2004

HARC

# Hawaii Agriculture Research Center

ANNUAL REPORT

# On the Inside



**Front cover images, clockwise, from top right corner:**

(1) The Experiment Station (Exp. Sta.) in Aiea, Hawaii from the west
(2) The Exp. Sta. from the north, with brown Aloha Stadium in background
(3) Ann Marsteller (Librarian) in the Exp. Station's reading room
(4) The Exp. Sta. from the north-east, with Pearl Harbor in the background
(5) Front of the 4-story Exp. Sta. from the north-west
(6) The Exp. Sta. from the south-west, constructed in 1976

**Background Image:**

Cultivars of Hawaiian sugarcane (*Saccharum* spp.) - photo courtesy of Maui Nui Botanical Gardens, Kahului, Maui


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# Director's Letter

The year 2004 was the 110th year of continuous research and service performed by the Hawaii Agriculture Research Center's staff. The work reported herein typifies the dedication and commitment to excellence demonstrated by our employees who continue to strive for a bright future for agriculture in Hawaii.

In 2004, the industry harvested 21,790 acres and produced 257,866 tons of raw sugar. The average tons sugar per acre was 11.83, a 10% decrease from the 13.15 tons sugar per acre produced in 2003. This is the first drop below 12 TSA for the industry since it consolidated around the 2 highest producing plantations. Wet weather with its negative impact on harvesting and sugar recovery and an increased disease incidence were considered significant contributors to this decline. An increased incidence of leaf scald, changes in herbicide practices and seed treatment and poor root development are being evaluated as well as variety reassessment.

This year's annual report includes progress on the development of a new antibody preparation against the Sugarcane Yellow Leaf Virus (SCYLV), use of the P0 gene from the SCYLV as a viral suppressor of post-transcriptional gene silencing and expression of biologically active proteins. Breeding and selection continued producing high yield potential varieties for Hawaii and other locations.

The recently discovered primitive Y chromosome in papaya received significant recognition by scientists interested in sex chromosome evolution as it shows only moderate degeneration and suppression for recombination and possibly holds the key to the origin of plant XY chromosome system. One of the regulatory genes for flowering in papaya has been cloned and characterized and others are in progress. Efforts continue to focus on developing tools to improve disease resistance in new papaya varieties and to contribute to a deeper understanding of plant disease resistance mechanisms. Data obtained on novel papaya disease defense genes demonstrated that expressing the *DmAMP*1 gene in papaya plants increases resistance against *Phytophthora palmivora* and that this increased resistance is associated with reduced hyphae growth of *P. palmivora* at the infection sites.

We transferred the first selected seedlings from our coffee breeding program to a commercial field for yield testing. Other sites have been identified and will also be receiving selected plants for field evaluation and cup quality evaluation.

There are reports that mortality in Hawaii's premier hardwood tree, *Acacia koa,* caused by *Fusarium oxysporum* f. sp. *koae* is occurring in natural stands at higher elevation as well as at low elevation. The origin of the pathogen is currently unknown. At Maunawili, Oahu, of the thirty Acacia koa families (seed sources) tested, survival ranged from a low 4.0% to a high 91.6% at 48 months. Family variation in *A. koa* in field trials strongly suggests the presence of resistance.

Collaboration with the USDA Pacific Basin Agricultural Research Center scientists located at HARC and scientists in China began on the molecular characterization of lychee to more accurately identify genetic relationships among the many cultivars.

Optimizing transformation procedures for several tropical crops continues to ensure the availability of genetically engineered systems should the recombinant DNA process be needed for problem solving or value enhancement in the future.

While successful in selecting a Hawaii fungus providing significant degradation of PCBs in liquid culture, attempts to demonstrate this activity in soil tests were not successful.

During this year, I completed 10 years as director of HARC. It has been a pleasure to be associated with the dedicated members of HARC's Board who tirelessly struggle to maintain a viable sugar industry, with the loyal staff and employees of HARC who are committed to problem solving and value creation, and with all our continuing and new partners working to make agriculture successful in Hawaii. I want to thank all of you for helping Hawaii Agriculture Research Center ensure its future in the twenty first century.

Major efforts are focused on the molecular biology of papaya including the cloning and characterization of flower development, investigating systemic acquired resistance and disease and pest resistance through gene insertion, and discovering a primitive Y chromosome.

For coffee, a genetic map was constructed, transgenic nematode-resistant plants are being developed and improved quality is targeted both in the breeding and selection work as well as in shading effects.

A vascular wilt disease was identified as the cause of dieoff of koa trees. This could be of very significant concern as it has been found on all the islands and at many elevations. Our prior field trials with koa suggest that there is some resistance within the population. Before initiating future field trials, seedlings will be screened for resistance to support our goal to select the best seed sources for the expansion of this high-value, uniquely Hawaiian crop.

The agronomic work in taro in the past years was followed this year with the first report of successfully inserting a useful gene into taro demonstrating that this technology can improve taro's resistance to diseases. Also in taro, a botanical extract was found to successfully control an extremely economically damaging pest, the apple snail.

Contract services have become a small but important part of our goal to develop new agricultural businesses or bring them to the state. We are proud that this year the potato certification business we have been building for 5 years was successful and passed on to farmers making room for us to develop and nurture another.

In this day and age when few people know where their food comes from, outreach is taken seriously by all the staff. They participate through assisting in the State Science Fair, the island farm fairs, visitor presentation and facility tours and schools and business meeting presentations. I am deeply appreciative of their volunteered time in this area and in their flexibility in the continuing transition both internally to this organization and externally in the agricultural community.

I also want to thank the sugarcane industry for its continuing support and commitment to research, to other commodity groups we are working with and to our clients on the mainland and other parts of the world.

Respectfully,

Stephanie A. Whalen
President and Director

# 100 Years Ago: 1904

Results of a survey to 45 plantation managers on keeping the Experiment Station, HSPA. Twenty-six were in favor of continuing the Station, 10 in favor of abolishing it, 4 in favor of a Hilo branch, 2 had no opinion, 3 had not replied.

At that time, the Experiment Station operated three separate divisions, each with its own director and staff. There was a Division of Agriculture and Chemistry, a Division of Entomology (formed in response to the serious damage caused by the sugarcane leafhopper), and a Division of Pathology and Physiology (organized in response to the growth failure of "Lahaina" cane variety).

Land that had been leased was purchased and a new building erected (see photo). Two substations (Waiakea and Laupahoehoe) were inaugurated to provide areas of agricultural experimentation in locations more representative of some plantations.

Crop losses of 48, 7, and 19 thousand tons of sugar from Hawaii, Maui, and Oahu, respectively, were primarily attributed to the leaf hopper.

The Waikiki Aquarium, the third oldest public aquarium in the US, was founded.

The construction of a can-making plant and a cannery in Haiku, Maui heralded that island's first pineapple cannery operations.

Mr. M.M. O'Shaugnessy began the construction of the Koolau Ditch to bring water from Honokane Stream, at 1,030 feet above sea level, 22 miles west to Hikapoloa. It passed intermittently through 17 miles of tunnels dug into the mountain, mostly by Japanese immigrant workers. Between 1904 and 1905, the old Hamakua Ditch was abandoned and replaced by the New Hamakua Ditch.

For about 1 week, Japanese sugarcane plantation workers engaged in the first organized strike in Hawaii.

*Building erected in 1904 to house the Agriculture and Entomology departments*

Ralph Hosmer was hired as the Territory of Hawaii's first forester. The first forest reserves were created to protect upper watershed areas by fencing, feral animal elimination, and reforestation with native and exotic tree species.

Wahiawa, then known as "The City of Pines", was considered the hub of the pineapple industry in the world.

In comparison to the 20 cars in Honolulu, the number of riders on the new electric streetcars averaged 18,327 per day.

The first motion pictures were shown in Hawaii.

- Blake Vance

# Sugarcane Research

## *Genetic Diversity in Native Hawaiian Saccharum officinarum*

Commercial sugarcane hybrid cultivars currently in production are high yielding, disease resistant, millable canes and are the result of years of breeding. In Hawaii, these commercial hybrids are quite distinct from the Saccharum officinarum canes that were brought to the islands by the ancient Polynesians and propagated for use as sweet juice and for chewing. The canes themselves are of varied bright colors, often with stripes. Perhaps for this reason they have been maintained in household gardens around the islands. The flowers are infertile, which precluded their use in the original Hawaiian commercial cultivar development. A collection of the extant Hawaiian "noble canes" was made by Moir (1932) and much of this collection is still available for study at the HARC sugarcane breeding station.

A project was initiated to establish the relationships among the presumed native *S. officinarum* canes with Hawaiian names and their possible genetic relationships to other *Saccharum* species. The taxonomy and speciation within the genus *Saccharum* is complex and uncertain. Most authors agree that *S. spontaneum* and *S. robustum* are ancestral wild species and that the whole group *(S. spontaneum, S. robustum, S. officinarum, S. barberi and S. sinensis)* is interfertile (Berding and Roach, 1987; Irvine 1999). Chromosome number and ploidy vary greatly and there is much overlap between them.

A total of 43 Hawaiian S. *officinarum* accessions and three later *S. officinarum* imports (Yellow Caledonia, Lahaina and Kokea) were collected for DNA fingerprinting, along with two early *Saccharum* hybrids (H52 and H109, circa 1925), five current commercial hybrids, and one sample each from *S. robustum* and *S. spontaneum* added as outgroups. DNA was extracted from leaf blade tissue. Each sample was digested with *Eco*RI and *Hind*III. About 7.5 g of DNA per lane were run on a gel. Southern blotting, radioactive labeling and autoradiography were as described (Chittenden et al. 1994). Thirty-eight genomic and cDNA probes derived from sugarcane and sorghum were selected for fingerprinting. For each probe, the polymorphic restriction fragments found from all varieties were numbered from high to low molecular weight. Each fragment (marker) was scored as present or absent. The data were formatted for the NTSYSpc cluster analysis software. The RFLP marker data were used to compute pairwise Dice coefficients (Dice 1945). Cluster analysis was performed on the similarity matrix using the "unweighted pair group method using arithmetic means" (UPGMA) algorithm.

*Native Hawaiian sugarcane 'Mahaiula'*

A phenogram (see figure, page 8) based on simple matching similarity coefficients was constructed. The cophenetic correlation coefficient r = 0.95. The results showed seven clusters with two or more closely related

accessions which could be distinguished from the outgroups samples. A cluster was defined as sharing 90% or more identical markers. One cluster of very closely related clones, all with historic Hawaiian names, can be seen at the top of the diagram. All of the names within this cluster are cited in one or more of the references to be of native Hawaiian origin or to be mutants of them. Only the names Kalaoa, Keauhou and Koula are not mentioned by any of the early authors. It is possible that cluster I represents a series of somatic mutants from a single original introduction by the ancient immigrants to the Hawaiian islands, as Mangelsdorf (1956) suggested. Some of the other canes that were thought to be of native Hawaiian origin shared less than 80% of the markers with the above-mentioned cluster. Whether we still have the correct canes so named or have other misnamed canes is unknown. Our Kokea sample is so different that it may not even be *S. officinarum*.

Other than the core group, there is quite a diversity among the Hawaiian clones, indicating that they represent many different introductions. As expected, the known foreign imports of *S. officinarum,* Lahaina and Yellow Caledonia, as well as the early commercial hybrids H52 and H109, share less than 80% of their markers with the core cluster. Lahaina and H109 share nearly 80% of their markers and are known to be parent and progeny. The native Hawaiian canes are seen to be a clearly distinct group from the modern commercial cultivars. It is also clear from the dendrogram that *S. officinarum* is more closely related to the *S. robustum* clone than it is to the *S. spontaneum* clone tested in this study.

*- S. Schenck, M.W. Crepeau, K. K. Wu, P. H. Moore (USDA ARS PBARC), Q. Yu and R. Ming*

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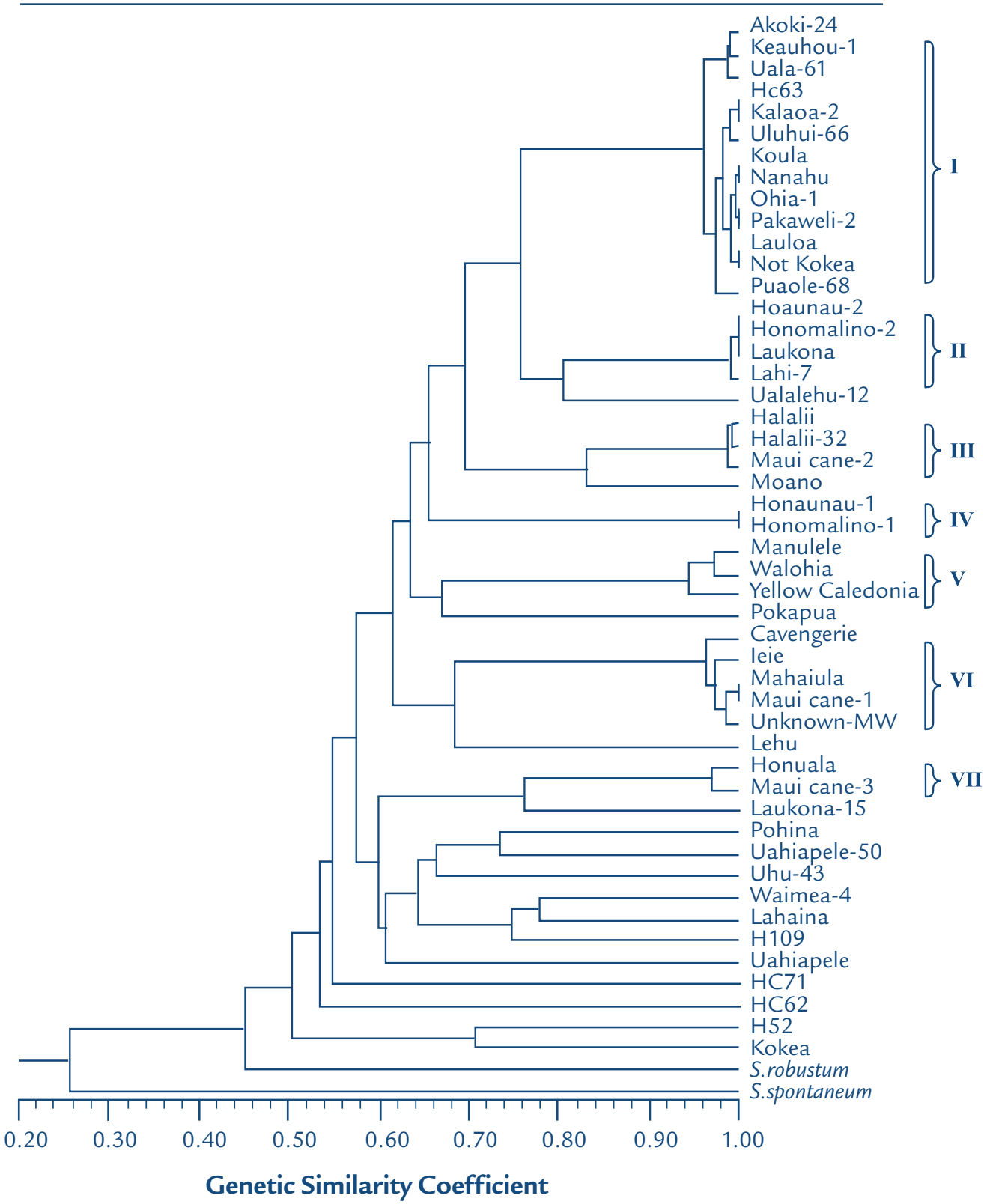
*Moir, W. W. G. 1932. The native Hawaiian canes. Bulletin 7. Proc. IV Congr. ISSCT: 1-8.*


Akoki-24
Keauhou-1
Uala-61
Hc63
Kalaoa-2
Uluhui-66
Koula
Nanahu
Ohia-1
Pakaweli-2
Lauloa
Not Kokea
Puaole-68
Hoaunau-2
Honomalino-2
Laukona
Lahi-7
Ualalehu-12
Halalii
Halalii-32
Maui cane-2
Moano
Honaunau-1
Honomalino-1
Manulele
Walohia
Yellow Caledonia
Pokapua
Cavengerie
Ieie
Mahaiula
Maui cane-1
Unknown-MW
Lehu
Honuala
Maui cane-3
Laukona-15
Pohina
Uahiapele-50
Uhu-43
Waimea-4
Lahaina
H109
Uahiapele
HC71
HC62
H52
Kokea
S.robustum
S.spontaneum
I
II
III
IV
V
VI
VII
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Genetic Similarity Coefficient

## Development of a new antibody against Sugarcane Yellow Leaf Virus.

Ongoing research with the Sugarcane Yellow Leaf Virus (SCYLV) and screening of commercial sugarcanes requires use of a tissue-blot immunoassay with an antibody specific for SCYLV. An effective antibody was produced from purified whole virus preparations by Dr. B.E.L. Lockhart and S. M. Scagliusi (Scagliusi and Lockhart, 2000). Over time, most of the antibody allotted to Hawaii has been used up and more was needed. Purification of SCYLV in sufficient quantity is very difficult because it exists in very low titre in infected plants. The virus is a polerovirus and is phloem limited which makes isolation and purification difficult in addition to the low titre. Impure preparations result in production of antibodies with background reactions to sugarcane tissue which interferes with the tissue-blot reactions. Production of antibody using cloned virus coat protein polypeptide does not necessarily react to the epitopes on the surface of whole virus particles deposited on tissue blots. Therefore, a new approach was tested.

Some guesswork led to identification of several possible short peptides that might prove to be the epitopes on the virus coat protein surface. These were injected into rabbits and the subsequent bleeds produced one that had an antibody active against SCYLV particles and did not react to uninfected sugarcane blots. This antibody is now being tested for reactivity to SCYLV isolates from locations other than Hawaii and for specificity to SCYLV as opposed to other related viruses.

*- M-L. Wang, S. Schenck and H. Albert (USDA ARS PBARC)*

Reference:

*Scagliusi, S. M. and B. E. L. Lockhart. 2000. Transmission, characterization, and serology of a luteovirus associated with yellow leaf syndrome of sugarcane. Phytopathology 90:120-124.*

## Developing Transgenic Sugarcane for SCYLV Resistance

Sugarcane Yellow Leaf Virus (SCYLV) infections in Hawaii have resulted in yield losses in susceptible cultivars. Virus infection is still present and widespread and has been shown to be transmitted by certain common aphid species. Infection within the plant spreads first to the juvenile, growing plant parts and young leaves and is eventually found throughout the entire plant. The virus also spreads by planting infected seed pieces. In infected commercial cultivars, SCYLV leaf yellowing symptoms usually appear under certain environmental stress conditions, especially cool temperatures and drought. However, preliminary tests in Hawaii and Louisiana indicated that the infection is harmful to the plants' performance even when the plants are symptomless. The reduction in growth rate, tillering and sucker formation appears to be greater in young plants. Since all plants of the susceptible cultivars in plantation fields contain the virus, no clear-cut comparison of infected and virus-free plants of the same cultivar at the same location have so far been possible. Recently, through meristem tip culture, virus-free plants of susceptible cultivars were produced and are ready for comparative studies. Extensive field tests with four important commercial varieties are planned to evaluate the effect of SCYLV infection on growth, biomass production and sugar yield.

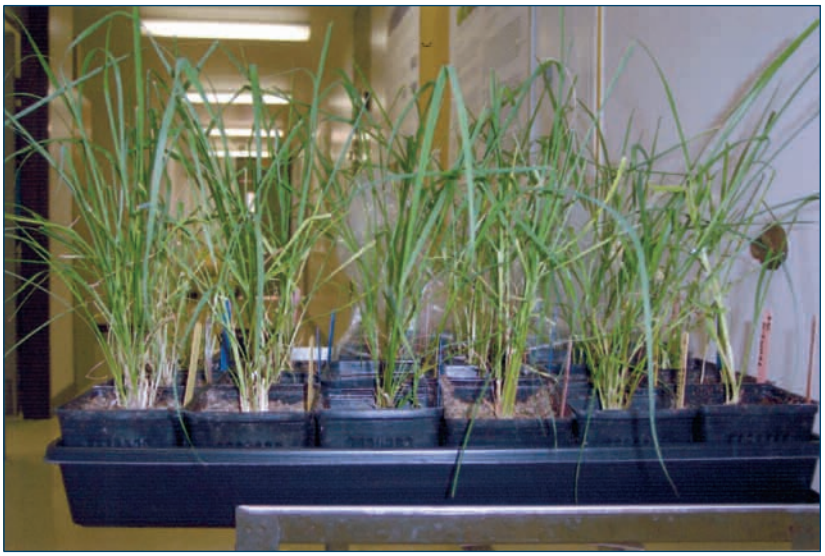
This project is intended to demonstrate that transgenic sugarcane with an untranslatable SCYLV coat protein gene can improve sugarcane resistance. Gene silencing seems to be a universal mechanism for plant resistance to viral infection and post-transcriptional gene silencing has already been achieved in

sugarcane by transformation with an untranslatable DNA sequence of Sorghum Mosaic Virus. A similar approach was attempted in this project with SCYLV. The SCYLV genome and the functions of the viral proteins are known so the strategy for production of a SCYLV-resistant cane will be straightforward. Sugarcane cultivar H65-4671 has been transformed with a non-translatable DNA sequence piece of the viral coat protein which should lead to gene silencing of viral RNA upon infection. Sugarcane seems to have a very powerful gene silencing system and the method appears likely to be successful.

About 12 independent transgenic lines (see figure) derived from variety H62-4671 were produced and PCR analysis has been carried out to verify the presence of coat protein gene and selectable marker gene, *NPT*II. A virus challenge assay to test the resistance level has been carried out on the greenhouse-grown transgenic lines. About 30 plants were inoculated with viruliferous aphids and plants were left in the greenhouse until new leaves developed. A tissue blot immunoassay was used to detect the virus in the inoculated plants. Southern blot analyses showed the PCR positive plants to contain from 1 to 10 copies of the *SCYLVcp* transgene based on the intensity of bands, which further confirmed the transgene integration.

The transgenic plants inoculated with virus were scored for the presence of virus, compared to uninoculated controls. The parent line H62-4671 plants were negative for virus before the test and all became positive for virus after the inoculation in three separate experiments, indicating that the parent line is highly susceptible for virus and that the aphids transmitted the virus. The resistance level from seven transgenic lines varied from complete resistance to slight resistance. The control transgenic plants, containing *NPT*II selectable marker only, also tested positive in most of the blots, indicating that *NPT*II transgene does not improve plant resistance to virus. H65-7052, another Hawaii cultivar, tested positive for the presence of virus in only some of the tests, possibly indicating a mild tolerance to virus.

*- Y. J. Zhu, H. McCafferty, G. Osterman, C. Moritomo, R. Agbayani, A. Lehrer, S. Schenck and P. Moore (USDA ARS PBARC)*



*Transgenic sugarcane plants with SCYLV coat protein gene are propagated for virus challenging experiment.*

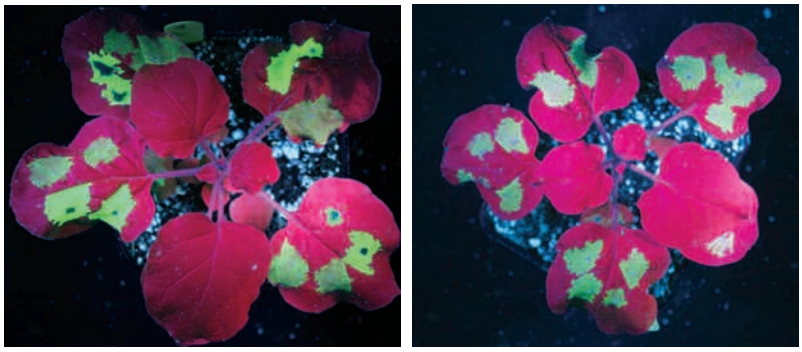
## *Mechanisms of Plant Gene Silencing and Viral Countermechanisms*

Gene silencing, including post-transcriptional gene silencing (PTGS), is an essential gene regulation mechanism which exists in plants, animals and all higher organisms. This process is important for regulation of endogenous genes and in plants, PTGS is an important defense mechanism against viral infection. Plants engineered for virus resistance use PTGS of a virus gene inserted in the plant genome to protect against that virus. Although there are many useful applications of PTGS, this process can create a major problem when trying to engineer plants for high level expression of a transgene.

We are studying the mechanisms of PTGS with one goal being to understand the basic biology of this important system. Our study of viral suppressors of PTGS is currently focused on the P0 gene of Sugarcane Yellow Leaf Virus (SCYLV). We previously tested this gene in transient expression experiments in corn and have now used it in the model plant *Nicotiana benthamiana*. Our experiments show that when introduced simultaneously with a silencing trigger, P0 can block local silencing of both transiently introduced (see figure) and stably integrated transgenes. Experiments to determine its effect on systemic silencing are currently underway.

Viral suppressors of PTGS offer a system which might be used to protect expression of transgenes; the challenge is to find a suppressor which protects against PTGS of a transgene without interfering with PTGS regulation of endogenous plant genes. This type of gene regulation is essential for normal plant development and growth. We have introduced the P0 suppressor of SCYLV and the Hc-Pro suppressor from Sorghum Mosaic Virus (which infects sugarcane) along with a transgene encoding a high-value recombinant protein. The resulting plants will be analyzed for accumulation of the high-value protein and for possible alterations in growth and development.

*- T. Mangwende, M-L. Wang, S. Ancheta, T. E. Mirkov (Texas A&M), P. H. Moore (USDA ARS PBARC) and H. Albert (USDA ARS PBARC)*



*(A)* *(B)*

**SCYLV P0 acts to suppress PTGS of transiently introduced Green Fluorescent Protein. A.** *Leaves of Nicotiana benthamiana were agrobacterium infiltrated with a Green Fluorescent Protein (GFP) expression construct (left leaf half) and GFP plus P0 expression constructs (right leaf half). After 3 days, GFP expression diminishes due to PTGS; where P0 was included in the infiltration, PTGS is suppressed and GFP fluorescence remains bright.* **B.** *Leaves were agrobacterium infiltrated with a GFP expression construct (left leaf half) and GFP plus a mutant (deletion) P0 expression construct (right leaf half). Unlike the full-length P0, the deletion mutant does not suppress PTGS.*

## *Breeding, Selection and Yield*

The sugarcane breeding season began on December 1, 2003 and was completed in the first week of January 2004. A total of 42 biparental and 495 polycrosses were made using more than 1,600 tassels. Seed harvesting was finished in February 2004.

During 2004, 56,640 seedlings were raised from the harvested seeds and transplanted in an FT1 trial located in Maui; about 52,000 plants in 2003-FT1 trial were cut and ratooned; 2,878 ratoon plants were selected from 2002-FT1 ratoon trial and their stalk cuttings, the clones, were planted in FT4 (2004-FT4); 577 selected clones from 2003-FT4 will be advanced to FT5, FT6 and FT7 in the future.

In 2004, 9 FT5, 9 FT6 and 22 FT7 tests were installed; 300 clones in 19 2002-FT7 tests were harvested, analyzed, and results were evaluated, of which 103 were identified as promising for further FT7 tests.

Cultivars with commercial potential identified are: H93-4068 and H93-4398 for Kauai; H83-7061, H87-5794 and H95-4655 for Maui. By the end of 2004, 633 acres of H87-5794 had been grown for more than 12 months while 235 acres of H83-7061 had been grown for less than 12 months. They will be harvested in 2005 and 2006, respectively.

From commercial fields, crops in 21,790 acres were harvested and processed during 2004. Six major cultivars were harvested: H65-7052, H77-4643, H78-3567, H78-4153, H78-7750 and H87-4319. They produced a total of 1,978,821 tons of net cane (or 90.81 tons cane per acre) and 257,866 tons of 96DA sugar (or 11.83 tons of sugar per acre). The top 4 cultivars in percent of total harvested area were H78-7750 (52.5%), H77-4643 (19.5%), H65-7052 (14.8%) and H78-3567 (9.0%).

At the end of 2004, 37,756 acres of crops were standing in commercial fields at various ages and are scheduled for 2005 and 2006 harvesting. The top 4 cultivars in percent of total cane area were H78-7750 (41.7%), H65-7052 (26.3%), H77-4643 (14.7%) and H78-3567 (10.0%). H65-7052 gained more acreage in 2004 and ranked second.

*- K. K. Wu*

# Tropical Fruit

## *Papaya SUPERMAN Ortholog Expressed Differentially in Dioecious Flowers*

Although all three sex forms found in papaya are genetically determined, the phenotypic sex expression of papaya is influenced by environmental factors, including temperature, nutritional status and moisture. Instability of papaya flower sex expression is common, and sex reversal occurs in all three sex forms of papaya flowers, especially in hermaphrodite and male flowers (see figure). The instability of papaya flowers can result in unmarketable, malformed fruit. Cloning major genes controlling flower development in papaya is the first step towards solving this problem. According to the widely accepted "ABC" model, "B" function genes are required for both petal and stamen formation. The *SUPERMAN (SUP)* gene is reported to be an upstream negative regulator of the two Arabidopsis "B" class genes *APETALLA3* and *PISTILLATA*. We have cloned and characterized the regulatory gene in papaya, *PSUP*.

The floral regulatory gene *SUP* is reported to act in the third whorl to establish a boundary between stamen and carpel by regulating cell division. The role of *SUP* in dioecious flowers is unknown. At the protein level, the papaya *SUP* ortholog, *PSUP* shares 43% identity and 52% similarity with the *Arabidopsis thaliana SUP (AtSUP)* protein, as well as 40% identity and 50% similarity to the *Petunia hybrida SUP (PhSUP1)* protein. Both the *AtSUP* gene and the *PSUP* gene encode for a $C_2H_2$ zinc finger protein. All zinc finger proteins contain a conserved zinc finger motif that acts as a DNA binding domain. Although the overall amino acid sequence identity between *PSUP* and *AtSUP* is low, their zinc finger domains and C-terminal regions are highly conserved. Within the zinc finger and flanking regions, *PSUP* shares over 97% amino acid sequence identity (only one amino acid difference) with *AtSUP*. Because the *PSUP* protein contains a zinc finger motif, which is a common motif in many different genes, initial BAC Southern analysis showed *PSUP* to have 4 different copies of different sizes. Upon closer analysis, *PSUP* was found to have only one copy in the papaya genome.

Functional analysis of the *PSUP* has been studied using quantitative RT PCR and in situ hybridization. Expression data generated by quantitative RT PCR showed a very high level of expression in female floral tissue over both male and hermaphrodite tissue. In female flowers, *PSUP* was expressed at a level sixteen fold higher than in male flowers and four fold higher than in hermaphrodite flowers.

In Situ results showed that the gene is being expressed in all three sex forms in Kapoho, SunUp and Drew genotypes. The gene appears to be expressed differently at various flower development stages from very young to mature but unopened. The very young flower buds appear to have strong expression all along the top of the primordia, whereas the expression in the older flower buds is weaker and concentrated in the stamens.

*- C. M. Ackerman, Q. Yu, P. H. Moore (USDA ARS PBARC), R. E. Paull (Univ. HI), D. L. Steiger and R. Ming*

*Male papaya flower*

## *Systemic Acquired Resistance (SAR) in Papaya*

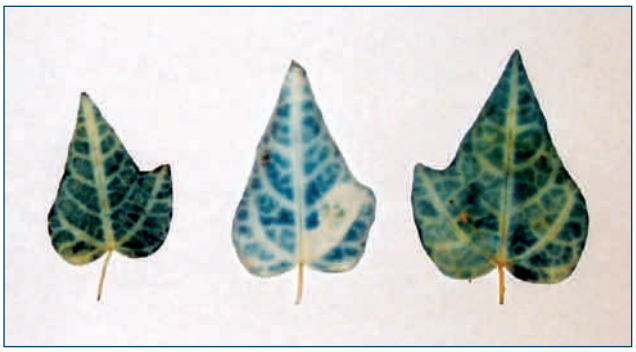
Plants have natural defense responses against pathogens which can be induced by avirulent pathogens. An attack by one avirulent pathogen at one point on the plant can trigger enhanced resistance against a broad spectrum of pathogens throughout the plant body; this response is termed systemic acquired resistance (SAR). SAR has been studied extensively in model plant systems like arabidopsis and it has been shown that SAR can be induced by application of salicylic acid or structurally similar chemicals like benzothiadiazole (BTH).

Using suppression subtractive hybridization (SSH) technology, we have identified 25 papaya genes which are induced systemically by BTH treatment. Many of these genes are papaya counterparts of genes known from other systems to be involved in plant defense. Others however are novel; genes not previously known to have any defense related role. The novel genes include three with potential roles in signal transduction and three with potential roles in establishing reducing conditions in the cell. In the model plant arabidopsis, the establishment of reducing conditions plays an important role in inducing defense genes.

To elucidate the function of these novel genes we are working to develop a gene silencing system for papaya. Because few gene mutations are known in papaya, a system like that used in *Nicotiana benthamiana* (see "Mechanisms of plant gene silencing and viral countermechanisms" p. 11) would be a very useful functional genomic tool. By doing *Agrobacterium* leaf infiltration with a GUS gene expression construct, we have been able to induce local silencing of a stably integrated GUS transgene. Current efforts are directed at achieving systemic silencing and silencing of endogenous genes.

This project is developing the tools to enable analysis of disease resistance in new papaya varieties and further contributes to a deeper understanding of plant disease resistance mechanisms through data obtained on novel papaya defense genes.

*- P. Guan, X. Qiu, M-L. Wang, P. H. Moore (USDA ARS PBARC) and H. Albert (USDA ARS PBARC)*



***Local gene silencing in papaya.*** *Leaves of transgenic papaya expressing a GUS transgene, which produces a blue precipitate when treated with a test substrate, were infiltrated with agrobacterium carrying three different expression constructs: negative control (left leaf), a GUS silencer construct (middle leaf), and a silencer construct for an unrelated gene (right leaf). The white area in the middle leaf indicates the GUS transgene has been silenced in the infiltrated area.*

## *Activation of Systemic Acquired Resistance in Papaya by Over-Expressing NPR1 Gene*

Systemic acquired resistance (SAR) is a general response by plants activated by pathogen exposure. In *Arabidopsis,* it was found that *NPR*1 (non-expresser of pathogenesis-related genes) controls the onset of SAR and over-expression of this gene leads to enhanced resistance with no obvious detrimental effects. In addition, *NPR*1 needs to be activated by a signal, i.e. - salicylic acid (SA), 2,6-dichloroisonicotinic acid (INA), or a pathogen, to become active, which then results in pathogenesis-related (PR) gene expression. This characteristic of being inducible is an advantage to the plant because it takes less energy than does constitutive expression of resistance. Thus, NPR1 could be an ideal target for engineering broad-spectrum disease resistance.

The objective of this study is to enhance the immunity of papaya by over-expressing a key regulator of the SAR signaling pathway, *NPR*1. To over-express *NPR*1 in papaya, we transformed papaya embryogenic callus with a construct containing the *AtNPR*1 gene *(Arabidopsis thaliana NPR1)* using biolistics. Transgenic papaya plants were then challenged with *Phytophthora palmivora,* a fungal pathogen, to determine the change in the level of resistance to the pathogen.

We conducted polymerase chain reaction (PCR) and genomic DNA blot hybridization to confirm the presence of the *AtNPR*1 gene in the transgenic plants. Also, leaf discs from independent lines were inoculated with *P. palmivora*. The results showed that the transgenic papaya plants developed lesion sizes significantly smaller than those of the non-transformed controls. This result indicates that over-expression of *AtNPR*1 gene in papaya can lead to enhanced resistance to *P. palmivora*. Seeds are available from a number of the transgenic lines and further analysis will be done for segregation of the enhanced resistance.

An *NPR*1 gene homolog from papaya was also successfully cloned; the gene product was shown to share 67% amino acid similarity with *AtNPR*1. Two expression vectors containing the papaya *NPR*1 (*ppyNPR*1) were constructed and were used to transform papaya. To date, *NPR*1 gene homologs have been identified in a number of plants (tobacco and tomato) but only the *AtNPR*1 gene has been used in previous studies. This particular transformation was done to determine the effect of over-expressing papaya's own *NPR*1 gene. This may lead to better disease resistance compared to the *AtNPR*1 gene.

*- R. Agbayani, W. Nishijima (UH), H. Albert (USDA ARS PBARC), P. Moore (USDA ARS PBARC) and Y. J. Zhu*

*Papaya leaf challenged with P. palmivora culture to test the resistance of transgene, NPR1.*

## *Genomic and Functional Analyses of the Male Specific Region on the Primitive Y Chromosome in Papaya*

Sex chromosomes in animals are ancient – about 300 million years old, and Y chromosomes are genetically eroded. Flowering plants appeared later, about 130-200 million years ago, and plant sex chromosomes evolved more recently. The *Silene* genus with its estimated 20 – 25 million year ancestry has generally been thought to contain the most recently evolved XY system known in eukaryotes. Yet 90% of the Y chromosome in *Silene* is degenerated and suppressed for recombination. The recently discovered primitive Y chromosome in papaya shows much more moderate degeneration and suppression of recombination. It has a small male specific region (MSY) comprising only about 10% of the Y chromosome. Thus, the papaya system appears to be the most recently evolved XY system in evolutionary terms and as such, may hold the key to answering a major question - how development of the plant XY system began.

We have sequenced 10 to 15 random subclones from 25 non-redundant bacterial artificial chromosomes (BACs) on the MSY. One hundred pairs of sequence specific primers were designed from random sequences of 23 of these BACs to test the three sex forms of three papaya varieties, 'Kapoho', 'SunUp' and 'AU9'. Among the 100 pairs of primers tested, 23 were male specific, two were co-dominant (both hermaphrodite and female genomic DNA amplified but produced different sized fragments), 68 were non-specific (same size of fragment amplified in both sexes), and 7 failed to amplify. Based on the male-specific markers, 12 of the 23 BACs were confirmed to be male specific. Among the 53 SCAR markers on the 12 male specific BACs, 23 SCARs were male specific (Y-like), two co-dominant, 24 non-specific (X-like) and 4 not amplified. Eleven of the 12 male specific BACs contained both Y-like and X-like sequences, reinforcing our previous conclusion that the MSY in papaya is a mosaic of conserved and diverged sequences. The 12th BAC 93O24 appeared to contain mostly male specific sequences.

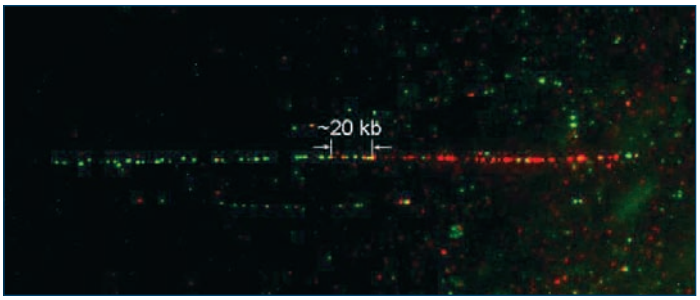
Five pairs of overlapping BAC clones based on Southern hybridization were subjected to fiber-FISH mapping by the Jiang lab (see figures). Two pairs of BACs (41F24 and 57B23; 54H01 and 1I11) showed overlapping of 20 kb and 25 kb BAC DNA sequences, respectively. One pair (76M08 and 89I15) showed a gap of 30 kb, whereas another pair (85B24 and 89M06) contained abundant repetitive sequences and showed a large gap of 400 to 500 kb. The fifth pair (79C23 and 71E16) was not on the same chromosome in meiotic pachytene. One interphase nuclei, 79C23 showed only a single FISH site (on a single chromosome), whereas 71E16 showed the expected two sites (on a pair of homologous chromosomes), suggesting that 79C23 is strictly Y specific without a counterpart on the X chromosome. This work demonstrates that fiber-FISH mapping is a crucial part of accurate DNA sequence analyses of the MSY and the corresponding region on the X chromosome.

From the partial sequences of the 12 male specific BACs, 14 putative genes were identified by searching the GenBank and EST databases from eight male specific BACs. DNA sequences from the remaining four male specific BACs yielded no match to any known genes. Sequence specific primers were designed from five putative genes and used to examine the expression of these five genes in hermaphrodite, male and female papaya plants. Quantitative RT PCR analyses showed that all of these five genes were expressed in all three sex types at similar expression level.

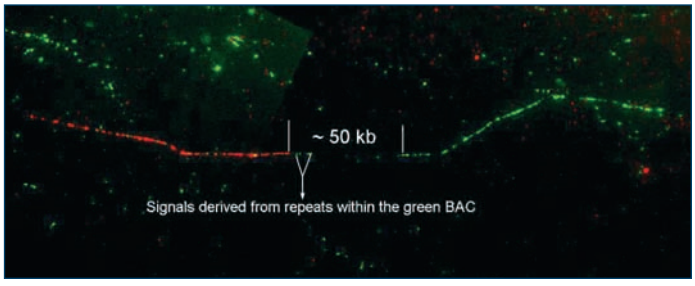
The partial sequences from BACs on the MSY provided an opportunity to test whether these BACs are truly male specific. Twelve of the 23 BAC were proven to be male specific, and the other 11 BACs could be either conserved region on the MSY or on the different chromosome. The mosaic structure of Y-like and X-like sequences on all but one male specific BAC verified the recent origin of the primitive Y chromosome in papaya. The conservation of five functional genes in all sex types added further support to this notion.

Pachytene and fiber-FISH analyses are essential for verifying the location, order and distance of male specific BACs. Although all the BACs on the MSY were mapped by Southern hybridization using male specific markers or BAC ends during chromosome walking, chromosomal duplication and repetitive sequences can cause misplacement of BACs or inaccurate estimate of physical distance between BACs. Chromosome FISH mapping confirmed two pairs of overlapping BACs and corrected the physical distances of three supposedly overlapping BACs, including a BAC that was not on the MSY.

*- Q. Yu, W. Jin (Univ. WI), P. H. Moore (USDA ARS PBARC), Z. Liu, R. Ostroff, C. M. Ackerman, M. R. Jones, J. Jiang (Univ. WI), A. H. Paterson (Univ. GA) and R. Ming*




*Fiber-Fish image of two overlapping BAC clones on the MSY in papaya. BAC clone 41F24 (130 kb) was labeled as red and BAC 57B23 (155 kb) as green. These two BAC clones overlapped approximately 20 kb.*




*Fiber-Fish image of two non-overlapping BAC clones on the MSY in papaya. BAC clone 76M08 (175 kb) was labeled as red and BAC 89I15 (180 kb) as green, these two BAC are separated by approximately 50 kb. The short green signal within the gap (close to the red signal) may be derived from a repetitive DNA element that is on BAC 89I15.*

## *Improvement of Papaya Pest Resistance through Genetic Engineering*

Papaya (*Carica papaya* L.) has a number of natural enemies These include nematodes, aphids, leafhoppers and mites. In this study, we have concentrated on two, the Carmine spider mite *(Tetranychus cinnabarinus)* and Stevens leafhopper *(Empoasca stevensii)*. Both these pests cause losses to the papaya industry in Hawaii through defoliation of the trees. The aim is to produce papaya plants with improved resistance to these pests.

A biolistic gene gun procedure was used to introduce a gene encoding a protein with known entomotoxic activity. The protein used was a lectin, GNA, from snowdrop, *Galanthus nivalis*. This gene has been incorporated into a number of plants and has given resistance to sap-sucking insects and nematodes.

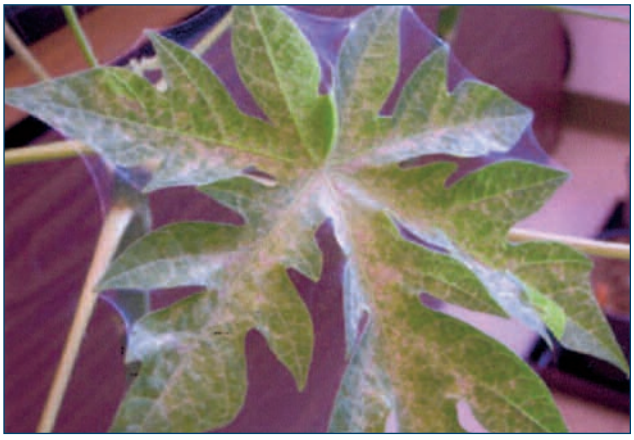
A number of independent papaya lines expressing the GNA protein have been generated. Six of these have been studied in depth. Molecular analysis has been carried out to detect the presence of the transgene. The recombinant GNA protein has been shown to be biologically active using a haemagglutination test with rabbit blood.

Experiments are under way to examine differences in expression levels of the lines. It remains to be seen if there is a relationship between lines showing high protein expression and those with high biological activity in the haemagglutination assay. Previous work has shown that a low level of recombinant GNA protein can improve resistance to sap-sucking insects.

Future experiments will investigate the resistance of the transgenic papaya plants to pest attack.

*- H. McCafferty, P. Moore (USDA ARS PBARC) and Y. J. Zhu*

*Lower surface of papaya leaf showing damage caused by spider mite feeding. The feeding tends to be concentrated around veins.*



*Papaya leaf showing chlorosis and covered with spider mite webs. The mites use the webs to facilitate movement and for protection.*

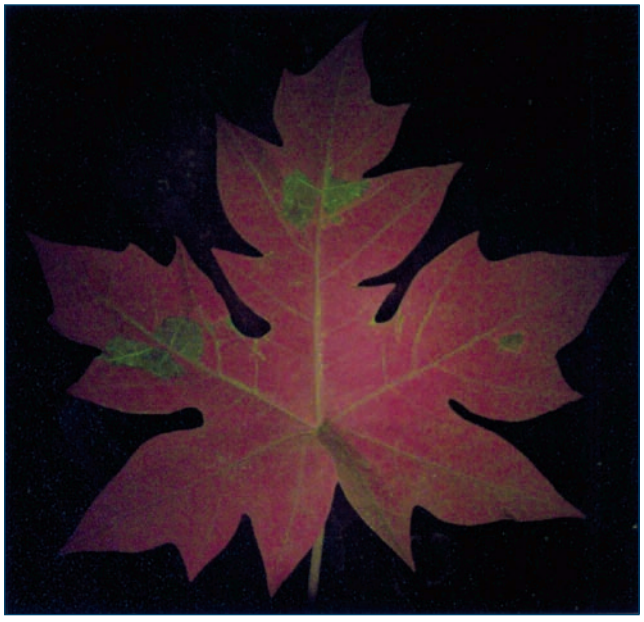
## *Identification and Characterization of Disease Resistance Genes in Papaya*

Recognition of invading pathogens and subsequent activation of defense responses is controlled by resistance genes in plants. These genes in resistant plants recognize specific elicitor compounds produced by certain pathogenic organisms. The elicitors trigger active defense responses in the plant that ultimately inhibit the pathogen. A peptide elicitor of defense responses in parsley, Pep-13, has been previously described from *Phytophthora sojae*. Mutational analyses done within the Pep-13 amino acid sequence showed that specific amino acids are indispensable for the defense-eliciting activity of the elicitor. It was also shown that this particular peptide is conserved in most *Phytophthora* species.

The Pep-13 gene was cloned from *P. palmivora,* the most important cause of fungal disease in papaya. This gene will be inserted into an *Agrobacterium*-infiltration vector along with green fluorescent protein (GFP) as a reporter. The *Agrobacterium* expression system will be used as a tool for the identification of disease resistance genes (see figure).

In addition, we are interested in understanding the signal transduction network that controls the activation of defense response in papaya. It was reported that some defense responses are activated by signal transduction networks that require jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) as signal molecules. Different pathogens are limited to different extents by SA-dependent responses and by JA/ET-dependent responses. Research is planned to elucidate the relative sites of action of the elicitor, Pep-13, in papaya.

*- R. Agbayani, P. Moore (USDA ARS PBARC) and Y. J. Zhu*



*Green fluorescent protein (GFP) used a reporter to monitor the expression of defense gene in Agrofiltration*

## *Antimicrobial Peptide from Dahlia merckii Inhibits Phytophthora palmivora Growth in Papaya*

*Phytophthora* spp., one of the more important casual agents of plant diseases, is responsible for heavy economic losses worldwide and papaya (*Carica papaya* L.), an important fruit crop of the tropics, is highly susceptible to *P. palmivora*. Heavy yield losses associated with root rot, severe decline, and death of papaya trees have been attributed to *P. palmivora,* particularly in poorly drained areas during the cool and rainy winter season. Control of this pathogen is needed to decrease dependence on fungicides, increase productivity, and improve pre- and post-harvest fruit quality. In addition, papaya is being developed as a model fruit crop system because of its relatively small arborial stature, short seed-to-seed life cycle, and its small genome size (372 Mb which is 10% smaller than the rice genome). Tools that have been developed include well-established cloning and transformation systems, a high quality BAC library, several cDNA libraries, and a high density genetic map. These resources will contribute to studies on the interaction of papaya with *P. palmivora* to suggest strategies on how *Phytophthora* disease might be controlled.

Defensins are cysteine-rich antimicrobial peptides that are widely distributed among plants, insects, and mammals. As potent defenders in protecting plants from pathogenic fungal attack, plant defensins are presumed to play an important role in the innate immunity of plants and are expected to find broad ranged applications in the production of transgenic crops. The defensin gene *DmAMP*1, cloned from *Dahlia merckii,* has been introduced as a transgene into a range of species to increase host resistance to pathogens to which they were originally susceptible. However, the mechanism of interaction of the *DmAMP*1 peptide with *Phytophthora* spp. has not been clearly characterized in planta. In this study, we expressed *DmAMP*1 in papaya (*Carica papaya* L.*),* a plant highly susceptible to a root, stem, and fruit rot disease caused by *Phytophthora palmivora,* to study the interaction of the *DmAMP*1 defensin with *P. palmivora.* Our results demonstrate that expressing the *DmAMP*1 gene in papaya plants increased resistance against *P. palmivora* and that this increased resistance is associated with reduced hyphae growth of *P. palmivora* at the infection sites.

*- Y. J. Zhu, R. Agbayani and P. Moore (USDA ARS PBARC)*

## *Genetic Transformation of Pineapple with Nematode Resistance and Flowering Control*

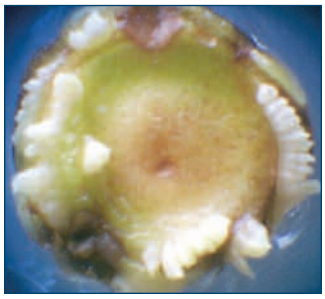
With collaboration from the Hawaii pineapple industry, the University of Hawaii, and Leeds University (UK), we set out to use genetic engineering technologies to solve two serious problems faced by the pineapple industry - nematode damage and precocious flowering (HARC Annual Report 2003 p.14).

The first transgenic line for flowering control (Line #51) was generated in 2003. In 2004, approximately 350 micropropagated plants each of Line #51 and non-transformed control were transplanted to soil and grown to maturity in the greenhouse. The flowering patterns are currently under observation. A second transgenic line (Line #2) was produced in early 2004. There are approximately 1000 plants of Line #2 maintained in cultures. To date, more than 100 randomly collected samples from Lines #51 and #2 were PCR positive for the transgene.

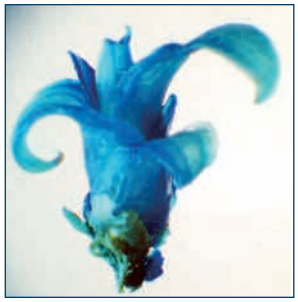
During the second half of 2004, our effort was focused on improving the transformation procedure by optimizing parameters related to *Agrobacterium* infection, plant regeneration, and antibiotic selection. The protocols in all three factors have been significantly improved based on the results of transient expression and regeneration efficiency. The time required to identify transformants has been shortened to about 3 months. We have initiated large-scale transformations to produce more transgenic lines for both flowering control and nematode resistance using the modified method.

*- M-L Wang, G. Uruu (UH), R. Paull (UH), B. Sipes (UH), M. Hao (UH), J. Hu (UH), K. Cheah (UH), J. Buenafe and C. Nagai*

*Adventitious buds induced from bases of pineapple leaf*



*Adventitious buds induced from segments of pineapple stems*



*A fully transformed pineapple plant stably expressing the reporter gene GUS*

# Coffee Research

## *Molecular Markers in Coffee: Fruit and Seed Characterization of Arabica Coffee Mapping Populations*

A segregating mapping population of Arabica coffee (*Coffea arabica* L.) was developed from a cross between the cultivars Mokka Hybrid (MA2-7) and Catimor (T 5175-7-1). Catimor is a variety developed in Central America from a hybrid between *C. arabica* and *C. canephora* and backcrossed to *C. arabica*. Catimor trees typically have large, round leaves, resistance to rust, high cherry yield and large size beans, but known to have lower cupping quality. Mokka, a mutant of variety Bourbon, was developed in Brazil and has small, thin leaves, small beans, susceptible to rust, and excellent cupping quality. In June 2001, 135 seedlings of the population were planted at the HARC Kunia Substation together with selfed progenies of both parents. This population was used for constructing the first Arabica genetic map with amplified fragment length polymorphism (AFLP) markers. It is used for mapping quantitative trait loci (QTL's) controlling coffee yield and quality. Morphological data for tree height and width, leaf characteristics was collected in 2002 (HARC Annual report 2001-02).

Yield, fruit and seed characteristics were determined among 60 progeny of the population in 2003-04 season. Fruits from individual trees were harvested and processed by a wet method. Yield was determined by immature, mature and over ripe fruits of 5 representative branches of each tree. Fruit and seed size, seed weight and density were calculated.

Fruit and seed weight analysis of the progeny showed a significant increase over the Mokka parent. The mean fruit weight of the progeny was 36% greater than Mokka. Seed weight in the progeny surpassed both parents, with values 15% greater than the Mokka parent and 13% greater than the Catimor parent. Yield analysis showed a four fold overall greater yield in the progeny as compared to Mokka. With the large increase in yield and significant increase in green bean weight, the pseudo F2 population shows hybrid vigor of a true F1 population. Cupping tests for both Mokka and Catimor parents were conducted at Cathy Cavaletto's laboratory at the University of Hawaii. However, no difference was detected.

Chemical composition, five organic acids and three types of sugar, of seeds from parents and progeny was analyzed by HPLC method. No difference was found among 10 progeny and parental samples.

A true F2 population of two F1 (00-20-25 and 00-20-41) was obtained. Seventy-five trees of each individual were planted along with ten trees of each parent at HARC Kunia Substation in 2003. The true F2 population started to show a larger segregation in tree morphology. This population will be used to map QTLs controlling tree morphology and fruit quality.

*- M. R. Jones, C. Nagai and R. Ming*

## *Quality Aspects of Shade Grown Coffee*

Traditionally coffee was grown as a shade crop and in some parts of the coffee growing world this is still the case. Shade grown coffee has been associated with higher quality at cupping. Therefore, a collaborative effort is underway between HARC and the University of Hawaii to determine what level of shade might be suitable for growing high quality coffee in Hawaii.

This project was started in 2003. Trials are now in place at three locations on two islands. Tree shade and artificial shade are being compared. The first harvest will be undertaken at the end of 2005. Analytical strategies have now been worked out and will entail the use of solid phase microextraction (SPME). When the coffee samples are cupped, the compounds in the vaporous phase and in the liquid phase will be sampled using SPME at the exact time that the trained cupper tastes the sample. This will allow for a direct comparison between cupping and chemistry. Analytical results will be obtained in late 2005 and early 2006.

*- M. C. Jackson, T. Idol (UH) and H. C. Bittenbender (UH)*

# Forestry

## *Family Variation in Field Survival of Acacia koa: Prototype Testing for Variation in Genetic Resistance to Koa Wilt (Fusarium oxysporum f. sp. koae)*

*Acacia koa* is the most important native hardwood tree in the State of Hawaii from both an economic and environmental perspective, but there is high mortality of young trees at low elevation sites due to a vascular wilt caused by *Fusarium oxysporum* f. sp. *koae*. A recent report also indicates that mortality is occurring in natural stands at higher elevation and the origin of the pathogen is currently unknown. At Maunawili, Oahu, of the 30 *Acacia koa* families tested, survival percent ranged from a low 4.0% to a high 91.6% at 48 months. The average family survival percent was 35.4%. However, the two best families had survival percentages of 91.6% and 75% respectively. Family variation in *A. koa* in field trials strongly suggests the presence of resistance to *F. oxysporum* f. sp. *koae*. To confirm whether genetic resistance exists, a short-term greenhouse test involving artificial inoculation was initiated. A subset of seed lots from the Maunawili trial were selected based on seed availability and represented a large range in survival. The results from this screening trial provide the first formal evaluation of existing genetic variation within koa for disease resistance. It is anticipated that this trial will also provide the prototype for a larger operational resistance screening and development of resistant populations of koa.

*- N. S. Dudley, R. Anderson (USGS), R. A. Sniezko (USDA) and A. Oguchi (CA Polytechnic State University)*

### Reference

*N. S. Dudley, R. Anderson, R. A. Sniezko and A. Oguchi. (2004) Family Variation in Field Survival of Acacia koa: Prototype testing for variation in Genetic Resistance to Koa Wilt (Fusarium oxysporum f. sp. koae). International Union of Forestry Research Organizations, Division 2. Nov. 1-5, Charleston, SC.*

## *Degradation of Chlorinated Organic Compounds Using Saprophytic Fungi from Hawaii's Forests*

Following studies undertaken in 2003, funding was obtained from Environet Inc. to study the feasibility of degrading polychlorinated biphenyls (PCBs) in soils in Hawaii. PCBs are insulating compounds used in electrical components. They are extremely long-lived, toxic, and tend to bind to soils into which they may leak as a result of discarding electrical equipment.

Some soils in Hawaii contain multiple part per million contamination with PCBs and required decontamination. This study looked at the possibility of using saprophytic fungi, found in Hawaii and known to degrade PCBs in liquid culture, to degrade these compounds in soil.

Three fungi, selected for their ability to degrade PCBs in liquid culture were used in this study. As these fungi utilize de-lignification of wood to obtain glucose as their primary energy source, soil contaminated with PCB was mixed in varying ratios with sugarcane bagasse. In 2003, studies showed that bagasse was a preferred lignin-containing substrate for these fungi. Each fungus was then added and allowed to grow. The PCB content of the test mix was analyzed periodically using gas chromatography (GC) to determine which fungi were causing degradation. The overall aim was to determine which fungus and which ratio of soil to

bagasse was most suitable. The tests were run for a total of 8 months.

Despite significant degradation of PCBs in liquid culture, none of the soil tests showed any PCB degradation. This may have been due to a number of reasons:

1. The PCB concentration (approximately 50 ppm) was too high and was toxic to the fungi.
2. Competition from other microbes present in the soil may have prevented the fungi from growing in enough quantity to allow for observable PCB degradation.
3. The PCB in the soil may have been so strongly bound to the soil that it was not available for degradation.
4. Some other compound in the soil may have been acting to inhibit fungal activity.

This study was completed in late 2004. Further studies are required to determine what is inhibiting these fungi from degrading PCBs in soil. Funding to continue this work is being sought.

*- M. C. Jackson*

# Miscellaneous Crops

## *A Fungal Biocontrol Agent for Control of Nematodes in Soil*

A project testing the efficacy of *Paecilomyces lilacinus* strain 251 (MeloCon WG, Prophyta Biologischer Pflanzenschutz GmbH) for control of nematodes on tomato (Burpee, "Orange Pixie") and cucumber (Ferry-Morse "Marketmore 76") was installed at the HARC Kunia Substation. This fungal product in a carrier substance is expected to be cleared by the EPA for commercial use in 2005. The test was installed in field plots infested with root-knot, *Meloidogyne incognita,* and reniform, *Rotylenchulus reniformis,* nematodes.

The treatments were 1) *Paecilomyces* 0.2 g/plant, 2) Vapam nematicide 65 ml/plot, and 3) untreated check. There were four replicate plots of each treatment on both tomato and cucumber. Tomato plots contained 10 plants each and cucumber plots contained 6 plants each. Yield data were taken as total fruit weight per plot of small, medium and large fruits in each harvest round. Nematode counts per plot and a rating of root damage were taken after harvest. Data were analyzed using Statistix 7 computer program giving ANOVA for each total and average. Comparison of means was performed using LSD at the 95% level.

Results of the tomato harvest showed the fruit numbers and weight to be highest in the *Paecilomyces* treatment (Table 1). The Vapam treatment was a close second and the untreated check was lowest. Due to the rather large variation in the test, only the medium size average fruit weight showed a significant difference between means. However, the consistency of the results indicated that the *Paecilomyces* had a real effect in protecting the tomato plants against nematode damage. The visual root galling ratings agreed with this. Soil nematode counts at harvest gave high numbers of root-knot nematodes and low to high numbers of reniform nematodes in all treatments.

The cucumber harvest yields had no significant differences between treatments (Table 2). Postharvest evaluation of plant roots showed severe nematode damage in all treatments. Soil populations of root-knot and reniform nematodes were low to moderate for all treatments. This is probably due to the severe root damage having drastically reduced nematode feeding sites and then leading to falling nematode populations. It appeared that the cucumbers were so susceptible and intolerant of the nematodes in

this study that neither the Vapam nor the *Paecilomyces* were able to overcome the damage.

The overall results indicated that *Paecilomyces lilacinus* was as effective as Vapam soil fumigant and significantly better than no treatment for control of nematodes in tomato. *Paecilomyces* was isolated from the MeloCon-treated plots at harvest, but one month later none was found.

*- S. Schenck*

## Table 1. Tomato Yield

*Comparison of means of average fruit weights (kg) per plant*

| treatment | small | medium | total wt./ trt. |
|---|---|---|---|
| Paecilomyces | 0.18 a | 1.35 a | 43.25 |
| Vapam | 0.15 a | 1.16 ab | 41.75 |
| control | 0.11 a | 0.92 b | 32.5 |

*Comparison of means of average fruit number per plant*

| treatment | small | medium | total wt./ trt. |
|---|---|---|---|
| Paecilomyces | 8.3 a | 36.5 a | 1274 |
| Vapam | 7.0 a | 31.1 a | 1219 |
| control | 4.4 a | 25.7 a | 964 |

means in the same column followed by the same letter do not differ significantly

## Table 2. Cucumber Yield

*Average fruit weight (kg) per plant*

| treatment | small | medium | large | total wt./ trt. |
|---|---|---|---|---|
| Paecilomyces | 0.4 | 1.1 | 0.7 | 42.2 |
| Vapam | 0.3 | 1.1 | 0.6 | 41.6 |
| control | 0.3 | 1.3 | 0.7 | 42.5 |

*Average fruit number per plant*

| treatment | small | medium | large | total wt./ trt. |
|---|---|---|---|---|
| Paecilomyces | 2.7 | 4.5 | 13.5 | 170 |
| Vapam | 2.9 | 5.9 | 12.4 | 199 |
| control | 2.7 | 5.5 | 12.8 | 9.7 |

## *Growing Stevia in Hawaii: Optimizing Glycoside Yield*

*Stevia rebaudiana* L. Bertoni is native to Paraguay, where it grows as an annual. Stevia prefers long daylight hours (>14) for vegetative growth. It has been grown as an annual in northern California, Washington State and Canada. Efforts have been made to grow stevia in Hawaii, without great success. Due to Hawaii's relatively short daylight hours, the plants tend to progress towards flowering within 70 days after planting. Another issue with the cultivation of stevia is that there is conflicting evidence about what happens to glycoside levels as the plant matures.

In this project, stevia lines that had been selected in California for delayed flowering characteristics were compared with a control line which had not. The top and middle thirds of plants were harvested at various stages during the maturation of the plant, including the pre-flowering vegetative stage, full flowering and at the post-flowering stage. Biomass of leaves and stalks and glycoside content in leaves was determined at each harvest. It was found that all of the plant lines, including those selected for delayed flowering, began to flower an average of 73 days after planting. At this stage, all plants were less than 1 foot in height. Consequently, biomass was lower than that found in Northern California, Washington State and Canada. The increase in biomass over the maturation of the plant was mainly due to stem growth and not to leaf proliferation. Glycoside content appeared to increase until the onset of flowering and then remained constant through the flowering period. The relative concentrations of the two main glycosides, rebaudioside A and stevioside, remained constant through flowering; however, a significant decline in rebaudioside A content was observed post-flowering. In some lines, this fell to almost zero.

Given the 73 day vegetative period for stevia grown in Hawaii, it is possible that 5 harvests per year could be achieved, assuming the top two thirds of the plant were harvested and the remaining third left to regrow for 73 days. When this cultivation scenario was calculated through, it was projected that leaf biomass yield would compare favorably with the yield obtained from current commercial cultivation in Northern California. However, the glycoside yield was projected to be lower. It was concluded that growing stevia in Hawaii would not be economically feasible unless lines were selected that had delayed flowering characteristics under Hawaii's environmental conditions.

*- M. C. Jackson*

## *Genetic Diversity of Lychee Germplasm Assessed by AFLP Markers*

A native evergreen fruit tree of China, lychee (*Litchi chinensis* Sonn.) is an important commercial fruit throughout China, India, and Southeast Asia. Global distribution and cultivation of lychee throughout tropical and subtropical regions has occurred over many centuries. Translation of cultivar names from Cantonese and Mandarin to English has resulted in confusion over the naming of the cultivars; frequently, many cultivar names are synonymous causing more difficulty. Phenotypic characterization through tree morphology and fruit quality is much of the foundation for cultivar naming. Movement away from phenotypic classification towards molecular characterization of lychee has occurred in order to more accurately identify genetic relationships.

Very few molecular studies have been carried out for genetic identification of lychee cultivars. Genetic diversity of lychee has been evaluated through isozyme analysis, RAPD markers, and microsatellite markers thus far. However, the amplified fragment length polymorphism (AFLP) technique has proven to be a reliable process with superior or equal resolution and reproducibility with a decrease in cost and time. Thus, AFLP allowed for the rapid screening of the lychee germplasm.

Genetic diversity was evaluated among 83 lychee accessions collected from Hilo, Hawaii using AFLP. The germplasm in Hilo includes cultivars from Florida, Hawaii, Thailand, and three areas of China. One-third of the samples originate from Chia Yi, Yunnan, and Guangxi in China. Markers were identified with the goal of clarifying genetic relationships of the lychee accessions by comparing results with a known pedigree. A total of 337 AFLP markers, with an additional 64 monomorphic markers, were used to identify genetic similarity based on the Dice similarity coefficient. We found substantial diversity among the lychee accessions with no identical samples found. The average genetic similarity among samples was 0.55, ranging from 0.05 to 0.94. The Kaimana accession differed from its sister line with genetic similarity of 0.52, which suggests a high level of genome heterozygosity within their parental varieties. The relationship between different cloning techniques, grafting and air-layering, of the same cultivar was also studied. The grafted sample was radically different from the air-layered sample with a genetic similarity of only 0.18. Further studies to more clearly define their relationship will be needed. It was also established that most of the germplasm originating from Florida are either related or similar to Hak Ip from Guangxi, China. Overall, these findings continue to confirm the extensive genetic diversity in lychee and offer a better understanding on the origin of each cultivar. Using molecular markers provided a more reliable way to identify genetic relationships. Understanding these relationships will improve breeding programs and commercial production and management.

*- M. R. Jones, F. T. Zee (USDA ARS PBARC), P. H. Moore (USDA ARS PBARC), M. S. Kim and R. Ming*

## *Transformation of Anthurium for Improved Disease and Pest Resistance*

A procedure for the genetic transformation of anthurium was previously reported by a group led by Heidi Kuehnle at the University of Hawaii. In order to refine that procedure to be able to generate sufficient numbers of transgenic anthurium in an efficient time frame, we are investigating a modified method.

Two commercial cultivars of standard form anthurium, "Marian Seefurth" and "Midori" have been selected for transformation. A number of genes are available for use in *Agrobacterium*-mediated transformation. The genes selected target the two main problems faced by anthurium growers in Hawaii, i.e. - the burrowing nematode (*Radopholus similis*) and bacterial blight, caused by *Xanthomonas campestris pv. dieffenbachiae*.

Previous publications include antibiotic levels using Kanamycin for selection of transgenics. In this case the antibiotics being used are Hygromycin and Geneticin. Prior to transformation, an antibiotic kill curve was produced to determine a suitable antibiotic level for selection.

Preliminary transformations have been carried out using anthurium callus material and two strains of *Agrobacterium*. Molecular analysis of the regenerating plantlets has begun. A number of putative transgenics have been selected and plantlets recovered. These will continue to grow and undergo further molecular analysis. The transformation procedure is currently being optimized.

*- H. McCafferty and Y. J. Zhu*

*(A)* *(B)* *(C)*

*Two commercial varieties of anthurium selected for the transformation study.*
*(A) Marian Seefurth inflorescence*
*(B) Midori inflorescence*
*(C) Marian Seefurth in tissue culture*

## *Expression of Biologically Active Protein in Plants Using a Plant Viral System*

This project is aimed at developing a system to transiently express high level of foreign protein in plants without going through tissue culture procedures. Plants offer several advantages over other, more traditional expression systems, for the production of high value products. It has been demonstrated that plants can express, fold, assemble and process complex foreign proteins. There may be significant economic benefits in the production of bulk quantities of valuable pharmaceutical products in plants as compared to animal cell lines or transgenic animals, and there may be fewer safety concerns associated with use of plant expression systems.

Several studies have demonstrated that plant viruses can be used as vehicles to introduce and express foreign proteins. Many plant viruses multiply to high levels, leading to concomitantly high levels of foreign protein expression of any gene incorporated in the viral construct. Virus delivery does not lead to permanent incorporation of the transgene into plants, nevertheless, depending on which virus is used, virus multiplication and gene expression can continue for long periods (weeks or months). Plant virus expression vectors have several other potential advantages over the more commonly used transgenic plant technology. In this study we used as a model, the human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene to evaluate expression and functions of the foreign gene.

The human GM-CSF is a glycoprotein with important clinical applications for the treatment of neutropenia and aplastic anemia and for reducing infections associated with bone marrow transplants. We evaluated the potential for using a potato virus X (PVX) viral vector system for efficient expression of the biologically functional GM-CSF protein in *Nicotiana benthamiana* leaves. The GM-CSF gene was cloned into PVX viral expression vector (PVX201) driven with the *CaMV 35S* promoter and terminated with a 3' 6xHis tag for efficient protein purification. Gene transfer was accomplished by inoculating plants with the plasmid DNA of PVX vector containing the GM-CSF gene. The expression level of the recombinant GM-CSF protein was determined with ELISA and its size was confirmed by Western blot analysis. The results showed that: 1) Leaf age significantly affects GM-CSF protein concentration with younger leaves accumulating 19.8 mg $g^{-1}$ soluble protein which is about twice the concentration in older leaves. 2) Recombinant protein accumulation is temporally regulated within a given leaf to reach a maximum concentration at 11 days post-inoculation. 3) The two leaves immediately above the inoculated leaves play an important role for GM-CSF accumulation in the younger leaves. Protein extracts of infected *N. benthamiana* leaves contained recombinant human GM-CSF protein in concentrations of up to 2% of total soluble protein, but only when the pair of leaves immediately above the inoculated leaves remained intact. The recombinant protein actively stimulated the growth of human TF-1 cells suggesting that the recombinant human GM-CSF expressed via PVX viral vector was biologically active. Thus, the plant PVX viral vector system has potential for eventual production of biologically active recombinant proteins.

*- F. Zhou, M-L. Wang, H. Albert (USDA ARS PBARC), P. Moore (USDA ARS PBARC) and Y. J. Zhu*

## *Snails*

Hawaii taro production in 2003 was estimated at 5.0 million pounds; an 18% decrease from 2002 (Hawaii Agricultural Statistics Service, Feb 9, 2004). This is the lowest ever recorded production and was in large part due to the effects of the Golden Apple snail *(Pomacea canaliculata)*. The value of sales in 2003 was about $2.7 million, compared with $3.294 million the previous year. Golden apple snail was introduced into Hawaii, Japan and many other countries in South-east Asia from South America as a source of food in the early 1980s. However, after its commercial markets had failed, discarded and escaped snails invaded taro and rice ecosystems and have been causing significant economic damage. In Hawaii, these snails were also purposely introduced into taro paddies (lo`i). The reasoning being that they could be harvested for food. However, the consequences of this action were not fully understood at the time. The snails are voracious, fast growing and have a huge reproductive potential. A single female can produce as many as 15,000 offspring per year, and can thrive in water at a density of 1,000 snails per square meter. They mature within 60 – 85 days and spawn at weekly intervals and have been described as the most damaging pest ever to hit neotropical areas. The snails very quickly spread throughout taro lo`i, via the extensive and interconnected irrigation system.

In 2003, the Hawaii Agriculture Research Center (HARC) received a grant from the Western region SARE program to conduct limited field studies of two botanical extracts. Extracts were made from plants that had shown promise in toxicology studies in HARC's laboratory. The field trial was initiated in December 2003.

Of the two extracts tested, application of the neem extracts resulted in an average snail mortality rate of greater than 90% compared with the untreated control plots, 66 days after the initiation of the trial. The majority of the snail deaths were observed within 15 days after initiation of the test. In February 2004, these data were communicated to the Environmental Protection Agency (EPA). Even though efficacy was demonstrated, and given the fact that this is a botanical extract, not a synthetic compound, it was still felt that a full registration study would be required. Unfortunately, this would be prohibitively expensive and a financial sponsor willing to fund the required studies could not be found. The study was therefore terminated in December 2004. However, new studies will be commencing in 2005 to determine the efficacy of an iron salt that is currently registered for use on terrestrial snails.

*- M. C. Jackson*

## *Marketing Hawaii's Specialty Crops*

Hawaii's agricultural industry needs export markets for its crops. The local market alone is viable, but is limited in the quantity of products that it can absorb. With the high production costs and additional export shipping costs, Hawaii agricultural produce cannot easily compete in the worldwide commodity markets. However, our specialty crops and value-added products are unique and can command higher prices that can make export sales profitable. The challenge is to locate markets, overcome trade barriers, and develop new marketing strategies. HARC is focusing on these problems for state agricultural producers.

Cut flowers and nursery products sales were valued at $92.1 million in 2002 and the industry has the capacity to expand. It comprises a valuable sector of the agricultural industry. One barrier to the export of fresh produce and cut flowers is the quarantine against pests and diseases. This is an important concern and needs to be addressed. A project is currently underway in cooperation with University of Hawaii scientists to satisfy the quarantine regulations pertaining to the cut flower industry. One method for eliminating insect pests that may be harbored in cut flowers is to pass them through irradiation treatment. A treatment facility is already in place and could be used to treat flowers if a suitable procedure could be established. This project was designed to test various pre-irradiation treatments and irradiation exposure times and dosage rates to find a procedure that will eliminate pests while not damaging the cut flowers or reducing their shelf life. Several different flower types are being tested with encouraging results so far.

Another project is in progress for the formation of a marketing consortium for export of Hawaiian specialty agricultural products to China. Recent changes in Chinese world trade regulations have created new opportunities for US products. Hawaiian growers now produce a large and diverse number of high value, low volume crops and value-added products. The critical issue is the lack of export market outlets and an efficient, statewide collection, shipping and marketing contact system. These products include: packaged coffee, teas, specialty sugars, macadamia nuts, candies, specialty cooking oils, packaged vanilla beans and extract, honey, jams, jellies, condiments and wines. Two trips to Hong Kong, Guangzhou, and Shanghai to meet with potential importers, buyers and to introduce the products at trade shows have so far met with success. The Chinese are receptive to the value-added products they were shown and are attracted especially to the idea of the Hawaiian theme. They look for attractive packaging and a guarantee of healthful ingredients. We are now in the process of getting together a group of producers and setting up the import procedure with an agent. Outlets and buyers have been located and we hope to soon be exporting Hawaiian value-added agricultural products to China.

*- S. Schenck*

# Services

## Environment

Despite its unique geographical and climatological situation, environmental laws which were designed to regulate mainland farming also apply to Hawaii's agriculture. HARC assists local farmers in understanding and applying these challenging mandates to their farming activities.

Every year, HARC cooperates with local and national environmental regulatory agencies and the State legislature to help develop appropriate policies, legislation, and regulations regarding agriculture.

Watershed planning activities throughout the islands were especially interesting this year, as local communities are getting more involved in coordinating plans to maintain and improve their natural environment. HARC worked with numerous advisory groups to obtain the best available research to make informed planning decisions based on good scientific data.

*- J. Ashman*

## Quality Assurance Unit

HARC continued to participate in the pesticide registration process under the Environmental Protection Agency's Federal Insecticide, Fungicide, and Rodenticide Act. A Quality Assurance Unit (QAU) is maintained to inspect and audit studies that must comply with these regulations. Two field trials were audited, a separate sugarcane field trial was inspected for a magnitude of residue study and 2 analytical laboratory reports were audited for compliance with Good Laboratory Practices. The QAU also participated in a second sugarcane magnitude of residue study. My term limit as a member of the Hawaii Pesticide Advisory Committee was reached; this position had enabled me to provide input from the sugarcane industry to the State Department of Agriculture.

*- B. Vance*

## Computer System Administration

The local area network at the Experiment Station has common components shared between HARC and the USDA/ARS. New users from both organizations are provided with an introduction to the system and policies. Anti-malware software at the user level was promoted. The LAN was upgraded with new switches. The file server and frame relay components were relocated to a more secure room. An infrequent yet annoying glitch in our telephone system was finally resolved.

*- B. Vance*

## Safety

A biosafety policy was appended to our safety manual. A monthly log of chemical wastes continues to be maintained.

*- B. Vance*

## Administration and Support Staff

Stephanie Whalen, President and Director
Kuo Kao Wu, Vice President and Assistant Director
Janet Ashman, Environmental Specialist
Florida Chow, Human Resources
Becky Clark, Bookkeeper
Elon Clark, Buildings and Grounds Superintendent
Ladislao Gonzalez, Watchman, Maintenance
Anthony Lannutti, Secretary-Treasurer, Controller
Ann Marsteller, Librarian
Cynthia Pinick, Executive Secretary
Blake Vance, Assistant Administrator

## Staff

Nicklos Dudley, Forester
Mel Jackson, Director of Product Development and Services
Ray Ming, Plant Molecular Geneticist
Chifumi Nagai, Plant Breeder, Biotechnologist
Jerry Pitz, Chemist
Lance Santo, Agronomist
Susan Schenck, Plant Pathologist
Ben Somera, Sugar Technologist
Kuo Kao Wu, Sugarcane Breeder
Aileen Yeh, Hawaii Coordinator
Qingyi Yu, Plant Molecular Biologist
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Susan Ancheta, Laboratory Technician
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Meghan Jones, Research Assistant
Elaine Rondez, Research Assistant
Josienellie Rosete, Laboratory Assistant
Sachiko Saito, Laboratory Assistant
Erin Yafuso, Laboratory Assistant
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## Kunia and Maunawili Substations

Hilario Alano, Experimentalist
Rudy Dizor, Mechanical Operator
Angel Galvez, Mechanical Operator
Roland Fernandez, Experimentalist
John Rockie, Experimentalist
Roger Styan, Experimentalist, Supervisor

## Maui Substation

Albert Arcinas, Maui Farm Manager
Artemio Bacay, Field Worker
Teodoro Bonilla, Field Worker
Romeo Cachola, Field Worker
Orlando Castres, Field Worker
Luis Dela Cruz, Weighing Machine Operator
Wilson Galiza, Foreman
Domingo Vallecera, Field Worker

## Kauai Substation

Fernando Garcia, Field Worker
Narciso Garcia, Field Worker

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Moriah Eustice, University of Hawaii
Linda He, University of Hawaii
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Jim Carr, Biological Laboratory Technician, USDA, ARS
Maureen Fitch, Plant Physiologist, USDA, ARS
Paul Moore, Plant Physiologist, USDA, ARS

# Sugar Production

| COMPANY | ACRES HARVESTED | TONS RAW SUGAR (96•) | TONS SUGAR PER ACRE |
|---|---|---|---|
| **2001** | | | |
| Gay & Robinson, Inc. | 4,193 | 54,691 | 13.04* |
| Hawaiian Commercial & Sugar Co. | 15,101 | 191,512 | 12.68 |
| Totals & average | 19,294 | 246,203 | 12.76** |
| **2002** | | | |
| Gay & Robinson, Inc. | 4,754 | 54,196 | 11.40* |
| Hawaiian Commercial & Sugar Co. | 16,557 | 215,888 | 13.04 |
| Totals & average | 21,311 | 270,084 | 12.67** |
| **2003** | | | |
| Gay & Robinson, Inc. | 4,191 | 55,267 | 13.19 |
| Hawaiian Commercial & Sugar Co. | 15,660 | 205,742 | 13.14 |
| Totals & average | 19,851 | 261,009 | 13.15** |
| **2004** | | | |
| Gay & Robinson, Inc. | 4,903 | 59,111 | 12.06 |
| Hawaiian Commercial & Sugar Co. | 16,887 | 198,755 | 11.77 |
| Totals & average | 21,790 | 257,866 | 11.83** |

* Includes Kekaha salvage cane
** Weighted average

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