with his analytical engineering analyses. (Work on improving soil sampling productivity is also being pursued here in the USA.) Other rumored Aussie work includes work in Western Australia and Queensland on yield mapping. Karl Wild (excellent Ph.D. student) reports that in Germany: "Working group Auernhammer (Weihenstephan) got the first on market available LBS- System (Agricultural Bus System) with tractor and fertilizer spreader for tests on site specific treatment. Schnug (FAL Braunschweig) offers together with 4 companies complete techniques for local resource management. Working group Ehlert (Potsdam - Bornim) applied nitrogen site specifically on 200 ha."

The Marshfield Clinic in Wisconsin is investigating the use of GPS to track farm equipment operators' positions and prevent them from getting injured. That's not svAg, but certainly would use related technologies. ♢

Pineapple News from Australia

The Pineapple Industry Farm Field Day, co-sponsored by Queensland Fruit & Vegetable Growers, Golden Circle Inc., and Queensland Department of Primary Industries, was held on 21 July, 1995 at the industry farm at Beerwah. Some of the presentations are abstracted below.

Can we Make More Informed Decisions on When to Apply Nematicides

Graham Stirling, Principal Nematologist, Department of Primary Industries (DPI), Brisbane

Dr. Stirling noted that community concerns about pesticide usage likely will require that farmers apply pesticides only when necessary to prevent serious economic losses. Where nematodes are concerned, this would mean applying nematicides only when nematode populations are high enough to indicate the possibility of economic losses. Points made by Dr. Stirling in his 1994 presentation were reviewed (see Pineapple News Vol. I, No. 2). Nematode monitoring was done on 20 fields on three growers' properties for 21 months to try to improve the reliability of the monitoring program. The results show that:

- some fields are more prone to nematode problems than others. In one case, low populations were associated with soil type but no consistent association between soil characteristics and nematode populations was mentioned, and
- no large and unexplainable changes in nematode populations have occurred in any field.

Field trials are being used to obtain better information on the relationship between nematode populations and crop losses. The data obtained to date confirm previous results that show that nematodes do not markedly reduce plant crop yields (their greatest effect is on the ratoon crop). When collecting nematode samples, growers were cautioned to keep samples cool so nematodes would not be killed by high temperatures prior to analysis. Because nematode analysis is relatively costly, it was suggested that growers might consider pulling a representative sample of plants to check roots for nematode damage. The root knot nematode is the most serious problem in Queensland, so visible galling provides a good indicator of the extent of a nematode problem. ♢

Sources of Magnesium Preplant Fertiliser

Doug Christensen and Eric Sinclair, Golden Circle Ltd.

Sources of magnesium differing is size and solubility (Granomag, 54% Mg; Dolomite, 8%; Serpentine, 18%; Grow-mag, 8%; Mag Feed, 56%; Kiserite, 16%) were applied in three different experiments. Amounts of pre-plant magnesium applied ranged from 100 to 432 kg/ha in the three trials, but no differences in

response were observed. The lack of a difference was attributed in part to foliar applications of magnesium made after planting. The authors noted that it was up to the grower to decide which product to use on the basis of pre-plant soil analysis, for example the quantity of calcium needed, and fertilizer cost, availability, and management criteria. It was concluded that pre-plant applications could not replace boom spray applications of magnesium sulfate except where soils were high in magnesium. ♢

Quality, Efficiency and Yield

Doug Christensen, Golden Circle Ltd.

A number of principles related to the effects of plant arrangement, spacing, and density on pineapple yield and fruit quality were reviewed. On average, fruit weight decreased as population density increased in all studies. Over the range 20,000 to 25,000 plants per acre (49,400-61,750 plts./ha), fruit weight decreased about 55 to 60 g for each 1,000 plant per acre (2,470 plants/ha) increase. The percentage of black heart (internal browning due to cool weather) in fruit harvested in July and August ranged from 15 to 76% and increased as density increased from 15,000 to 30,000 plants per acre (37,050-74,100 plts./ha). Increased incidence of blackheart and acidity and reduced sugars were associated with ripening during these months. Regardless of month of harvest, ripening in Queensland is delayed one day for each increase in population density of 1,000 plants per acre (2,470 plts./ha), the spread of fruit maturity increases (more pickings are required), and variation in fruit size is greater. The greatest impact is at densities above 20,000 plants per acre (49,400 plts./ha).

Spacing between plants in the row is very important and should not be less than 23-25 centimeters, while spacing between beds is critical. Fruit weights will be more uniform and weights may be greater where spacing between plants is more nearly equal on all sides (orchard like). It is recommended that the area per plant (average distance between plants times average distance between rows) be 0.2 m^2 in winter and not less than 0.14 m^2 in summer. ♢

Ed. Note. The above results are consistent with Hawaii data except the variation between winter and summer is not so great. Spacing between plants is usually not less than 23 cm and spacing between the centers of two-row beds typically is not less than 112 cm in high density plantings destined for fresh fruit, but may be greater when fruit will be processed.

Pineapple News From Brazil

Notes on Pineapple Crop in Brazil

Natural Flowering of Pineapple. Precocious natural flowering is causing many losses to pineapple growers in Brazil. Its occurrence is becoming more and more frequent. Results of a preliminary study have shown the possibility of using growth regulators for the inhibition of pineapple flowering, mainly those which have the ability to block the ethylene biosynthesis. It is suggested that new studies should be carried out using other growth regulators and concentrations and also cultural practices related to the mineral nutrition and development of the pineapple plant. Cunha, G.A.P. da. Preliminary trial on the control of natural flowering of pineapple. Rev. Bras. Frutic., Cruz das Almas/BA (Brasil), v. 11, no 3, p 59-62, 1989. ♢

Current Pineapple Research Projects in Brazil

Irrigation of pineapple is becoming common in Brazil, not only in the traditional producing regions, but also in new ones, mainly under semi-arid conditions. This has created a great demand for information about this practice. There aren't any data available from local studies to help the growers. Thus, EMBRAPA/CNPMF - Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical is carrying out a project (Management of irrigated pineapple crop), in order to develop local technologies on this subject.

Fusarium and wilt are the main problems of pineapple in Brazil. It is common knowledge that the genetic base of the crop is narrow and the most widely cultivated varieties are 'Perola' and 'Smooth Cayenne'. In order to face those problems, EMBRAPA/CNPMF is developing a project with the objective to obtain cultivars resistant to pests and diseases for different ecosystems of the country. ♢

Pineapple News From Cuba

Somatic embryogenesis in pineapple (Ananas comosus (L.) Merr)

M. Daquinta, A. Cisneros, Y. Rodríguez, M. Escalona, C. Pérez, I. Luna y C.G. Borroto. Centro de Bioplantas, Carretera a Morón Km 9, Ciego de Avila CP 69 450, CUBA.

Abstract: Different concentrations of dicamba were evaluated for callus formation from the base leaves obtained from *in vitro* growing plantlets. In these explants were obtained a slow growing, hard, nodular and pale yellow callus. The embryogenic characteristic of callus and embryos were determined by histological examination.
Keywords: Pineapple - Somatic embryogenesis - Callus culture - Histology Tissue culture.
Abbreviations: 2,4-D - 2,4 dichlorophenoxyacetic acid. BA - 6 benzyladenine.

In pineapple, *Ananas comosus* (L.) Merr Wakasa et al. (1978) established the formation of callus from young syncarps, axillary buds, crown and slips. There are other reports of callus formation from hybrid embryos (Srinivasa Rao et al., 1981), *in vitro* grown plantlets (Mathews and Rangan, 1981), lateral bud and meristem tips of crowns (Liu et al., 1989), crown sections of pineapple with and without buds (Lapade et al., 1989), and crown apices (Fitchet, 1990). In spite of these callus cultures, the regeneration of plants in pineapple was by indirect adventitious organogenesis and not as the result of somatic embryogenesis

(Fitchet, 1990). The present report demonstrates the possibility to achieve somatic embryogenesis in pineapple from leaf cultures of *in vitro* grown plantlets.

The youngest leaves were removed from *in vitro* pineapple plantlets (Smooth Cayenne and Red Spanish) cultured on Murashige and Skoog (1962) solid medium as modified by Daquinta et al. (1994). The pH of the medium was kept at 5.8 by addition of 1 N KOH prior to adding agar (0.8 %) and then autoclaved at 121 $^{\circ}$C and 1.5 kg/cm^2 for 15 minutes. A histological study of callogenesis was performed by following the technique described by Johansen (1940).

Callus initiation and proliferation from the base leaves were affected by dicamba concentration in the culture media. The presence of a large number of active cells in the area of the basal and primary thickening meristem made it an attractive zone from which to secure vigorous cells.

Fitchet (1990), was unable to obtain callus from more than 1% of cultures with isolated leaves from *in vitro* cultured plantlets. However, as in other monocots the potential for dedifferentiation is restricted to immature tissues.

In most plant tissues and especially in monocots, 2,4-D is the hormone that confers embryogenic competence to tissue-cultured plants (Ammirato, 1983). Although in some plants, like pineapple, there were no responses. Nevertheless, other synthetic auxins such as dicamba and picloram have been used successfully to initiate embryogenic cultures.

The combination of 2.5 mg l^{-1} dicamba and 0.5 mg l^{-1} BA was the best for callus formation from pineapple leaves, and its plant regeneration ability was higher than the others, when the callus was transferred to an auxin-free medium.

Embryogenic callus culture of *Zingiber officinale* Rosc was induced in young leaf segments from *in vitro* shoot cultures, and dicamba at 0.6 mg l^{-1} was the most effective (Kackar et al., 1993). Histological studies showed that the proliferating embryogenic aggregates were composed of meristematic cells in active division and there was close similarity between zygotic and somatic embryos.

In previous approaches, neoformed tissue from leaf explants was of perivascular origin, formed by meristematic cells with a dense cytoplasm, a large, centrally positioned nucleus, a single and quite large nucleolus and small starch granules like those described by Michaux-Ferrière and Schwendiman (1992) in coffee and oil palm. These examples show clearly that callus quality is primordial in the somatic embryogenesis process and that it can only really be judged by histological examination.

Even though integrated root and shoot meristems were seen in somatic embryos, simultaneous development of root and shoot was only infrequently observed (Kackar et al., 1993).

Somatic embryogenesis has been obtained easily in certain plants but with much more difficulty in others (Ammirato, 1983). As far as we know, our report is the first on *in vitro* studies of somatic embryogenesis in pineapple.

The successful induction of somatic embryos in a range of crops shows that the phenomenon of embryogenesis is universal and that the totipotency to produce somatic embryos exists in diverse tissues.

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Estudio Comparative entre dos Cultivares de Piña (Ananas comosus (L.) Merr)

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Resumen: Como forma de conocer las diferencias existentes entre los cultivares de piña Cayena lisa Serrana, colectada en Ciego de Avila, y la Cayena lisa de Martinica, colectada en Pinar Del Río en cuanto a su adaptación a nuestras condiciones edafoclimáticas, fue realizado un ensayo comparativo entre éstas, para lo cual se estudió la dinámica de crecimiento y desarrollo, y algunos parámetros del rendimiento y calidad de las frutas. Cayena lisa Serrana mostró los índices más favorables, demonstrado estar más adaptada a nuestras condiciones ecológicas.

Abstract: Comparative study between two pineapple (*Ananas comosus* (L.) Merr) cultivars. A comparison between a Smooth Cayenne Serrana collected in Ciego de Avila and from Martinique was carried out in Ciego de Avila, Cuba. Local Smooth Cayenne grew faster and fruit quality and yield were higher than Smooth Cayenne £rom Martinique in our climatic conditions.

Introduccion: La piña (*Ananas comosus* (L.) Merr.) está clasificada como una de las frutas más finas de las regiones

tropicales. En la actualidad es estimada en todo el mundo, aunque su cultivo se limita a las regiones tropicales y subtropicales.

Existen cinco grupos de piña, siendo el más importante la Cayena lisa, pues en el se encuentran las mejores variedades, tanto para la industria como para el consumo (Py y col, 1987).

Es por ello que a partir de 1988 se comienzan a introducir en Cuba, Cayenas de Guinea y Martinica, por la provincia de La Habana: sin embargo, no se ha logrado con ello en la práctica los éxitos deseados (Treto y Guzmán, 1979).

Por otra parte no es hasta 1990, cuando comienzan los primeros estudios con Cayena lisa en Ciego de Avila, región de mayor producción piñera en el país, donde Esapañola roja ha sido su principal cultivar desde hace más de cinco décadas debido a su rusticidad y adaptación a las condiciones de cultivo.

Se hace necesario entonces seguir fomentando el cultivo de variedades reconocidas en el mercado por su rendimiento y cualidades organolépticas que puedan aportar al país recursos financieros y abastecer el mercado interno, es por ello que se hace necesario estudiar un grupo de caracteres que permitan conocer la adaptación ecológica de los cultivares de Cayena lisa Serrana y Martinica, para su posterior introducción en la producción.

Materiales y Metodos: El trabajo fue realizado en el Centro de Bioplantas, para lo cual se seleccionaron posturas de piña de Cayena lisa colectadas en la zona Serrana en Ciego de Avila y de Martinica, proveniente de Pinar del Río, la cual había sido introducida desde Martinica en 1968.

Se utilizaron 96 hijos claveles (200-300 g), tratados con una mezcla de Policarbaxín (7.27 g/L) y Xitol (0.72 %), de cada uno de los cultivares, y fueron sembrados en Marzo de 1992 en la Unidad de Ciencia y Técnica *Juan Tomás Roig*, del Instituto Superior Agrícola de Ciego de Avila, sobre un suelo Ferralítico rojo compactado, en hileras dobles con 24 plantas (de las cuales se evaluaron 20 plantas) cada uno (90 x 40 x 30 cm), sin arrope, y replicado 4 veces en un diseño de bloque al azar.

La preparación del terreno, siembra de los hijos, así como la inducción de la floración con la mezcla de Flordimex (ácido-2-cloroetil-fosfórico), Urea y Carbonato de sodio, a los 11 meses de efectuada la plantación se realizó según MINAG (1989).

Observaciones realizadas: Para el estudio de la dinámica del crecimiento y desarrollo de la planta, se registró la altura de la planta (cm), largo y ancho de la hoja D (cm), hojas emitidas y diámetro de la planta; las cuales fueron observadas cada dos meses a partir del segundo mes de realizada la plantación (Teran, 1983).

La longitud y diámetro del pedúnculo (datos registrados a 3 cm por debajo del fruto, cm), número de hijos (basales, claveles y criollos), peso del fruto con corona y sin corona (g), número de coronas, y el porciento de frutos fasciados, fueron analizados al momento de la cosecha, entre los 5.5 y 8 meses de realizada la inducción artificial de la floración.

El porcentaje de frutos fasciados se determinó como: [(número promedio de coronas - 1)/número de plantas evaluadas)] * 100. Como índices de calidad se determinaron el contenido de vitamina C (%), ácido cítrico (% g), y sólidos solubles (°Brix) del jugo de los frutos.

A los seis meses de realizada la cosecha se procedió al conteo del número de hijos claveles y criollos, así como su longitud (cm), en los dos cultivares estudiados.

La humedad relativa (%), precipitaciones (mm), así como las temperaturas promedios mensuales (°C), fueron registradas durante todo el desarrollo del experimento en el puesto de observación meteorológica de la propia Unidad de Ciencia y Técnica (Tabla 1).

Table 1. Registro de las condiciones climáticas durante el desarrollo del experimento.

	1992			1993		
	Precip	Hum Rel	Temp	Precip	Hum. Rel	Temp
Meses	mm	%	C	mm	%	C
Enero	8.5	83	22.2	27.3	82	23.7
Febrero	97.2	81	22.7	21.2	79	21.5
Marzo	3.5	79	23.4	80.3	80	23.4
Abril	96.9	79	24.4	219.2	79	24.4
Mayo	81.3	77	24.8	23.5	79	24.8
Junio	475.3	80	27.2	92.6	81	27.5
Julio	150.9	80	28.2	57.0	79	24.7
Agosto	152.5	82	27.9	115.5	81	28.1
Sept.	277.5	82	27.1	198.4	82	27.2
Octubre	91.9	82	26.4	106.8	84	26.2
Nov.	17.1	83	25.8	78.6	84	24.7
Diciem.	2.0	84	22.9	44.0	84	23.8

Todos los datos fueron procesados estadísticamente mediante un análisis de varianza simple con ayuda del utilitario CSS (Complete Statistical System).

Resultados y Discusion: La dinámica de crecimiento y desarrollo se ilustra en las Figuras 1A, 1B, 1C y 1D, donde se observa una curva de crecimiento sigmoidal tal como describen Vázquez y Torres (1982), en cada uno de los caracteres analizados.

En la altura de las plantas (Figura 1A), ya a los dos meses comienzan a diferenciarse los cultivares, alcanzando la Cayena lisa Serrana los mayores valores.

De forma similar ocurre con el largo y ancho de la hoja (Figuras 1B y 1C), aunque las diferencias entre ambos cultivares no fueron significativas. Cuando se analizan las hojas emitidas (Figura 1D), se encontró que Cayena lisa Serrana emite de forma general 1.82 hojas promedio más que Martinica, y en el mes de Enero de 1992 de 2.64 hojas.

Solo el diámetro de la planta (Figura 1E), no tuvo una caída brusca en el mes de Enero de 1993; sin embargo, cuando se analiza su incremento, éste fue muy discreto de Noviembre de 1992 a Enero de l993 (8 cm en Serrana y 3.97 cm en Martinica, como promedio), en comparación con el período evaluado de Septiembre a Noviembre de 1992 (38.22 cm en Serrana y 28.1 cm en Martinica).

Una caída del crecimiento generalizada en los parámetros analizados se observó de Noviembre de 1992 a Enero de 1993, donde las escasas lluvias registradas en ese período tuvieron un peso fundamental (Tabla 1).

Si se analizan las variaciones de temperatura durante el ciclo de crecimiento y desarrollo, podemos asegurar que los promedios de ésta (Tabla 1), le fueron favorables a los cultivares estudiados, ya que se conoce que por debajo de los 21 °C y por encima de los 38 °C se detiene el crecimiento (Bartolomew y Kadzimin, 1977), con un óptimo de 24 °C (Dalldorf, 1990).

Aunque Py y col (1987), señalan que la piña es resistente a la sequía, ésta necesita entre 1000 y 1500 mm anuales de lluvias (Samson, 1980), para su normal desarrollo. Los datos registrados

durante el desarrollo del experimento indican que el total de lluvia caída de Marzo a Diciembre de 1992 fue de 1348.9 mm, lo cual debió ser favorable para el cultivo, sin embargo éstas no estuvieron bien distribuídas por meses, nótese que sólo se acumularon 19.1 mm durante los meses de Noviembre y Diciembre de 1992, muy por debajo de los 60 mm indicados por MINAG (1970), lo cual provocó la caída de la curva en los parámetros anteriormente mencionados.

Los parámetros cuantitativos analizados al momento de la cosecha en los dos cultivares aparecen en la Tabla 2. En cada uno de los indices se destaca Cayena lisa Serrana, excepto en el número promedios de coronas, donde ésta presentó 0.06 coronas promedios más que Martinica, lo cual representa un 0.075 % de fasciaciones.

Tabla 2 Caracteres cuantitativos del rendimiento, analizados al momento de la cosecha en dos cultivares de piña.

Cultivares#	Pedúnculo		Fruto		
	Long. cm	Diám. cm	Peso con corona, g	Peso sin corona, g	Número coronas
Serrana	16.67 a	1.97 e	1111.87 a	919.68 e	1.06
Martinica	19.92 b	1.69 b	649.03 b	470.76 b	1.00
Es A	0.354	0.044	8.183	6.424	0.023

#Cayena lisa

Se aprecia menor longitud y mayor diámetro del Pedúnculo en Serrana, parámetros buscados en la selección de individuos promisorios en un programa de mejoramiento genético en piña (Cabot, 1988), pues ellos presuponen resistencia al acamado del fruto y rendimientos superiores.

Altas diferencias se observaron en el peso del fruto, donde los frutos de la Cayena lisa Serrana entran en la designación *B*, por sus 1111.87 g de peso promedio, y Martinica en la designación *pequeña*, de acuerdo a la clasificación Colombiana de calidad (Gallo, 1993).

La producción de hijos basales en piña está bajo control genético (Py y col, 1987), y es uno de los parámetros utilizados para la diferenciación de las variedades; Martinica produjo como promedio 0.96 hijos basales al momento de la cosecha (Tabla 3), mientras que en Serrana estuvieron ausentes, coincidiendo con MINAG (1989).

Aunque fueron producidos muy pocos hijos, Serrana presentó 0.125 hijos claveles y ningún criollo, por su parte Martinica no presentaba ninguno de éstos.

Tabla 3. Número de brotes en dos cultivares de piña, evaluados al momento de la cosecha.

Cultv.#	Promedio de brotes emitidos		
	Basales	Claveles	Criollos
Serrana	-	0.1250	-
Martinica	O.9600	-	-
Es A	0.0043	0.0021	-

#Cayena lisa

En la evaluación realizada 6 meses después de realizada la cosecha, se obtuvieron similares resultados que IRFA (1986), pues el número de brotes aumentó significativamente (Tabla 4), no existiendo diferencias en el número de claveles entre los dos cultivares, pero sí en cuanto a su longitud, donde los hijos tipo claveles de La Serrana mostraron 25.4 cm de longitud más que los de Martinica, aspecto éste que presupone una segunda cosecha en Serrana con mayores rendimientos.

Tabla 4. Número y longitud de los brotes en dos cultivares de piña, luego de seis meses de realizada la cosecha.

Cultiv.#	Número promedio de brotes y su longitud, cm			
	Claveles	Longitud	Criollos	Longitud
Serrana	1.05	51.7	0.62	16.1
Martinica	1.02	26.3	0.15	2.3
Es A	0.045	0.92	0.06	0.85

#Cayena lisa

Cayena lisa de Martinica emitió muy pocos hijos, lo cual se corresponde con los estudios realizados por Teran (1983), quien encontró que la Cayena lisa proveniente de Martinica presentaba muy baja producción de brotes.

Prácticamente en todos los componentes cuantitativos del desarrollo vegetativo y reproductivo se destacó el cultivar Serrana sobre el de Martinica. Este cultivar, colectado en áreas campesinas de la provincia de Ciego de Avila y cultivado durante más de cinco décadas en la zona, muestra por tanto una adaptación local respecto al cultivar de Martinica. Este efecto Sobre la influencia adaptativa del medio ambiente sobre los genotipos *norma de reacción* (Dubinin, 1981), es reconocido y juega un importante papel en la regionalización de variedades.

Los indices de calidad (Figura 2), indican que son los frutos del cultivar Martinica los que presentan mayor contenido de vitamina C, no existiendo diferencias en cuanto al contenido de ácido cítrico.

Se alcanzaron altos valores de sólidos solubles con los dos cultivares, donde Serrana presentó 1.37 °Brix más que Martinica, y una relación sólidos solubles/acidez de 1.19, no llegando a alcanzar los 16 °Brix que se requieren para el mercado fresco (Crochon y col, 1981, citado por Py y col, 1987), pero si para la exportación de fruta fresca de primera calidad para Costa Rica (Saborio y Camacho, 1993).

Las diferencias encontradas en los índices de calidad entre los dos cultivares estuvieron determinados por el tamaño del fruto, por existir una relación directa entre el tamaño de éste y la concentración de azúcares o ácidos (Gallo, 1993).

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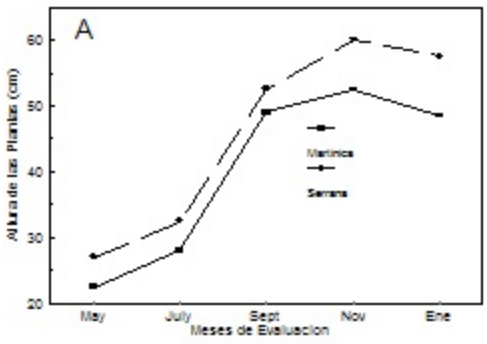

Figura 1A. Dinàmica de crecimiento y desarrollo en dos cultivares de piña (Altura de las plantas.)

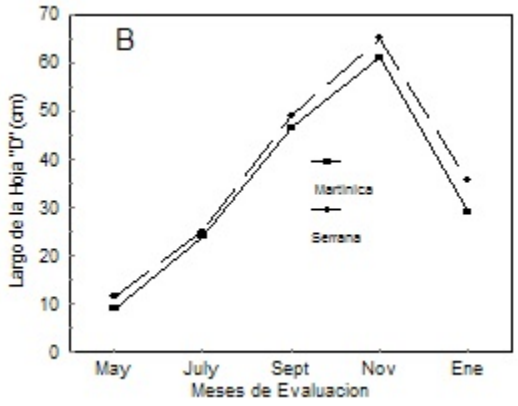

Figura 1B. Dinàmica de crecimiento y desarrollo en dos cultivares de piña (Largo de la hoja D).

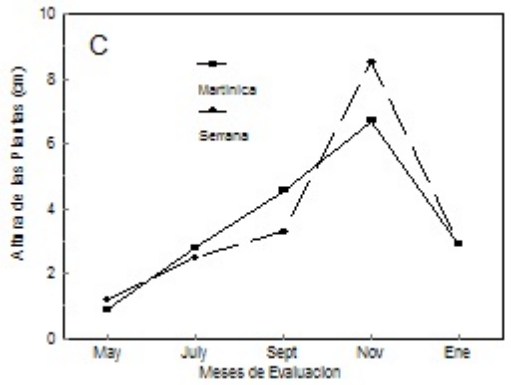

Figura 1C. Dinàmica de crecimiento y desarrollo en dos cultivares de piña (Ancho de la hoja D).

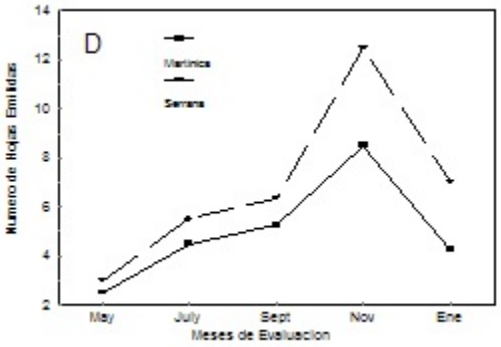

Figura 1D. Dinàmica de crecimiento y desarrollo en dos cultivares de piña (Numero promedio de hojas emitidas).

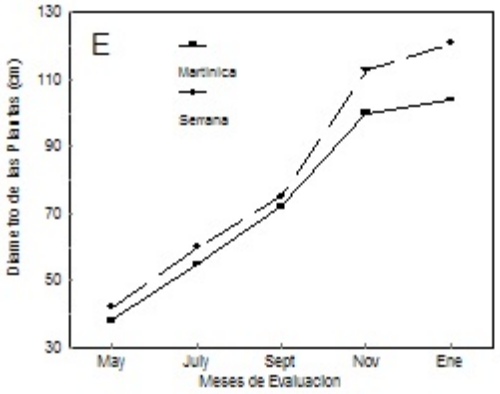

Figura 1E. Diànamica de credimiento y desarrollo en dos cultivares de piña (Diàmetro de las plantas).

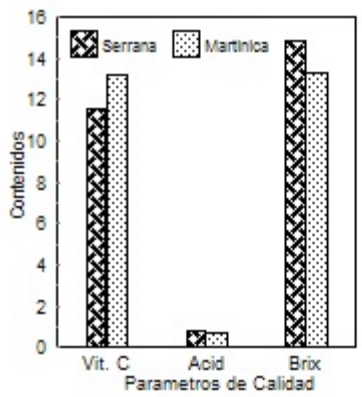

Figura 2. Paràmetros de calidad analizados al momento de la cosecha [1, vitamina C, %; 2, contendio de adico citrico, %g; 3, solidos solubles (°Brix)].

Germinación in vitro y formación de callos en semillas híbridas de piña (Ananas comosus (L.) Merr.)

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Resumen: Se probaron diferentes medios para la germinación *in vitro* de las semillas de piña, obtenidas en un programa de mejoramiento genético del cultivo llevado a cabo en Ciego de Avila; se analizan parámetros como la dinámica de germinación, porcentaje de conversión, y peso fresco de las plántulas. El medio agua destilada y sales + vitaminas MS, resultaron más favorables en los parámetros evaluados, la formación de callos de los embriones híbridos se obtienen cuando se adiciona al medio 1 mg/L de ANA.

***In vitro* germination and callus formation in pineapple (*Ananas comosus* (L.) Merr) hybrid seed**.

Abstract: Different media for the *in vitro* germination of pineapple seed were tested. They had been obtained from a genetic improvement program carried out in Ciego de Avila, Cuba. The germination dynamics, conversion percentage and the plantlet fresh weight were analyzed. The distilled water medium and salts + MS vitamins medium, were more advisable in the characters analyzed. Callus formation from hybrid embryos was observed with 1 mg/L ANA.

Introduccion: Los trabajos de hibridación artificial en piña, son fuentes de nuevos cultivares para la producción. En las hibridaciones que resultan de la combinación de los diferentes cultivares no se garantiza totalmente la transmisión de las características deseables (Fitchet, 1990), por lo que es necesario obtener una gran cantidad de individuos y lograr altas segregaciones para conseguir la combinación deseada (Cabot, 1987), lo cual resulta engorroso y encarece el método.

Los porcentajes de germinación de las semillas de piña son muy bajos, debido a la dureza de la cáscara (Miles y Holmes, 1930), dependiendo la brotación de éstas de los procedimientos físicos-mecánicos y de las condiciones en que se pongan a germinar (Weaver, 1982). Se le ha prestado atención en los últimos tiempos al uso de las técnicas de cultivo de tejidos para inducir variación (Lakshmi y col., 1974). La variación somaclonal en las plantas de piña regeneradas de cultivo de callos, es un método de obtención de plantas en cultivares específicos que podrían ser difícilmente obtenidas por otras vías (Fitchet, 1990), siendo además una técnica de interés en el cultivo de la piña.

En este trabajo se estudia la dinámica de germinación *in vitro* y formación de callos a partir de semillas híbridas de piña en diferentes medios de germinación.

Materiales y Metodos: El trabajo fue realizado en el Centro de Bioplantas de Ciego de Avila, para lo cual se utilizaron semillas híbridas de piña obtenidas del cruzamiento realizado entre los cultivares Cayena lisa Serrana y Perolera (material donado por el CNPMF/EMBRAPA, en Cruz das Almas. Brasil).

A los tres meses de extraídas las semillas de los frutos, fueron escarificadas con ácido sulfúrico concentrado durante 30 - 40 segundos, enjuagadas con agua destilada estéril y desinfectadas con $Ca(ClO)_2$ al 1 % durante 10 minutos. A continuación se sembraron en tubos de ensayos (120 x 25 mm) en la mesa del flujo laminar en cinco medios de germinación *in vitro* gelificados con 0.5 % de agar (Tabla 1), con 30 repeticiones para cada variante.

Tabla 1. Medios para la germinación y formación de callos en semillas híbridas de piña.

Medios	Constituyentes
1	Sales + vitaminas MS (1962), Cabot (1989).
2	Agua destilada (Miles y Holmes, 1930).
3	MS (1962) al 50 % + ANA (1 mg/L).
4	MS (1962) al 50 % + ANA (0.3 mg/L) + BAP (0.2 mg/L).
5	MS (1962). Control.

Cada una de las réplicas se colocaron en condiciones controladas de temperatura 25 +/- 2 °C, y 1 500 Lux de iluminación y alta humedad relativa en el cuarto de luz artificial.

Se realizaron observaciones cada dos dias con el objetivo de determinar la dinámica de germinación de las semillas, y se registraron los datos relativos al porcentaje de conversión de plantas y peso de la masa fresca (mg) de los distintos tejidos formados a los 45 días.

El porcentaje de conversión de plantas (*Cp*), se calculó usando la ecuación: $Cp = [(c/G)*100)]$; donde c, es el número de semillas que convirtieron a plantas a los 45 días, y *G*, es el número máximo de semillas germinadas.

Todos los datos fueron procesados estadísticamente mediante un análisis de varianza simple, con ayuda del utilitario CSS (Complete Statistical Systems).

Resultados y Discusion: Al observar la dinámica de germinación de las semillas de piña en los diferentes medios (Figura 1), se

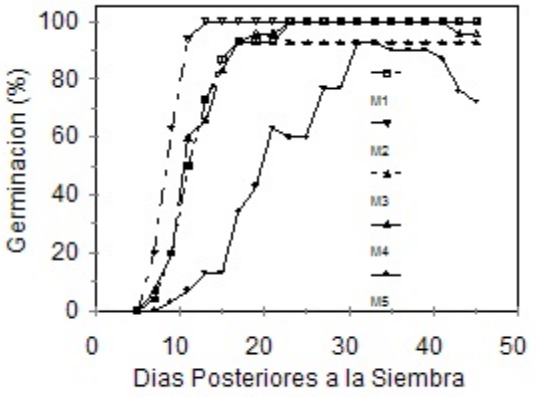

Figura 1. Dinàmica de germinaciòn *in vitro* de las semillas hibridas de piña en diferentes medios.

aprecia que los porcentajes de germinación *in vitro* de las semillas resultaron dependientes del tipo de medio utilizado.

Altos valores de germinación se obtuvieron con los medios agua gelificada (medio 2) y sales + vitaminas MS (medio 1), donde se favoreció la rápida imbibición del agua por el tegumento de la semilla (Vázquez y Torres, 1987).

Se logró mayor precocidad en la germinación con la utilización del agua gelificada, ya que a los 7 días el 20 % del total de las semillas habían iniciado la germinación, alcanzándose el 100 % a solo 13 diás de sembradas en comparación con el medio de sales + vitaminas MS, donde a los 7 días germinaron solamente el 3.33 % y se logró el 100 % a los 23 días. La utilización de 1 mg/L de ANA (medio 3), y la relación ANA/BAP (medio 4) en el medio, aunque lograron altos porcentajes de germinación, no alcanzaron los valores máximos obtenidos en los medios 1 y 2, con el 93.33 % y 96.66 % respectivamente a los 45 días.

La concentración osmótica, provocada por la sacarosa y la presencia de las sales de Murashige y Skoog (1962) en el control, dificultó la normal germinación de las semillas. Se apreciaron crecimientos y decrecimientos en la dinámica de germinación, debido a la muerte de plántulas ya germinadas.

Smith y Greeg (1991), indican en *Capsicum annum* una correlación de - 0.98 entre la germinación de las semillas y la concentración osmótica de la solución en que eran embebidas.

Pierrik y col., (1984), hallaron diferencias de respuestas en la germinación de semillas de tres *Bromeliaceae* cuando añadían al medio diferentes concentraciones de ANA. Por otra parte Mekers (1977), en la propagación de *Tillandsioideae*, plantea que es la sacarosa la responsable de los bajos porcentajes de germinación de las semillas.

La sacarosa (sustancia osmóticamente activa), unida a los nutrientes minerales reduce la imbibición del agua por el tegumento de la semilla por aumento del potencial hídrico del medio, lo que conlleva a menor entrada de agua a la semilla, siendo éste el primer paso metabólico de la germinación (Vásquez y Torres, 1987).

Afectaciones en el porcentaje de conversión de plantas ocurre con la adición al medio 3 de 1 mg/L de ANA y el medio 5 (Tabla 2). El ANA provocó un 3.7 % de formación de callo, del tipo compacto, originado del embrión híbrido de la semilla, que imposibilita la formación de plántulas, y otro callo del tipo friable surgido de los tejidos de la base de las plántulas.

Por su parte, los mayores valores de peso fresco en hojas + tallos, se obtienen con la utilización de las sales + vitaminas MS, pero poca formación de raíces, donde el balance de sus constituyentes favorece la elaboración de sustancias para la formación de tejidos hacia la parte aérea: caso contrario ocurre con las plántulas que se desarrollaron en el agua gelificada, donde existe un mayor peso de las raíces y peso total de las plantas. La combinación ANA/BAP en el medio 4, favoreció la formación de brotes y total de peso fresco, por su parte con el control se obtienen los menores valores.

Tabla 2. Conversión do plantas (Cp) y peso fresco de las plántulas desarrolladas en cinco medios de germinación.

Medios	Cp(%)	Peso fresco, mg				
		Tallo + hojas	Brotes	Raíces	Callos	Total
1	100.0 a	67.0 a	_	9.2 b	_	76.2 b
2	100.0 a	53.4 b	_	31.1 a	_	84.6 b
3	89.6 b	30.9 c	_	_	18.3	49.3 c
4	96.6 ab	29.3 c	71.28	_	_	110.8 a
5	78.5 c	13.8 d	_	0.7 c	_	4.5 d
Es A	1.763	0.22	0.08	0.16	0.12	1.21

La necesidad de lograr la máxima conversión de plántulas es requisito para que un medio de germinación sea efectivo. Al porcentaje de conversión de plantas se le presta especial interés en los trabajos de semilla sintética, donde ésta depende de la calidad de los embriones, de la fitohormona y de otros compuestos inorgánicos con que son inducidos Stuart y col., l985; citados por Harada y col., 1990).

Formaciones de callos a partir de embriones híbridos fueron obtenidos por Pierrik y col. (1984), cuando experimentaron diferentes niveles de ANA en tres especies ornamentales de *Bromeliaceae*, aunque las respuestas variaron en dependencia de las concentraciones hormonales y de la especie. Por su parte Srinivasa y col., (1981), lograron la formación de callos en embriones híbridos de piña (Kew x Queen), pero con la utilización de IBA o una combinación de IBA + BAP.

El cultivo de tejidos ha contribuído al mejoramiento genético de los cultivos, donde la variación somaclonal proveniente de la inestabilidad genética de las divisiones celulares no homogéneas de los tejidos de callos, implican la regeneración de individuos variados (Novak, 1989); variaciones somaclonales en piña han sido obtenidos por (Wakasa, 1989), utilizando tejidos de diferentes orígenes. La posibilidad de regenerar individuos a partir de los callos de los embriones híbridos obtenidos en este trabajo, eleva por tanto la tasa de variación esperada de estos descendientes, incrementando las potencialidades del mejoramiento en las selecciones futuras.

Abreviaturas: AIB (Acido Indolbutírico), ANA (Acido Naftalenacético), BAP (Bencil Amino-purina), MS (Murashige y Skoog)

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Pineapple News from India

Assam

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In Assam, pineapple is grown commercially under rain-fed conditions. Natural flowering of pineapple occurs during February-March and extends to June-July. The peak harvesting season of pineapple is June to September. However, fruits are sporadically harvested in other seasons of the year. Assam produces 37.8 thousand tonnes of pineapple annually from an area of 37.8 thousand hectares. Though it is a commercial crop of Assam, the yield is very low averaging only 14.91 tonnes per ha. However, with the use of proper agrotechniques, the yield of pineapple has been augmented in recent years to a quite satisfactory level.

Pineapple is ratooned in Assam, but economical yields are only obtained up to the second ratoon in the plains due to heavy infestation of weeds and poor management practices, particularly the lack of fertilization. Perennial cultivation of pineapple even up to 50 years in some hill slopes of Lakhipur area of Cachar district and Deuthor area of Karbi Anglong district of Assam are the unique sight of commercial cultivation. Considering the importance and development of pineapple research in this region, the All India Coordinated Research Project on Pineapple was established at Assam Agricultural University Jorhat. The centre is engaged in various aspects of pineapple research and development. The broad objectives of the project are:

1. Collection, conservation and evaluation of pineapple germplasm for its utilization as cultivars or as a source of desirable traits in a hybridization programme.
2. Standardization of production technology viz. population density, nutrition, use of plant growth regulators, and weed control.
3. Standardization of crop protection technique through efficient management of insects, pests, and diseases.

The salient findings under the All India project are:

1. NPK dose of 12:2:12 g/plant for the main crop is recommended under 30 x 45 x 90 cm spacing.
2. Suckers weighing 501 to 750 g and slips weighing 301 to 400 g are superior planting material.
3. Diuron a 3 kg a.i./ha can be effectively used for controlling weeds.
4. Trench planting is not suitable for Assam conditions.
5. Ethrel at 25 ppm with 0.04% $CaCO_3$ plus 2% urea enhances flowering.
6. Planofix at 200 ppm applied two months after flowering increases fruit size.
7. Crown splitting (sectioning) is the most suitable method for rapid multiplication of planting material.
8. Split application of nitrogen into 6 g basal plus 2 g spray at the 5th months plus a 2 g spray at flowering and a 2 g spray after flowering increases fruit yield.
9. A ratoon crop of "Kew" requires that 40% of the nutrients be applied to the main crop. ♢

West Bengal

Effect of plant density on growth, yield and fruit quality of pineapple

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In order to standardize the optimum plant density of pineapple cv. Kew under North Bengal conditions, the effect of plant population densities ranging from 51,282 to 76,923 per ha on growth and yield were studied. The higher plant density reduced the percentage of flowering by about 10.6 % and also delayed inflorescence emergence by about 20 days compared with the lowest density. Higher plant density increased yield per unit area but decreased the individual fruit weight. The yield of fruit per hectare increased from 61.7 to 70.3 tonnes as density increased from 51,282 to 76,923 plants/ha. The percentage of juice, total soluble solids, total sugar, non-reducing sugar, and sugar/acid ratio of the fruit decreased as density increased while canning ratio and fruit acidity increased at higher densities. A plant density of 64,000 plants/ha is considered optimum for cultivation of pineapple cv. Kew in the terai region of West Bengal based on yield, acceptable fruit size and quality. ♢

N and K requirements of pineapple under rain-fed conditions

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In order to standardize the optimum amount of nitrogen (N) and potassium required for pineapple under rain-fed conditions in North Bengal, two separate experiments were carried out using a plant density of 64,000 plants/ha.

In the first experiment, N was applied at 6, 12, or 18 g per plant while holding P_2O_5 (P) constant at 8 g and K_2O (K) constant at 12 g per plant. Plant growth increased with increasing N. Percent flowering was 12% higher when 18 g N was applied relative that at 6 g N. An increase in N from 6 to 12 g increased average fruit weight (1.66 kg), and yield (71.2 tonnes/ha); increasing N to 18 g reduced fruit weight to 1.51 kg and yield to 67.0 t/ha. Maximum yield was associated with a leaf P:N ratio of 1:11 and an N:K ratio of 1:2.3 at 9 months after planting.

At 12 g N/plant, percentage juice was 54.2 and total soluble solids (TSS) was 14; However, total, reducing, and non-reducing sugar contents were decreased compared to those from the 6 g N/plant treatment. Fruit acidity increased with increasing N from 6 to 18 g/plant but the effect of N on fruit quality attributes was not significant.

In the second experiment, 6, 12, and 18 g K/plant were applied while keeping P constant at 8 g and N at 12 g/plant. At flowering, plant height was maximum at 138.1 cm and leaf number at 52.2/plant with 18 g K applied. Flowering was delayed by about 9 days with 18 g K relative to that at 6 g K/plant. Percentage of flowering was maximum at 72.8 in the 18 g K treatment. There were no significant effects of K level on fruit size, weight, or total yield. Average fruit weight (1.43 kg) and yield (64.3 t/ha) were slightly higher in the 12 g K treatment. The highest yield was associated with a leaf P:N ratio of 1:10.1 and an N:K ratio of 1:2.2 at 9 months after planting. Application of 12 g K/plant resulted in maximum percentages of juice (59.5), TSS (14.85), total sugar (12.49), and non-reducing sugars (9.36) in fruit. The sugar/acid ratio of fruit was also maximum (10.72) with 12 g K/plant. At 18 g K/plant, fruit TSS and total sugars were lower while acidity was higher. The results indicated application of N and K at 12 g/plant per year for Kew pineapple in the terai region of West Bengal.

Assessment of the stage of physiological maturity of plant for forcing

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The experiment objective was to identify the optimum time after planting to apply Ethrel. Plants were forced by applying 50 ml of a solution containing 0.25 ml/l Ethrel, 2% urea, and 0.04% calcium carbonate in the core of the plant at intervals of 20 days from 300 to 380 days after planting.

The percentage of flowering increased from 69 to 94 by delaying forcing from 300 to 380 days after planting. Treatment at 380 days after planting resulted in appearance of the inflorescence at 118 days after forcing while application at 300 days delayed emergence up to 202 days. Fruits matured about 30 days earlier by forcing at 380 days after planting relative to the control. Delaying forcing until 380 days resulted in greater fruit weight (1.82 kg) and increased yield by 35 tonnes relative for forcing at 300 days and by 45 tonnes relative to the control. Forcing at 380 days after planting resulted in maximum percentages of juice (66.0), TSS (13.99), total sugars (11.65), and non-reducing sugars (8.38), the lowest fruit acidity (0.87), and the highest sugar/acid ratio (13.39) in the fruit at maturity.

Pineapple News from Israel

Propagating Plants with Paclobutrazol

Yigel Natav of IKN Agric Services Ltd. mentioned in a brief letter that he had used paclobutrazol, commercial name Cultar®, to propagate pineapple. I asked him if he would mind sharing his experiences with others who might have access to paclobutrazol but would be unable to obtain the more commonly used Maintain®, which has been widely used to propagate pineapple in Hawaii. Yigel writes:

We have successfully multiplied [pineapple] by tissue culture lines of Queen and several lines of Smooth Cayenne. Our difficulties were only in the variety brought from Hawaii (probably 198). We do not know whether these difficulties are associated with the variety, with the lab, or some other factor.

The paclobutrazol was used one day after the second spraying of Ethrel (at 100 cc (1st spraying) and 200cc (2nd spraying)) -- one spraying of paclobutrazol at 100 ppm in 300 liters of water per hectare. The plants were sprayed at 10-11 months of age. It seems that the age of the plants has some importance, but the direction of the influence is not clear.

Within the next 4-6 months, we harvested 20-40 plantlets from each plant. The size of approximately 50% of the plants was such that they could be planted immediately in the field, while the others had to be planted in a nursery. Once the harvest of the plantlets was finished, suckers developed and we continued the harvest of the suckers like after harvesting the fruits.

It should be mentioned that we have harvested as many suckers as plantlets from each plant and the large suckers were the best multiplication material. The many suckers were probably the result of relatively young mother plants. ♢

Ed note: I would appreciate hearing if others have had difficulty propagating some lines or groups of pineapple in tissue culture but not others. Also, I am not aware of a clone or line numbered 198.

Pineapple News From the Martinique Symposium

A Brief Overview on Pineapple Breeding Work from the Communications Presented at the Second Pineapple Symposium

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Since the beginning of the century, pineapple genetics and breeding have constituted a significant part of the effort invested in research and development although results have seemed disappointing, as the main commercial varieties are still the same as those selected by native Americans before Christopher Columbus' voyages. In our second symposium, in Martinique, there were communications on most aspects of genetics and breeding, covering the themes of genetic resources, genetic markers, varietal identification, Mendelian genetics of qualitative traits, quantitative genetics, somaclonal variation and mutagenesis, and intermediate results of selection programs were presented. This session gave the opportunity for a rapid overview of the progress and prospects of pineapple genetic improvement.

Conventional breeding

Clonal selection: Clonal selection is tedious and rigorous work. It has been the object of constant efforts by modern pine growers. As a result, 'Smooth Cayenne' potential has been maintained at its best. The importance of clonal selection to avoid the accumulation of negative quantitative and qualitative variation is illustrated by the comparison of different strains of the 'Smooth Cayenne' (Benega et al., 1995b). But clonal selection is not a creative method. Old 'Smooth Cayenne' clones in French Guyana are not very different from the most modern clones. Most mutations are deleterious and no significant positive new traits have appeared, with the possible exception of wilt resistance selected by our Mexican colleagues (Torres Navarro et al., 1989).

Clonal selection has generally been neglected in other varieties, with the exceptions of 'Queen' in Australia and South Africa and 'Espanola Roja' in Cuba and Puerto Rico. In most places, heterogeneity is the rule. Many cultivated clones of 'Espanola Roja' and 'Singapore Spanish' have reverted to the spiny condition and even such important varieties as 'Singapore Spanish', 'Perola' and 'Perolera', although well adapted to the conditions of important local markets and/or zones of production, suffer from strong defects as a proliferation of crowns and slips. If we want to maintain a minimal genetic diversity in this crop, we should select performing clones in varieties of regional importance.

What is needed for efficient clonal selection is a better knowledge on the selection parameters (importance of somaclonal variation, efficiency of clonal selection) and fast multiplication methods. On the first of these themes, published data are very poor despite the constant efforts of growers and breeders. The second theme was the subject of the session devoted to biotechnology. Micromultiplication and acclimatization techniques are commonly and satisfactorily used in pineapple now. Most of the work presented at the symposium was oriented to optimize efficiency and reduce production costs (Almeida et al., 1995; Daquinta et al., 1995; Dominquez et al., 1995; Firoozabady et al., 1995; Ventura et al., 1995), and to improve and complement acclimatization through inoculation with endomycorrhizal fungi (Guillemin et al., 1995) or Azotobacter (Gonzalez et al., 1995).

Somaclonal variation is generally limited. With standard *in vitro* techniques, its importance is probably more related to the multiplication rate per se than to the particular physiology of the plants in the growth chambers. However there remains some risks that the progress in homogeneity, quality and quantity obtained through the selection of specific subclones may be partially lost in the micropropagation process.

Hybrid breeding: Most pineapple varieties are the product of sexual recombination and the heterozygosity level observed by all breeders indicates their hybrid origin. Hybrid breeding naturally appeared the most promising way to a breakthrough. The first programs based on the virtues of sexual recombination were undertaken in Florida, Hawaii, Puerto-Rico and Taiwan. Continuing these efforts, similar programs are currently reported from Brazil (Cabral et al., 1993), Cote d'Ivoire (Atse, 1995), Cuba (Isidron, unpublished communication presented at the Primer Simposio Latinoamericano de Pinicultura), Philippines (Villegas et al., 1995), Malaysia (Chan, 1995), and Martinique. Honestly, we must recognize that all this represents a lot of work for meager results until now. The problem deserves to be rethought from the start.

The first necessary discussion should aim at a clear definition of the objectives. This seems trivial but it is frequently overlooked. The Hawaiian breeding program started with the idea, still valid, of the danger of monovarietal cultivation. But all the created genotypes were discarded because of some flaw (Williams and Fleisch, 1993). Or was it because it was too different from the 'Smooth Cayenne' which was at the same time the ideotype and only one of the genitors? Is the 'Smooth Cayenne' exempt of defects? Of course not, but we know how to manage them. There is some contradiction between the initial objective of diversification through the use of recombination and the rejection of genotypes unfitted for a production system based on a unique variety. We must go back to the definition of consistent breeding objectives. Do we need a "Super Cayenne" or do we need varieties for diversification, at least for the fresh fruit market? What about the phytosanitary risk? Let us imagine fusariose out of Brazil.

The second point is the need for definition of new breeding schemes. We have a good knowledge of floral biology and we know that interspecific crosses are compatible. The possibility of selfing should be explored and sexual reproduction studies completed in this sense. They have been resumed in Martinique. Could genetic recombination be controlled better by using partially homozygous genitors?

A third point, related to the definition of breeding schemes, is the necessary knowledge of the heredity of the numerous traits under selection. Our knowledge of the genetics of quantitative traits is still extremely limited. Dr. Chan's communication showed the importance of genotype x environment interactions. The situation looks better for qualitative traits. Since the work by

Collins and Kerns (1946) we can breed spineless varieties. Their genetic model was confirmed and we know more of the heredity of fusariose resistance and dark-red leaf colour (Cabral et al., 1995). Many negative dominant mutations have been studied by Collins and Kerns (1938) but these traits are not selected. Concerning screening procedures, new tools make it possible to screen genetic resources for resistance to nematodes (Sarah et al., 1995).

Genetic resources

The study of genetic resources is related to both clonal selection and hybrid breeding. The first could allow direct utilization of new germplasm. A review of past and present hybridization programs shows that their genetic base is mostly restricted to the six main commercial cultivars (Coppens d'Eeckenbrugge and Duval, 1994). Wide areas have been recently prospected, resulting in hundreds of new genotypes (Duval et al., 1995; Ferreira et al., 1995) and characterization and evaluation of this material is in progress. Resistance to widespread pests and diseases can be found in clones of local importance (Bello et al., 1995). DNA markers will provide us with a powerful tool for the study of these new genotypes and new traits (Noyer et al., 1995).

A better use of genetic resources implies the proper identification of varieties. An expert system (Gutierrez et al., 1995) and original genetic markers (Coppens d'Eeckenbrugge et al., 1995) have been proposed.

Special techniques

New techniques are now available to improve pineapple by methods that allow us to avoid or control recombination. The mutation rate for particular traits, as reduced spininess, could be increased with a new old technique, such as mutagenesis (Perez et al., 1995).

The isolation of protoplasts (Pinho et al., 1995) and the regeneration of plantlets from immature ovules (Benega et al., 1995a) open prospects for genetic manipulation.

No work or project has been presented on genetic transformation. Here also, the first step is the definition of clear objectives.

After the first observations of a closterovirus associated with wilt, the logical continuation would be a project to transfer the gene of the viral protein to confer resistance to the most widespread disease of the pineapple. However, the cause of mealybug wilt is still not clearly understood and we shall have to wait for more information from virologists before transformation work can be undertaken. Yet a badnavirus has been also found (Hu et al., 1995; Walkman et al., 1995). Another approach would be to make the pineapple resistant not to the virus but to its vector, the mealybug.

Other possible objectives for the application of transformation techniques have been presented by Australian scientists in their 1994 field day. The use of anti-sense gene constructs for polyphenol oxydase could allow to inhibit the enzymes associated with blackheart (Underhill et al., 1994). Blocking the genes for natural ethylene production would result in the complete inhibition of natural flowering (Sanewski, 1994).

There is also interest in transferring genetic systems for nematode control to pineapple (Stirling, 1994). No doubt these works will be followed with great interest by all pineapple specialists.

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Pineapple News from South Africa

White grubs in pineapples: a new strategy for management of the problem (Coleoptera: Scarabaeidae)

G.J. Petty, Agric. Res. Council, Inst. Tropical and Subtropical Crops, Private Bag X1, Bathurst, 6166 South Africa.

Pineapple root systems are attacked by the larval stages of a number of scarab beetle species commonly known as white grubs. The economic threshold for the melolonthid *Asthonopholis subfasciata* Blanch was determined by seeding first instar grubs at different levels of infestation into concrete lysimeters planted with Smooth Cayenne crowns. At a grub density of 0.5 per plant a significant 5.5% reduction in plant growth occurred by the time grubs had attained the third instar stage. To devise a strategy for post-plant chemical control of *A. subfasciata* (previously unattainable) seasonal development of instars and stadia was determined by biweekly soil sampling in a pineapple field. Transition from third (final) stage larvae to adult emergence occurred between October and December. Various isazophos 500 EC foliar spray treatments were applied in October and or November 1988 in an effort to control adult female beetles and prevent reinfestation by a subsequent generation of larvae. Two liters/ha applied in mid-October and again in mid-November resulted in a highly significant 78.5% grub reduction in the following year, judged by grub counts from an untreated control. ♢

Description and identification of white grubs (Coleoptera: Scarabaeidae) attacking pineapple crops in South Africa

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Pineapple crops host a variety of insects that lower crop yield. Considerable economic losses result from damage to the root systems of plants by several species of scarab beetle, including the larvae of *Asthenopholis subfasciata* Blanchard, *Trochalus politus* Moser, *Macrophyla ciliata* Herbst, *Congela valida* Péringuey and *Adoretus ictericus* Burmeister, and the adults of *Heteronychus arator* Walker.

Since the biologies of these species differ, rapid and reliable identification is desirable to assist in their control. Previously there was no readily available method of identifying them. We have provided two keys to the third instar larvae, one for use by extension officers and farmers in the field and the other for confirmatory diagnosis in the laboratory. Drawings were made to assist in identification.

The pattern of setae on the distal abdominal sternites is a good diagnostic field character, however setae may be rubbed off as the larva passes through the soil. For this reason, characters of the mouthparts should be used for confirmatory diagnosis in the laboratory. ♢

Pineapple News From Sri Lanka

Pineapple research activities at the Department of Agriculture

H.M.S. Heenkenda, Regional Agricultural Research and Development Centre, Bandarawela, Sri Lanka

1. Breeding of new varieties.

Hybridization of Maruitius, Kew, and smooth Cayenne has resulted in some attractive lines.

2. Evaluation of tissue culture plants.

A comparison is being made between performance of plants produced from suckers with those produced by tissue culture.

3. Sucker production by mechanical decapitation.

The best size of Kew plants for decapitation, and optimum timing and quantity of fertilizer required to produce the maximum sucker numbers are being tested.

4. Comparison of suckers and crowns as propagules for plants to be used for sucker production by decapitation.
5. Control of mealybugs.

Mealybug wilt of pineapple is very severe in the Western Province of the Island. There is need to develop an effective mealybug control program there.

6. Development of screening techniques to detect pineapple wilt virus (PWV).

Virus infected plantations will be detected and mapped. Also, the growers will be cautioned about the spread of disease after indexing plants.

7. Maintenance of germplasm.

Cultivars such as Kew, Mauritius, Mashmerah, Sarawak, Gal ananas (local semi-wild cv.), Wal annasi (wild cv.) and some ornamental bromeliads are being maintained. ♢

Pineapple News from the United States (Hawaii)

Hawaii Research Progress Reports for the period January 1 - June 30, 1995.

Development of Management Tactics for the Control of Plant-parasitic Nematodes in Pineapple

B.S. Sipes, M.P. Ko, D.P. Schmitt

Objectives:
1. Develop microorganisms or their products as biological control agents of nematodes.
2. Incorporate host-plant resistance and tolerance to reniform and root knot nematodes, *R. reniformis* and *M. javanica,* into cultivars of pineapple.
3. Evaluate selected crop plants for nematode control in pineapple.
4. Determine and capitalize on weaknesses in nematode life cycles to reduce initial population densities.

Progress Abstract: Three hundred eighty Randomly Amplified Polymorphic DNA markers have been identified in *Rotylenchulus reniformis*. These will provide a basis for assessing similarities among and within populations of the nematode. French marigold, sun hemp and sudex have been identified as potential cover crops to use during the inter cycle fallow period to reduce population densities of *Meloidogyne javanica* as an alternative to preplant fumigation.

Accomplishments and Results:

Research to develop and exploit *Pastueria penetrans* as a biological control of nematodes in pineapple has continued through federal funding. Differences among isolates from Hawaii are being determined. Factors affecting *P. penetrans* distribution and survival will be identified and then used to develop strains which are usable in the field.

Rotylenchulus reniformis females have been subjected to Randomly Amplified Polymorphic DNA (RAPD) analysis using PCR. One hundred twenty primers have been evaluated and 60% produced bands. The 72 primers which amplified nematode DNA gave 380 distinguishable bands which represent potential polymorphisms in *R. reniformis*. These polymorphisms will be used to determine within and among population variation of the nematode

Twenty five potential cover crops were tested in small field plots for their ability to suppress *Meloidogyne javanica* populations. The worst plants were cow pea, rye grass, and lana woolypod vetch, which were excellent hosts. These three plants increased nematode population densities more than ten fold. French marigold, sun hemp, and sudex suppressed nematode population densities the most and will be used in future field evaluations. ♢

Development of Nematicide Management Strategies for Control of Nematodes in Pineapple.

B.S. Sipes

Objectives:
1. Evaluate the efficacy of pre- and post-plant nematicides for the control of reniform *(Rotylenchulus reniformis)* and root knot *(Meloidogyne javanica)* nematodes in pineapple. a) Compare the efficacy of solarization, an oat cover crop, and bare fallow for reduction of population densities of *R. reniformis* during the inter cycle fallow period. b) Determine the rate of reniform and root knot nematode development on newly rooted pineapple plants in the greenhouse. 2. Develop and enhance application techniques and methodologies to minimize environmental impacts of nematicide application in pineapple production. a) Conduct a field scale comparison of an emulsifiable formulation of 1,3-D (XRM-5053) to the standard liquid formulation (Telone II) for efficacy, distribution in the soil, and volatilization into the air. b) Determine the toxicity of metam-sodium (Vapam) to reniform nematode in the laboratory and greenhouse. c) Determine the distribution and efficacy of metam-sodium in small field plots when applied with a chisel injection and drip irrigation method.

Progress Abstract: Inter-cycle cover crops that do not support nematode reproduction could be promising inter-cycle cover crops. These crops could replace preplant fumigation and assist in soil erosion control. XRM-5053, an emulsifiable formulation of 1,3-D, continues to be as effective Telone II and experiences less volatile losses. Metam-sodium is proving to be extremely toxic to *R. reniformis* and effective in the field when applied properly. Additional tests are needed to determine optimal application techniques to provide consistent field control with metam-sodium.

Accomplishments and Results:

Oats were evaluated last fall as an inter cycle cover crop and did not allow *R. reniformis* reproduction. Additional plant species *(Crotalaria, Desmodium, Indigofera, Lablab,*

Macroptilium, Neonotonia, Sesbania, Trifolium, Lolium, Paspalum, and *Triticum)* are now being tested for their susceptibility to *R. reniformis*. Data collection will be completed in October and a field evaluation initiated to test the most promising cover crops.

The initial period of root formation following pineapple planting appears to critically affect the population dynamics of plant-parasitic nematodes. A follow up experiment was initiated in January and will be completed in October to determine if plant age does affect nematode population dynamics. A thorough understanding of these effects could prove beneficial in development of nonchemical alternative control measures.

Soil population densities of *R. reniformis* continue to remain low (under 500/250 cm^3 soil) in Telone II, XRM-5053, and metam-sodium treated plots 6 months after planting. The XRM-5053 has been as effective as Telone II (both at 228 lb a.i./ac). Metam-sodium (50 gal a.i./ac) applied in the drip has also been very effective. The nematode population will continue to be followed at 2-month intervals for the next 6 months. Additional experiments to determine the environmental fate of metam-sodium are being initiated and will be completed by August.

Laboratory experiments have been undertaken to determine the toxicity of metam-sodium, 1,3-D, ethoprop, fenamiphos, and fosthiozate to *R. reniformis.* After a 24 hour exposure, metam-sodium was the most toxic compound to *R. reniformis.* Exposure to concentrations of 2.5 ppm reduced nematode reproduction by 80% compared to the untreated controls. No nematode control was achieved after 1,3-D exposure up to 100 ppm in a hatch assay or 30-day nematode reproduction assay. Toxicity to fosthiozate was evident at 1 ppm in the reproduction assay. Ethoprop and fenamiphos were not as toxic to the nematode after 24 hour exposures as fosthiozate. Longer exposure times, 48 and 72 hours, will be evaluated in the next series of experiments. ♢

Ecology and Control of Ants and Mealybugs in Hawaiian Pineapple Fields

Marshall W. Johnson and Hector Gonzalez-Hernandez,
Department of Entomology, Univ. of Hawaii - Manoa

Objectives:

1) Quantify the role of big-headed ant *(Pheidole megacephala)* and other pest ants as protectors of mealybugs from parasites and predators. Identify the species of parasites and predators attacking gray and pink pineapple mealybug populations in pineapple and evaluate their effectiveness.

2) Study the impact of big-headed ant *(Pheidole megacephala)* and other pest ants of pineapple on the searching behavior of natural enemies of pineapple mealybugs.

3) Determine the potential of a natural enemy augmentation program for control of pineapple mealybugs in the presence of ants:

a) Develop mass rearing procedures for pineapple mealybugs and associated natural enemies; and

b) Conduct natural enemy augmentation studies in ant-infested pineapple plantings to feasibility and success of mealybug control.

Progress Summary Abstract

A mass rearing system is being developed to produce pink pineapple mealybugs and their parasitoid, *Anagyrus anantis*, for field augmentation. Results to date are encouraging, but work still remains to increase the mealybug production and develop the parasitoid production techniques.

Progress and achievements:

Objective 1: ***N. bilucernarius.*** Laboratory studies were conducted to determine what life stages of the ladybug *N. bilucernarius* were impacted (egg, immature, adult) by the presence of the big-headed ant, *P. megacephala.* For this experiment, two arena cages were used: one connected to a laboratory ant colony (treated cage) and the other without a connection (control cage). Arena cages consisted of a transparent plastic shoe box (15 cm wide x 9 cm high x 29 cm long). To provide ants access to the arena, a plastic tube (5 mm diam.) was connected to another similar shoe box containing a *P. megacephala* colony. All laboratory experiments were conducted for 24 h at 24 ± 1°C and photo phase of 14 h. To observe the impact of ants on *N. bilucernarius* adults, two freshly cut pineapple leaves (approximately 3 cm at the base, 15-20 cm in length) were infested each with 100 pink pineapple mealybug (PPM) first instars. One infested leaf was placed into each arena cage. One hour later, an adult coccinellid was introduced into each experimental arena cage for one day. At the end of the experiment, the condition (live or dead) of the coccinellid and final number of mealybugs were recorded for each arena. To observe the impact of ants on *N. bilucernarius* immatures a similar procedure was followed with the exception that pineapple leaves were infested with 10 *D. brevipes* adults. Condition (live or dead) of coccinellid third instar larvae and number of consumed mealybugs were recorded at the end of the experiment. Impact of ants on *N. brevipes* eggs was also tested. Similar arena cages were used for this experiment. Ten coccinellid eggs were placed in a 4.0 cm diam x 0.6 cm petri dish cover and exposed for 24 hr to ants in the arena cage. A day later, the number of eggs remaining in the petri dish cover was recorded. All experiments for all coccinellid stages were replicated 10 times. An ANOVA was conducted to compare differences in consumed prey (between coccinellid adults, larvae, eggs) by the predator in the presence or absence of ants.

In the absence of ants, *N. bilucernarius* adults consumed an average of 53.6 *D. brevipes* crawlers in 24 hr, while in the presence of ants, only 14.6 crawlers were consumed ($F = 53.6$; $df = 1$; $P < .001$). Thus, mealybug predation was decreased 73% by the presence of ants. Ants killed three (out of 10) coccinellid adults. There were significant differences in the number of mealybugs killed by the coccinellid larvae in the presence and absence of ants ($F = 12.9$; $df = 1$; $P = 0.002$). In the presence of ants, coccinellid larval predation was reduced 33.3 % (a mean of 0.5 mealybugs killed) compared to predation in the ant-free arena (a mean of 1.5 mealybugs killed). Ants killed 8 (out of 10) coccinellid larvae. From 100 coccinellid eggs placed in the arena cages, ants consumed 79%.

Anagyrus ananatis. Laboratory studies were conducted to determine the impact of *P. megacephala* on searching *A. ananatis* adult females. The laboratory setup consisted of the same types of arena cages and ant nest colony used for

observing the impact of *P. megacephala* on *N. bilucernarius*. The only difference was the mealybug stages and number used. In this case, pineapple leaves were individually infested with 80 mealybugs (third instar or adult females). Once the pineapple leaf was infested with mealybugs, *P. megacephala* was given free access to the respective arena cage. A day later, a pair of male and female of *A. ananatis* adults were introduced to each cage for 24 hr. Afterwards, mealybugs were removed, placed in separate plastic vials covered with fine mesh, and held until mummies were formed. This experiment was replicated eight times and conducted for 24 h at 24 ±1 °C and photo phase of 14 hr. An ANOVA test was conducted to compare differences in *A. ananatis* parasitized hosts in the presence and absence of ants (Wilkinson 1990).

P. megacephala interfered with the searching behavior of *A. ananatis* adults. Significantly more mealybugs were parasitized by *A. ananatis* when ants were absent (15.9 mealybugs) than when present (8.3 mealybugs) ($F = 6.59$; $df = 1$; $P = 0.019$). Percent parasitization by *A. ananatis* in the presence of ants was 48% lower than in the absence of ants.

The impact of the big-headed ant is significant on the dominant parasitoid and predator species found attacking pink pineapple mealybug in pineapple. By reducing parasitization and predation by these species 48 and 79%, respectively, this releases the pink pineapple mealybug from its natural controls, thereby promoting increases in its population densities to economically important levels. This action combined with the theorized sanitation (removal of honeydew) provided for the mealybugs by the ants, leads to even greater mealybug densities.

Effectiveness of mealybug natural enemies

Earlier studies made by project personnel (H. Gonzalez-Hernandez & N. Reimer) showed that the parasitoid *Anagyrus ananatis* and the predatory ladybug *Nephus bilucernarius* were the dominant natural enemies attacking pink pineapple mealybug in pineapple plantings on Oahu and Maui. The pink pineapple mealybug was more common than the gray pineapple mealybug and was found in all plantings surveyed and sometimes in mixed populations with the gray pineapple mealybug. Studies were conducted on these species to determine their effectiveness as natural enemies of the pink pineapple mealybug. Studies on parasitoid and predator reproduction and developmental rates have already been reported.

***Nephus bilucernarius*, Prey species preference**. Choice and no-choice studies were conducted to determine which species of pineapple mealybug (pink or gray) were preferred by *N. bilucernarius*. In the no-choice experiment, pineapple leaves were individually infested with 10 mealybug crawlers of either pink pineapple mealybugs or gray pineapple mealybugs and kept in a 5 liter polyethylene container. One day later, mealybugs were exposed for 24 hr to a 2-5 day old adult female *N. bilucernarius*. The number of consumed prey was determined by counting the remaining live mealybugs and subtracting the total from the initial prey number. In the choice experiment, pineapple leaves were individually infested with 5 crawlers of each mealybug species. Time of exposure and determination of the number of consumed prey were identical to that conducted for the no-choice experiment. Data were analyzed with ANOVA and when necessary with a Tukey mean comparison test.

In the no-choice experiment, no significant preference was exhibited by *N. bilucernarius* for either mealybug species ($F = 0.05$; $df = 1$; $P = 0.82$). A mean of 7.3 pink pineapple mealybug crawlers were consumed as compared with 7.4 gray pineapple mealybug crawlers. Similarly, in the choice experiment, no significant differences among the number of pink and gray crawlers consumed by *N. bilucernarius* were recorded ($F = 1.73$; $df = 1$; $P = 0.20$). A mean of 3.0 and 3.7 crawlers of the pink and gray mealybugs, respectively, were consumed.

Prey stage preference. Choice and no-choice studies were conducted to determine which life stages of the pink pineapple mealybug were preferred by *N. bilucernarius*. In the no-choice experiment, fresh-cut pineapple leaves were infested with 10 mealybugs of either first, second, and third instars and adult females. One day later, mealybugs were exposed for 24 hr to an adult female predator. In the choice experiment, mealybugs were exposed simultaneously in groups of three individuals from each of the four prey stages to the adult female predator. For this experiment, time of exposure and assessment of number of prey consumed were determined as in the prey preference experiments. Each treatment was replicated 20 times. Data were analyzed with ANOVA and when necessary with the Tukey mean comparison test.

When only one stage of the pink pineapple mealybug was present (no-choice experiment), *N. bilucernarius* adults preferred the smaller mealybug stages. The predator had greater preference for the first instar than for all other mealybug stages ($F = 29.7$; $df = 3$; $P < 0.001$). The mean mealybugs consumed by this predator was 4.25 individuals in the first instar, and 0.95 in the second instar. When alone, third instars and adult females were not attacked or consumed by *N. bilucernarius* adults. In the choice experiment, *N. bilucernarius* attacked all mealybug developmental stages. There was greater preference for first (Tukey HSD; $P < 0.001$) and second (Tukey HSD; $P = 0.023$) instars than for the adult stage ($F=7.35$;$df=3$;$P<0.001$).

Functional Response. The functional response of a predator mathematically describes the change in number of prey (mealybugs) eaten as the prey (mealybug) density increases. At high prey densities when prey are easy to find, the numbers of prey eaten may be limited by a number of factors including "handling time" (time required to eat prey) and predator satiation (predator has eaten as many mealybugs as possible). When handling times are long (i.e., greater than 30 minutes), the potential number of prey that can be consumed in 24 hours may be limited. Laboratory studies were conducted to determine the functional response of adult *N. bilucernarius* when reared on pink pineapple mealybug. Pineapple leaves were infested with 1, 5, 10, 25, 50 and 100 mealybugs crawlers. Each infested leaf was maintained in a 5 liter polyethylene container. A day later, an adult female *N. bilucernarius* was introduced for 24 hr and then removed. Number of mealybugs consumed were recorded. There were 10 replicates for each mealybug density. Mean numbers of consumed mealybugs were plotted against mealybug densities.

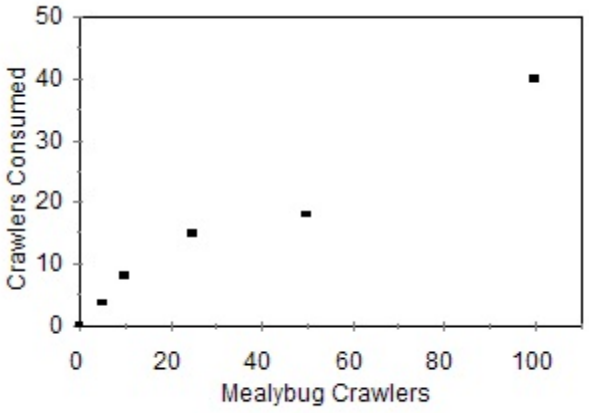

Figure 1. Functional response of the ladybug predator, *Nephus bilucernarius* as the density of the pink mealybug crawlers increases.

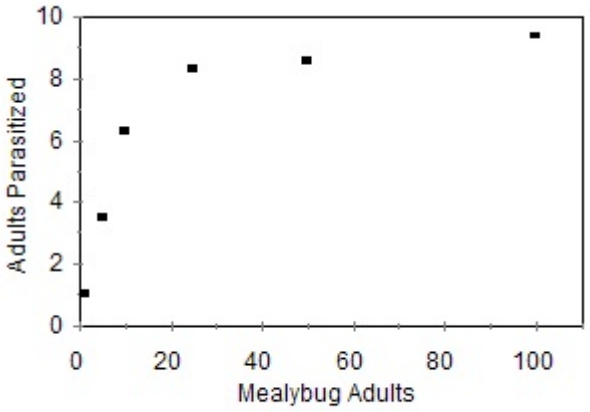

Figure 2. Functional response of the parasitoid *Anagyrus ananatis* as the density of pink mealybug adults increases.

The maximum number of mealybug prey that *N. bilucernarius* would attack is 40 mealybug crawlers in 24 hr at a prey density of 100 assuming replacement of consumed prey (Fig. 1). Comparison of the functional responses of *N. bilucernarius* and the parasitoid *A. ananatis* Gahan (Fig. 2) indicates that the predator consumes approximately 4.2-fold more mealybugs than *A. ananatis* parasitizes at 100 mealybugs per unit area. The response of *A. ananatis* plateaus at approximately 8.2 parasitized hosts at a host density of 25 mealybugs per unit area. *N. bilucernarius* starts to plateau around 100 mealybugs per unit area. However, attacks by *A. ananatis* result in reproduction for each mealybug parasitized. This is not true for the prey that *N. bilucernarius* consumes. Based on the information collected on this project, it takes approximately 15 mealybug crawlers per day for *N. bilucernarius* to produce a single egg.

The ability of the predator to find a prey could be another limiting factor on the rate of attacks. Brief observations on the searching behavior of this predator showed that it found and attacked a prey only when its maxillary palps came in close contact with a prey item. Most coccinellid predators detect prey only after physical contact.

Anagyrus ananatis. **Host species preference**. Laboratory studies were conducted to determine susceptibilities of the pink pineapple mealybugs and gray pineapple mealybugs to *A.ananatis* parasitization. Choice and no-choice experiments were conducted for this study. For the choice experiment, pineapple leaves were individually infested with five adult females of each mealybug species. In the no-choice experiment, pineapple leaves were individually infested with either 10 adult females of the pink pineapple mealybug or the gray pineapple mealybug. Infested pineapple leaves were individually kept inside a polyethylene container (5 liter capacity). One day after infesting leaves, an *A. ananatis* female and male (2-3 days old) were introduced into the container. After 24 hr, parasitoids were removed, and the mealybugs were transferred to a 37 ml polyethylene vial. Mealybugs were held in vials until they mummified or parasitoids adults emerged from parasitized mealybugs. Numbers of parasitized mealybugs from each treatment were recorded. Treatments were replicated 20 times in both sets of experiments. Data were analyzed with ANOVA and Tukey mean comparison test.

In the choice experiment, *A. ananatis* significantly preferred to attack the pink pineapple mealybug over the gray pineapple mealybug ($F = 59.2$; $df = 1$; $P < 0.001$), parasitizing 11.5 fold more individuals of the former versus the latter species. From exposed gray pineapple mealybugs, three mummies were produced, but adult parasitoids emerged from two mummies only. Similarly in no-choice tests *A. ananatis* attacked only the pink pineapple mealybug ($F = 40.9$; $df = 1$; $P < 0.001$), and did not parasitize a single gray pineapple mealybug.

Host stage preference. Laboratory studies were conducted to determine which developmental stages of the pink pineapple mealybug were susceptible to oviposition by *A. ananatis*. Choice and no-choice experiments were conducted. In choice experiments, a pineapple leaf was individually infested with 3 individuals each of pink pineapple mealybug first, second. third instars and adult females. In no-choice experiments, immature and adult mealybugs stages were exposed separately in groups of 10. Two day old adult *A. ananatis* females were used. After 24 hr exposure mealybugs and parasitoids were processed and analyzed as described above.

In the choice experiment, A. *ananatis* preferred to attack mealybug adult females, and in only one case was a mealybug nymph (second instar) parasitized. Analysis indicated that mealybug adults were preferred over all other stages ($F = 39.3$; $df = 3$; $P < 0.001$). In the no choice experiment, *A. ananatis* attacked mealybug second and third instars, and adult females with 67 % of the attacks being made on adult females. *A. ananatis* preferred to parasitize adult females the greatest followed by third instar mealybugs. Sex ratio of *A. ananatis* that emerged from parasitized third instars was 1.0: 0.6 (F: M), and from adult females was 1.0: 0.2 (F: M)

Functional response. The functional response of a parasitoid mathematically describes the change in number of hosts (mealybugs) parasitized as host (mealybug) density increases. At high host densities when hosts are easy to find, the numbers of hosts parasitized may be limited by a number of factors including "handling time" (time required to parasitize hosts). When handling times are long (i.e., greater than 30 minutes), the potential number of hosts that can be parasitized in 24 hours may be limited. Laboratory studies were conducted to determine *A. ananatis* functional response when using pink pineapple mealybug as a host. Pineapple leaves were individually infested with 1, 5, 10, 25, 50 and 100 adult mealybug females (2-5 days old). Each mealybug density was replicated 10 times. Each leaf was maintained in a 5 liter polyethylene container. Twenty-four hr later, one two-day old adult female parasitoid was introduced into the polyethylene container. Mealybugs were exposed to parasitoids for 24 hr. Exposed mealybugs were held on leaves until emergence of adult parasitoids.

The number of hosts attacked increased as mealybugs densities were increased from 1 to 25 individuals, and then plateau ed at densities above 25 individuals (Fig. 2). This observation was characteristic of the type II model of the Holling disk equation. A limiting factor that influences the final rate of host attack by the parasitoid may be related to the capacity of the parasitoid to produce eggs. Dissections of mated *A. ananatis* females, prior to exposure to suitable mealybug hosts, revealed that the maximum number of mature eggs found was 24. Observations indicate that *A. ananatis* stops ovipositing after a given number of hosts are attacked, even when it is provided with a new group of healthy, unparasitized mealybugs. Therefore, egg depletion may be a factor limiting the rate of host attack for this parasitoid and not handling time (time during which parasitoid is in contact with host).

Knowledge of the functional response of a natural enemy provides insights with regard to its effectiveness as a mortality agent for pest control. It also provides information useful in the development of mass rearing methods for augmentation programs. Information about *A. ananatis*' functional response indicates that optimal parasitization rates (70%) are at densities of 5 mealybugs per parasitoid. Thus, if one wishes to have *A. ananatis* parasitize 100 mealybugs in 24 hours, it would be best to confine about 30 parasitoids (= about 1 parasitoid per each 3.5 mealybugs) with the mealybugs. Fewer parasitoids would result in less parasitization whereas more probably would not increase parasitization rates greatly.

Objective 2

Techniques were devised to study the searching behavior of the parasitoid *Anagyrus ananatis* in the absence of ants and in their presence. Preliminary data analysis suggest that the parasitoid exhibits several behaviors typical of parasitic hymenoptera such as preening, drumming of antennae, and oviposition (stinging). The sequences of these behaviors are being determined and analyzed. Preliminary results suggest that ants interfere with the searching behavior of the parasitoids. A complete analysis will be available for the next report.

Objective 3a

Pink Mealybug Mass Rearing. Following adoption of the Kobocha pumpkin as a mealybug host, a new system was designed (Fig. 3; Ed. note: Please contact editor for a copy of Fig. 3.) to handle approximately 12 pumpkins in a rearing unit that allowed easy collection of mealybug crawlers. Three units were constructed and mealybug infested pumpkins were placed inside. The objective of the new rearing units was easier collection of the mealybug crawlers for use in reinfestation of pumpkins for mealybug rearing and provision of hosts for parasitoids. Efficient parasitoid production requires that the most optimum stage for parasitization be presented to the parasitoid in the highest numbers possible. Results of studies conducted under Objective 1 indicate that *A. ananatis* prefers to lay its eggs in 3rd instar nymphs and adults of the pink pineapple mealybug. Thus, it will be best if only these stages can be offered to the parasitoid. Mass infestation of pumpkins will permit the production of large numbers of mealybugs in the same developmental stage (3rd instars or adults) at any one time. Currently, we can collect about 200 mealybug crawlers from each rearing unit daily. Units are still not at a full capacity of 12 pumpkins per unit. Work continues on refining the techniques used to infest pumpkins with the crawlers. It appears that the crawlers are active for about 7 days before settling down to feed. This causes a problem when one wants the crawlers to settle on a given pumpkin. Work will continue on rearing mealybugs as well as the parasitoid in mass numbers (> 10,000 individuals).

Objective 3b: No results to report at this time, dependent on Objective 3a. ♢

Pineapple Pesticide Evaluation

Glenn Taniguchi, Research Associate
Scot Nelson, Asst. Plant Pathologist

Objectives:

1. Evaluate fungicides for pre-plant seed treatment.
2. Evaluate fungicides for control of post harvest fruit diseases.
3. Evaluate insecticides for control of mealybugs.
4. Evaluate insecticides/baits for control of ants.

Progress Abstract

Fruit rot trials with the fungicide Elite for control of *C. paradoxa* has shown that at 100 and 150ppm, the percent incidence was 4.4% and 2.2% respectively, compared to the standard fungicide, Bayleton of 150ppm with a percent incidence of 15 6%. Other fruit rot trials are being conducted currently with the fungicide Tilt.

Butt rot trials are also being conducted currently with Elite and Tilt. Some data has been collected but not sufficient for reporting at this time.

A re-testing of the ant bait Bushwhacker (Boric Acid) is in its final stages. At this time it appears that Bushwhacker is not able to control the Big-headed ant, *P. megacephala.* Ed. Note: Tilt was granted a label for the control of butt rot in 1994.

Accomplishments and Results:

Objective 1:

Efforts were concentrated on collecting efficacy data from the fungicides Elite (tebuconazole) and Tilt (propiconazol). These were selected as being the best source for control of butt rot

caused by *Chalara paradoxa*. Also, these two fungicides are backed with strong support by the chemical companies towards the pineapple companies to seek registration or to amend the label as need be. Tests are currently under way, though data collection incomplete at this time.

Objective 2:

The same fungicides from Objective 1 are being screened for control of fruit rot caused by *C. paradoxa*. Efficacy data are currently being obtained for Tilt. Efficacy data for Elite are complete. Tests were conducted at each pineapple plantation. Rates used were Elite at 25ppm, 50ppm, 75ppm, 100ppm, 150ppm, control and a Bayleton standard of 150ppm. Severity ratings of 0.5-4.0 (4.0 being very severe, flesh dark brown black and 0.5 being slight, flesh grayish to light brown) were used. Elite at 100ppm and 150ppm provided better control than the standard Bayleton at 150ppm. Mean severity ratings of 0.6 and 0.8 respectively were obtained compared to Bayleton score of 1.4. In terms of incidence, Elite at 100ppm and 150ppm had a mean percent incidence of 4.4% and 2.2% respectively, compared to Bayleton at 150ppm with a mean percent incidence at 15.6%.

Objective 3:

No new work. Results reported in progress report July 1, 1994-December 30, 1994.

Objective 4:

Re-testing of Bushwhacker (Boric Acid) for control of the Big-headed ant, *Pheidole megacephala* is in the final stages. Earlier testing with Amdro revealed some control. However statistically and academically it was shown to express inter-plot interference from Amdro. Presently data indicates that Bushwhacker is not effective in controlling *P. megacephala.*

Pineapple Root Rot in Hawaii: Etiology and Control

Scot Nelson, Assistant Plant Pathologist
Mr. Glenn Taniguchi, Research Associate

Objectives:

1.To determine the identity and distribution of pathogenic *Pythium* species attacking pineapple in Hawaii.

- 1a. To identify all pathogenic species
- 1b. To compare virulence, aggressiveness among species
- 1c. To determine species distribution on plantations
- 1d. To determine the effect of temperature on virulence

2. To determine the efficacy of pre-plant applications of Aliette and Ridomil for control of root rot caused by *Phytophthora* and Pythium spp.

3. To determine the efficacy of Aliette, Ridomil and Fluazinam for control of pineapple root rot caused by Pythium spp. under controlled conditions.

4. To assess the efficacy of Fluazinam for control of *Phytophthora* and *Pythium* root rot under plantation conditions.

5. To determine the optimum frequency of application of Aliette for control of pineapple root rot in Hawaii.

- 5a. To determine the rate of degradation of Aliette in pineapple crowns.
- 5b. To determine the potential for Aliette to control root rot after infection.
- 5c. To asses the effects of soil moisture and temperature upon efficacy of Aliette.

6. To determine if current levels of Aliette are causing pineapple phytotoxicity.

7. To determine if chemical plant stresses increase susceptibility to *Pythium* or other root-rotting fungi.

- 7a. To determine if the use of Ethrel during forcing increases pineapple susceptibility *to Pythium* and/or *Phytophthora* species.
- *7b.* To determine if phytotoxicity due to Aliette or use of Aliette increases pineapple susceptibility to *Pythium* species.

8. (new) To determine the comparative efficacy of Dimethomorph, Ridomil, WECO 42894 and Aliette for control of pineapple root rot caused by *Pythium* and *Phytophthora* spp. under field conditions.

Accomplishments and Results:

Objectives la and 1c.

Surveys for root pathogen distribution were continued at two pineapple plantations. A total of approximately 35 fields on the two plantations have been sampled. Five *Pythium* species have been identified as root pathogens at the Del Monte plantation at Kunia, Oahu: *P. aphanidermatum, P. arrhenomenes, P. aristosporum, P. splendens,* and *P. myriotylum*. *P. myriotylum* has not been reported previously as a pineapple root pathogen in Hawaii. The most common and widespread root rot pathogen at Maui Land and Pine is *P. arrhenomenes*. Maps of species occurrence and distribution are being produced.

A training program was established in October 1994 and is still continuing to enable employees of Maui Land and Pine to assay knockdown soil samples for the presence/absence of pathogenic *Phytophthora* and *Pythium spp.* Their personnel are becoming proficient at recovery and identification of these pathogen species.

Objectives lb and 1c.

A controlled environment plant growth chamber was purchased in October, 1994 to address these objectives but no results have been obtained yet.

Objective 2.

Block-size experiments were initiated in May and June, 1993 to accomplish this objective. Fungicides were applied as pre-plant dips in the following treatments: Aliette only, Ridomil only, and Aliette + Ridomil. Soil samples indicate that *Pythium* spp. are present. No plant death or disease symptoms were observed. Harvest of one replication was completed in December, 1994; the other replication was harvested in January, 1995. Disease symptoms were not observed in these fields during the plant crop. Preliminary analysis of the data indicated a treatment by location interaction with regard to total fruit weight. At one location (Del Monte Field 33) no significant differences were observed among treatments. However, at Field 31, significantly greater yields were obtained with the Aliette-Ridomil combination.

Objective 3.

This objective has been accomplished and reported upon in earlier progress reports. In summary, data indicate that the fungicide, Aliette, provides little to no control of root rot of

pineapple caused by *Pythium* spp. Ridomil provided little to no significant control of *Pythium* root rot under greenhouse conditions, depending on the species. Tests with Fluazinam were suspended due to its phytotoxicity to pineapple.

A field experiment was initiated in October 1994 at Maui Land and Pine to examine the efficacy of pre-plant dip and drip-tube applications of Ridomil for control of pineapple root rot caused by *Pythium arrhenomenes*. The experimental design includes replications of the study to be established at several locations which differ in temperature and amount of annual rainfall. Preliminary analysis of soil and tissue samples at two locations indicated that little plant infection had occurred, perhaps due to ineffective inoculation or an environment that was too dry. The experiments continue to be monitored. ♢

Effects of Environment on the Growth, Flowering, and Fruiting of Pineapple

Duane P. Bartholomew and Xiang-jia Min

Objectives:
1) To characterize the effects of environment on vegetative growth of pineapple and to define the minimum data set required for this characterization.
2. To characterize the effects of plant population and plant size at forcing on plant growth and yield.
3. To characterize the effects of environment on fruit development of pineapple.
4. To develop or refine computer-based models for the prediction of vegetative growth, fruit development, and yield.
5. To improve the control over the flower initiation process.
   a. To develop methods to inhibit natural flowering.
   b. To develop methods for improving control over growth regulator-induced flowering.

Progress Abstract:
Uniconazole, paclobutrazol, and 3-chlorophenoxyacetic acid (Fruitone) consistently inhibit natural flowering of pineapple in pot studies, with uniconazole and paclobutrazol being the most effective but vegetative growth inhibition is a side effect with unknown consequences.

Accomplishments and Results:
Objectives 1-4. No work was done during the period.
Objective 5.a. Ten month old potted plants were treated in October, 1994 with paclobutrazol (5 or 25 mg per plant), uniconazole (0.5 or 2.5 mg per plant), Fruitone (3-chlorophenoxyacetic acid) (0.5 or 2.5 mg per plant), and water (control). All plants in the control produced an inflorescence during February and March, 1995, approximately four months after treatment. The appearance of inflorescences on plants treated with 0.5 mg Fruitone was delayed about 30 days. Six of 8 plants treated with 5.0 mg paclobutrazol and the same number treated with 0.5 mg uniconazole produced an inflorescence but appearance was delayed about 21 days relative to the control. The other two plants in both treatments remained vegetative eight months after treatment. All plants treated with a higher concentration of compounds (2.5 mg Fruitone, 2.5 mg uniconazole, or 25 mg paclobutrazol per plant) remained vegetative eight months after treatment.

In a second experiment with potted plants, 9 of 10 plants in the control produced an inflorescence, while only 3 of 10 plants treated with 1.0 mg uniconazole per plant did. There was no difference in the time of appearance of the inflorescences among the control and plants treated with silver thiosulfate, an inhibitor of ethylene action, or aminoethoxyvinylglycine (AVG), and inhibitor of ethylene biosynthesis.

Two field experiments to test the efficacy of Fruitone and uniconazole as inhibitors of natural flowering were installed in the fall of 1994. A very low incidence of natural flowering and early forcing of the field on one plantation made it impossible to collect meaningful results.

The detailed results are part of the Ph.D. dissertation Physiological Effects of Environmental Factors and Growth Regulators on Floral Initiation and Development of Pineapple [*Ananas comosus* (L.) Merr.], recently completed by Dr. Xiang-jia Min.
Objective 5.b. No work was done on this objective.

Life of Project Summary
Objective 1.

Research on characterizing the effects of environment on vegetative growth of pineapple made it clear that the subject is very complex. At the outset, it was assumed that quantifying the relationships between environmental factors, particularly temperature, and vegetative growth would make it possible to develop a pineapple growth simulation model that could be used to predict vegetative growth in a particular environment. Because there is a high correlation between plant weight at forcing and fruit weight at harvest within a given location (environment), it was assumed that it would then be possible to predict average fruit weight and yield. However, even in the absence of stresses (water, pests, diseases), average fruit weight per plant varies with season to an unknown extent. This variation does not invalidate the correlation between plant weight and fruit weight, but it makes it less valuable for prediction because of the relatively large amount of variation. Detailed data are costly to collect and, with a long-term crop such as pineapple, many things can cause additional variation in average fruit weight, which further complicates the data collection process. Systematic date-of-planting and flowering induction studies in Australia confirm the relatively poor predictability of average fruit weight and yield. In those studies, poor predictability resulted both from seasonal changes in the relative productivity of the plants (plants of a given size produce larger fruit at one harvest date than at another with no obvious explanation for the variation), and from variation in the success in forcing flowering; e.g. when flowering was forced on very warm days, average fruit size was smaller than when plants were forced a few days earlier or later when conditions for forcing were much better. The intricacies of these interrelationships have not yet been adequately characterized.

The minimum environmental data set required to characterize the vegetative growth response of pineapple to environment is total daily solar radiation, and maximum and minimum air temperature. Work by E. Malézieux on the simulation model ALOHA Pineapple made it clear that rainfall data also would be

required if the crop is not irrigated. Plant data include total plant green leaf area and leaf, stem, peduncle, fruit and crown total fresh and dry weight. For reproductive development, it appears likely that some measure of diurnal wind speed may be required. Wind during the day appears to be important in cooling the fruit and thus preventing sunburn. Wind may also cool the fruit sufficiently to delay development but the extent of this effect has not been characterized. Additional studies are required to determine the full significance of wind.

Objective 2.

A study of the effects of plant population and plant size at forcing on plant growth and fruit yield was especially designed to determine if the efficiency of the pineapple plant in producing fruit was altered as plants of a given size were crowded more closely together. Data on plant growth collected systematically during that study showed that plant weight decreased as density increased.

Fruit yield per plant also decreased as density increased while yield per unit area increased with increasing density. While the fruit yield results were not unique, new data on dry matter distribution in pineapple, which are essential to characterization of the effects of density on plant development, were collected. The fruit weight/plant weight ratio, a measure of the efficiency of a plant in producing a fruit, did not change with density. Because plant growth in that experiment was thought to be below normal, a second density experiment was planted in a Del Monte field near Kunia Camp in August of 1991. The results of that trial are contrasted with those of the earlier study in Table 1.

Table 1. Effect of plant population density on pineapple plant growth and fruit yield.

Planting date	PPD†	LAI	Plant weight	Fruit weight	Fruit yield	Ratio
			-kg-	-kg-	Mg/ha	
8/22/91	3.9	8.07	0.85	2.96	115.8	0.53
	7.8	9.32	0.43	1.87	145.7	0.64
	11.7	9.75	0.30	1.28	149.4	0.68
6/15/89	2.6	4.54	0.89	2.12	55.3	0.59
	5.2	5.85	0.50	1.53	79.9	0.52
	7.8	7.63	0.39	1.33	104.1	0.52
	10.1	8.15	0.31	1.23	124.2	0.63
	12.8	9.52	0.27	1.10	140.2	0.55
8/15/89	2.6	4.03	0.67	2.03	52.7	0.61
	5.2	5.53	0.44	1.48	77.2	0.57
	7.8	6.65	0.30	1.17	91.5	0.58
	10.1	6.70	0.23	0.98	98.5	0.53
	12.8	7.30	0.20	0.70	90.3	0.61

†PPD, plants per m^2; LAI is m^2 leaves per m^2 ground area; Plant weight, dry weight basis; Fruit weight and yield (Mg = megagrams) on a fresh weight basis; Ratio, fruit weight/plant weight ratio, dry basis. Plants planted in 1991 were forced on August 1992; plants planted in 1989 were forced on September 1990.

Mean date to 50% fruit harvest was significantly later at high plant populations densities than at lower ones. Variability among plants in the main experiment, which was attributed to poor land preparation, precluded the collection of meaningful data for the ratoon crop. Observations made on sucker growth indicate that initiation and growth of suckers for ratoon crop production was delayed at the higher densities. It would be expected that the crop cycle would be delayed at the higher densities because of delayed harvesting and sucker growth.

Objective 3.

Water stress, water excess, and a night temperature of 30 °C all decrease average fruitlet number and average fruit weight of pineapple. The mean number of fruitlets per fruit on plants grown at a 20 °C night temperature and transferred to a 30 °C night temperature on the day of forcing was significantly greater than the fruitlets per fruit on plants grown at 30 °C, but significantly less than the number of fruitlets per fruit on plants grown at a night temperature of 20 °C. Water excess and water deficit stress and a 30 °C night temperature all significantly reduced leaf titratable acidity in the early morning, indicating that they reduce CO_2 assimilation, and ultimately, photosynthesis. Neither water excess nor water deficit stress induced natural flowering of pineapple, showing that water is not a major factor in natural flowering of pineapple. Water deficit stress reduced plant susceptibility to forcing with ethephon, perhaps due to the rapid onset and extreme level of stress that occurred in these potted plants.

Objective 4.

A computer simulation model, ALOHA Pineapple, was completed and incorporated into the decision support aid (DSSAT) produced by International Benchmark Sites Network for Agrotechnology Transfer (IBSNAT). The model can simulate pineapple vegetative growth, fruit growth, estimate fruit harvest date, and predict fruit yield. A water balance submodel is included in the model for use when the crop is grown with natural rainfall. The model needs further testing in real-world situations, so further evaluation is needed. Because quantitative information on how environmental factors influence fruit growth is quite limited, fruit harvest date prediction needs to be re-tuned for local conditions. With some modest revision, ALOHA Pineapple has the potential to be used on farms and plantations to predict fruit harvest date with greater accuracy than historical records, the current practice on most farms.

Objective 5.

Among the chemicals tested for their potential to delay or inhibit pineapple flowering, uniconazole, paclobutrazol and Fruitone delayed or inhibited pineapple flowering, while aminooxyacetic acid, aminoethoxyvinylglycine, silver thiosulfate, and daminozide did not. Fruitone, a synthetic auxin, stimulated ethylene production, but the mechanism by which it delayed flowering is not known. Uniconazole and paclobutrazol, growth retardants that inhibit gibberellin biosynthesis, inhibited leaf elongation and decreased leaf area. The activity of uniconazole in the inhibition of vegetative growth and flowering was approximately ten times stronger than paclobutrazol. Uniconazole and paclobutrazol significantly inhibited ethylene production by and aminocyclopropane-1-carboxylic acid oxidase activity of leaf tissue. Decreased ethylene production by leaf tissue could be one factor responsible for delayed flowering in treated plants. Based on the results of this research, further field evaluation of the effectiveness of uniconazole and paclobutrazol

in delaying or inhibiting natural flowering of pineapple seems warranted. Uniconazole, paclobutrazol, and Fruitone all can inhibit natural flowering of pineapple by some unknown mechanism. Field studies need to be done but the chances of success are limited where natural flowering in unpredictable.

Genetic Engineering of Pineapple

Robert Paull

A collaborative research project was recently established, involving the Hawaii Sugar Planters' Association, the University of Hawaii at Manoa - College of Tropical Agriculture and Human Resources, the Pineapple Growers Association of Hawaii, and the United States Department of Agriculture, Agricultural Research Service. The purpose of this collaboration is to develop methods for the genetic engineering of pineapple and then to improve pineapple by transforming it with agronomically useful genes (virus and nematode resistance, flowering control, fruit quality). Early goals of this project are to establish reliable tissue culture procedures and to develop efficient transformation protocols. Excised axillary buds from field-grown materials, have been established in tissue culture and are being manipulated hormonally for the production of either globular "protocorm-like bodies", or rapid shoot multiplication and rooting of the shoots. These tissues are being used to optimize biolistic gene gun parameters, to study the effect of different promoters regulating the expression of the GUS reporter gene, and to establish the selection system for transgenic plants.

References

This section contains references published since the last newsletter. Please help keep this section up-to-date by sending information on new publications or copies of those publications to the address given on page 1 of the newsletter. I regret that I have neither the time or resources to provide copies of the listed references. In some cases, reprints can be obtained by writing directly to the author(s). Please contact me if you are unable to locate the author's address as I may have it in the Pineapple News mailing list. Another source, though an expensive one, is:
Library External Services
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The charge is $14.00 plus postage for the first 20 pages and $0.25 per page over 20 pages.

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Directory of Professionals

If you wish to have your name listed here in a future issue, please send your name, address, and a brief resume. This listing is primarily for the convenience of those seeking the assistance of professionals with experience in one or more aspects of pineapple production and processing.

Brazil

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