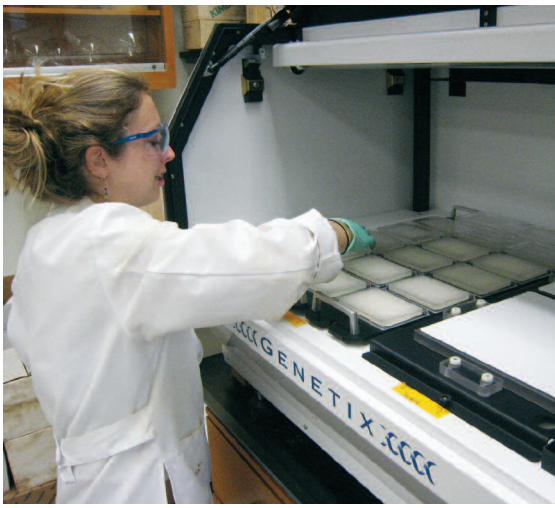
2006

HARC

Hawaii Agriculture Research Center



ANNUAL REPORT

On the Inside

Front cover images, clockwise, from top right corner:

(1) Anthuriums
(2) Annie Byers with molecular biology robotics
(3) *Acacia koa* selections
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Background Image:

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

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Director's Letter

Stephanie A. Whalen,
President and Director since 1994

To our Board of Directors, Members, Clients and Friends

The year 2006 was the 112th year of continuous research and service performed by the Hawaii Agriculture Research Center's research and support staff for Hawaii's agricultural sector. This year HARC again faced a significant challenge: the potential loss of its leased Oahu Kunia field experimental site when its owner put the property on the market. This station is considered vital to the continuity of HARC's research in leeward environments not to mention the existing infrastructure investment on the site. The decision was made to sell the Aiea facility to provide the funds to purchase the field station and co-locate the laboratory and field portion of HARC's activities. The organization is hopeful that in 2007 the many factors necessary to implement this action will be achieved. This provides the opportunity to once again have its primary research activities consolidated at one facility and reduce the inefficiencies created when, in 1975, it lost its former site to the city resulting in the separation of its laboratory and field activities.

In recent annual reports we've been reflecting on happenings 100 years ago. We are continuing this practice and in 1906 there were 54 plantations which produced 429,213 tons of raw sugar from 96,230 acres of sugarcane. In 2006, there were two plantations which produced 213,485 tons from 20,413 acres. With the increase in the national interest in bioenergy it is interesting to note that 100 years ago the organization prepared a report on the profitability of by-products which included the production of ethanol from molasses. This is not a new prospect for the sugarcane industry just one where the economics must once again be carefully considered. There have been no significant advances in the technology for converting molasses to ethanol, however there is a great deal of effort nationally in improvements in related technology as well as in the conversion of fiber to ethanol, a more efficient process. Also reported 100 years ago was the industry's strong influence and efforts in the biocontrol of pests in Hawaii. HARC's entomologist, Mr. Muir became a world traveler collecting beneficial insects for control of the introduced sugarcane leafhopper. The C&H Sugar Refining Cooperative in Crockett, California began operations. The Cooperative provided the industry with a vertically integrated business model allowing value-added margins to flow back to the growers. This helped to offset the higher costs associated with agricultural production in Hawaii and the distance to export markets.

This year's feature article is on progress in sugarcane molecular biology and the challenges for increasing yields.

This report includes an update on the breeding and selection programs and further molecular studies with the *Sugarcane yellow leaf virus* P0 protein and other viral suppressor proteins to advance our understanding of biological processes and help overcome the silencing of introduced high value proteins. Also reported is the successful transformation of sugarcane for resistance to the *Sugarcane yellow leaf virus* and information on the antibody produced to monitor the virus.

In papaya, research in several areas is reported: molecular approaches to reduce diseases caused by *Phytophthora palmivora, Oidium caricae* and *Colletotrichum gloeosporioides;* chromosome walking of the male specific Y region and determining the importance of the centromere in the evolution of this sex

chromosome; cloning of organ-identity genes in flower development; differential expression of the cloned lycopene ß-cyclase gene; and the creation of organ-specific promoters.

The coffee program's first Mokka hybrid families are demonstrating cupping improvements over yellow Catuai and Catimore. Work continues to identify QTL markers controlling tree morphology and fruit quality which will ultimately lead to a more efficient marker-assisted selection program. To advance the understanding of factors affecting quality a collaboration with Dr. Ishihara from Ochanomizu University was established. This work focuses on the metabolic pathways and concentrations of caffeine and trigonelline, two major alkaloids in coffee with quality, flavor and health impacts. To date, chemical markers have been identified that can discriminate among the various environments and conditions coffee is grown in, but so far cannot discriminate for quality.

A renewed interest in cacao production and a timely collaboration between HARC and the USDA ARS/SHRS to preserve and co-locate individual trees with good quality traits are leading to a cacao breeding program. Molecular analysis by SHRS and tasting by an established chocolatier suggested that Hawaii could be a source of high quality chocolate. Identifying superior genotypes from existing stands, reproducing them vegetatively, and establishing a germplasm base has been accomplished in the first steps for a cacao breeding program.

Recognizing the growing export cut flower industry as an important contributor to Hawaii's agriculture, HARC has partnered with the USDA Pacific Basin Agricultural Research Center and the Hawaii Anthurium Grower Association to tackle the two main problems faced by anthurium growers: bacterial blight and the burrowing nematode. This collaboration has resulted in optimizing an *Agrobacterium*-mediated transformation system and the development of several individual transgenic lines for greenhouse testing. HARC also partnered with the University of Hawaii to overcome quarantine barriers for cut flowers by exploring methods to mitigate irradiation injury. Successful protocols were developed for a number of flower species with varying sensitivities to irradiation.

Surveys of koa, *Acacia koa* are ongoing throughout Hawaii to determine the frequency and geographic distribution of natural resistance to *Fusarium* wilt. Artificial inoculation techniques were developed for resistance screening and 332 *Fusarium* isolates have been obtained from diseased koa. Their degree of virulence is being determined and they are being maintained in a special collection for future use.

I want to recognize HARC's loyal and hardworking staff that not only are instrumental in bringing in research dollars, but contribute countless hours volunteering their time and expertise in explaining the role of agriculture and agricultural research to society.

I want to thank HARC's supportive Board of Directors, their dedication to science and their recognition of its place in their companies' struggle to remain competitive in a very tough global agricultural market.

Respectfully,

Stephanie A. Whalen
President and Director

100 Years Ago: 1906

The year of the terrible San Francisco earthquake also ushered in some changes for Hawaii and the sugarcane industry.

Traveling entomologist, Mr. Muir of HSPA Experiment Station, went to Fiji and China on collecting trips to bring beneficial insects to Hawaii for control of the sugarcane leafhopper among other damaging insects. In addition to egg parasites of the leaf hopper, numerous colonies of other beneficial insects were distributed to the plantations. HSPA entomologists were among the first to envision biocontrol of harmful introduced insects by natural parasitoids.

Past experience had shown how very risky it was to import plant material indiscriminately without adequate inspection for unwanted diseases. HSPA took it upon itself to educate plantation managers on inspection procedures since no other organization in the territory was so well equipped to examine plant material. A considerable amount of diseased crop material besides sugarcane was found.

The HSPA Committee on Labor-Saving Devices reported that more labor could be done with the present labor force by painstaking and more skillful use of the horse and new labor saving devices. "'Extra help' does not always mean more men. In some cases, one horse will do the work of four men". One of the new labor saving devices was the innovative Portable Cane Loader, a wheeled vehicle powered by a four horsepower gasoline engine and carrying a jib crane capable of lifting 1,000 pounds. Promising work was reported on the mechanical Ginaca cane harvester.

A report on the profitable utilization of plantation by-products highlighted the possibility of the production of ethyl alcohol from molasses by fermentation and distilling.

That year saw the first Trans Pacific sailing race which has continued as a biennial event ever since. There were three yachts entered in the 1906 race which started near Los Angeles, CA and ended 2225 nautical miles away at Diamond Head Lighthouse on Oahu. The Lurline owned by H.H. Sinclair was first place winner in a time of 12 days, 10 hours. Anemone was second and La Paloma third. The current record time for the race is 7 days, 11 hours and 41 minutes, almost halving the time for sailing vessels over a 100 year period.

The first dog show held in the Islands was given by the Hawaiian Kennel Club. There were 105 entries with J.L. Fleming's pointer Tess winning best in show.

Other items of interest were: the opening of the Kohala Ditch and Wahiawa Reservoir (Lake Wilson), the Oahu Rail and Land company agreed to link the railroad line between Wahiawa and Honolulu, and the Oahu Country Club was formed. The Crockett Refinery commenced operation as C&H Sugar Refining Coop. in Crockett, California.

Sugar production amounted to 429,213 tons which was grown on 54 farms.

- S. Schenck

Sugarcane Research

Oxyfluorfen Herbicide Registered for Sugarcane

A new herbicide was registered for commercial use in sugarcane in late 2006. Goal 2XL© and GoalTender©, Dow AgriScience's products for the compound oxyfluorfen, had final registration requirements completed. Oxyfluorfen was already registered for use in guava, coffee, cacao, and papaya. Much of the registration work done in field trials for efficacy and residue testing was performed by HARC staff in the 1980's and 1990's. A small demonstration test for phytotoxicity on sugarcane of the new water-based formulation, GoalTender, was completed in 2006.

Oxyfluorfen works as a pre-emergent herbicide on most weeds and post activity on broadleaves. It provides control of many of the most problematic weeds in sugarcane fields: guineagrass, Aiea morning glory, and other broadleaf vines. It has great potential to be mixed with other commonly used herbicides such as Prowl (pendimethalin) and Roundup (glyphosate). Work will continue in 2007 on its potential for adoption in certain field conditions.

- M. Poteet

Plantation Review and Recommendations for Greater Productivity

In late 2006, a group of former and current HSPA and HARC staff conducted reviews of plantation agricultural practices for Hawaii's two remaining sugar plantations. The plantation evaluations were pursued at the request of the Board of Directors. It was expressed that determining the causes of a recent drop in total sugar production was vital to the management of each operation.

The evaluation was conducted by former HSPA President and Director Dr. Don Heinz, former Vice President of HARC Dr. Bob Osgood, current President and Director of HARC Stephanie Whalen, Agronomist Lance Santo, Sugarcane Program Manager Albert Arcinas and Assistant Agronomist Mike Poteet. The group visited each plantation and conducted interviews with staff, toured fields, and observed production practices. A comprehensive 78-page report detailing the findings from the evaluations was distributed to the industry.

The final report identified several issues for the plantations to address. Two of the production factors the plantations were recommended to address were age of the crops at harvest and the development of new varieties. Cane harvested less than 24 months of age, significantly contributed to lower yields. Much time and effort have already been spent by plantation staff to begin correcting this problem. The evaluation group advised that more diligent work on variety development be undertaken. Each plantation will require new varieties to maintain a schedule of variety rotation. Variety development must also be evaluated for the development of one-year canes to possibly supplement the recent spike in demand for bioenergy and biofuels in Hawaii. Other areas of concern were planting operations, weed control and availability of irrigation water for crops.

Weather conditions since 2004 also led to many of the problems witnessed for the 2006 crop. Extremely wet conditions restricted many field operations in 2004, leading to poor field preparation and delayed planting. Delays in planting schedules cause operations to be rushed. Rushed planting can lead to poor stands. Stand

establishment is a critical factor in good yields, and problems encountered at early stages are often magnified at harvest. Drought conditions were also experienced at some locations in 2005, limiting productivity during a high-growth period for some of the 2006 crop. During the early spring of 2006, Hawaii experienced historic rain events, as some areas within the State received measurable precipitation 56 consecutive days. Heavy rains can cause anaerobic conditions, leach fertilizer, reduce radiation received by plants and limit photosynthetic activities.

The committee was pleased with the initiative being taken by the plantations to address concerns. The present staff at each plantation has been working diligently to correct the problems outlined in the evaluation. Current staff members have been instituting new practices and research focuses on their respective plantations to address some of their own concerns. Modifications to some of the variety selection practices have been proposed. Plantation managers and staff have taken the lead in implementing recommendations from the committee's report. HARC will continue to provide support and consultation for plantation research and production activities.

- M. Poteet, A. Arcinas, D. Heinz, R. Osgood, L. Santo and S. Whalen

Sugarcane Breeding and Selection

Developing new cultivars by breeding and selection still remains an essential part of HARC's activities. Hawaii's sugarcane industry relies on the development of new replacements for commercial varieties that succumb to disease pressure or yield decline. Through cooperation with USDA sugarcane breeding stations and other research institutions worldwide, HARC has been able to support its breeding objectives by maintaining a large and diverse germplasm collection at the Maunawili Breeding Station on the island of Oahu. In addition to breeding for cane tonnage and sugar yield, the HARC breeding program is also focusing on the potential use of sugarcane as biofuel. New methods and technologies, including the use of molecular markers, are being adopted and are proving to be useful additions to traditional practices.

Sugarcane crossing for 2006 began on November 27 and was completed by January 3, 2007. Fifty-two biparental crosses were made using elite commercial or promising Hawaiian clones. Six biparental crosses were made of Louisiana commercial variety LCP 85-384 x Hawaiian commercial clones in an effort to introduce foreign commercial germplasm into a highly inbred Hawaiian breeding population. Polycrosses (360) were made from 1,065 tassels of 135 breeding clones.

There were 62,496 new seedlings produced from true seeds collected in the 2005-2006 breeding season that were planted at the HARC Variety Station on Maui. From the 51,984 clones planted in 2005 and ratooned in 2006, 2,388 were selected for advancement to FT4 trials in 2007. Of the FT4 clones installed in 2005, 873 were advanced to FT5 in 2006.

In 2006, we evaluated 357 seedling clones in 19 FT7 yield tests, of which 25% advanced to further testing. During the year, 16 new FT7 tests were installed to evaluate seedling clones selected in 2005.

Commercial varieties in 2006 were H65-7052, H77-4643, H78-3567, H78-4153, and H87-4319. H65-7052 is now the leading variety by acreage across the state of Hawaii and continues to increase. It is replacing

H77-4643 on Kauai, which will be terminated after 2006 due to several years of continuous yield declines. In 2005, H65-7052 increased to 37.1% of the state's total acreage as it replaced H78-7750 as the dominant variety in Maui. It is estimated that around half of the state's total sugarcane acres are planted with H65-7052. With the exception of H87-4319, which is on the increase in Maui, all other commercial varieties remain at roughly the same percentage of total acres as in 2005.

Three promising varieties, H87-5794 and H95-4655 in Maui and H93-4068 in Kauai are being evaluated in large acre block tests as potentially new commercial varieties. Upcoming challenges facing the breeding program are renewed interest in one-year cane production, revisiting selection strategies for drought tolerant cane, and the onset of a new national directive to develop sugarcane into a bioenergy crop.

- A. Arcinas

Ethanol Gives Hawaii's Sugarcane Producers New Potential Markets

On April 1, 2006, the State of Hawaii began enforcing a previously passed bill that required 85% of the State's gasoline be sold as a blend of 90% gasoline with 10% ethanol, also known as E-10. This E-10 mandate created a significant demand for ethanol in the State. To meet current demand, Hawaii is currently shipping in over 100,000 barrels of ethanol per month from all over the world. Nations supplying Hawaii with its ethanol are the United States, Jamaica, China, and countries in South America.

Ethanol has moved to the front of the global warming and fuel security debate in recent years. The United States produced almost 5 billion gallons of ethanol in 2006. A large majority of this ethanol is produced from fermentation of corn grain. The most efficient method for ethanol production by fermentation is to use sugar-producing crops such as sugarcane and sugarbeets. By the end of 2006, much of the talk across the nation centered on cellulosic ethanol. Cellulosic ethanol is made from the three main components of biomass: cellulose, hemi-cellulose, and lignin into liquid fuels. The development of cellulosic ethanol technologies is still some time away, but efforts are focused on two methods of production. One method is called gasification, where the solid biomass is heated and pressurized to form a synthetic gas which is then condensed and refined into ethanol. The second method is biological in nature, using enzymes to break down the biomass components into carbohydrates that can then be fermented like traditional ethanol processing.

The E-10 mandate has provided Hawaii's sugarcane plantations an opportunity to diversify operations to increase profitability in the future. Each plantation is moving forward on plans to construct new ethanol manufacturing facilities adjacent to current milling operations by the end of the decade. Ethanol production at sugarcane plantations can be based around waste molasses streams, raw sugar streams, a combination of the two, or, in the future, biomass utilization. Hawaii's sugarcane growers plan to capitalize on these possibilities by incorporating ethanol manufacturing into their current operations. Research into oil crops to help offset Hawaii's dependence on fossil fuels will expand. HARC is attempting to develop a sustainable research program in several oil crops that can benefit the public and private sector in the coming years.

- M. Poteet

The P0 Protein of Sugarcane Yellow Leaf Virus has Novel Activities that Suppress RNA Silencing

RNA silencing is an essential part of gene regulation in all higher organisms and is now acknowledged as critical to proper regulation of many biological processes. In 2006, the Nobel Prize for Medicine was awarded to two scientists for discoveries of specific RNA silencing mechanisms in nematodes. Plants use RNA silencing to regulate endogenous genes, and also as a defense mechanism against viruses. Viruses in turn have evolved suppressor proteins which block RNA silencing and thereby allow the virus to evade host defenses. We have previously shown that the *Sugarcane yellow leaf virus* (SCYLV) P0 protein acts to suppress PTGS in corn and in the experimental plant *Nicotiana benthamiana*. When RNA silencing is induced by a single-strand RNA, SCYLV P0 (P0SC) blocks RNA silencing both locally and systemically. Additionally in *N. benthamiana,* P0SC induces cell death. P0 proteins from dicot-infecting viruses (*Beet western yellows virus, Cucurbit aphid-borne virus,* and *Potato leaf roll virus*) suppress local silencing, but do not suppress systemic silencing, and do not induce cell death in the *N. benthamiana* system. It has now been shown that the dicot P0 proteins act by targeting an essential component of the plant's silencing machinery for degradation.

Using deletion analysis, we have defined those regions of the P0SC protein which contain the functional domains required for suppressor activity. A 15 amino acid region at the carboxy terminus of the protein was shown to be required for suppression of systemic silencing and for induction of cell death. Deletion of these 15 amino acids leaves the truncated protein with only the suppression of local silencing activity, similar to the dicot P0 proteins. This suggests the possibility that P0SC may contain functional structures similar to the dicot P0 proteins, but has one or more additional domains which impart the novel activities.

Viral suppressors of silencing may be applied for protecting transgenes from gene silencing, however this remains a long shot. The basic research we are doing now on this suppressor and more broadly on RNA silencing is helping to build a base for future applied crop improvement in the areas of plant development, gene regulation, and pathogen defense.

- M-L. Wang, S. Ancheta, E. Ahrendt, T.E. Mirkov (Texas A&M), W. Borth (UH), J. Hu (UH), P.H. Moore (USDA ARS) and H. Albert (USDA ARS)

Production of Recombinant Proteins in Sugarcane

Sugarcane has several important advantages for transgene/product containment that may position this crop as a very "secure" platform for production of high-value recombinant proteins. In Hawaii, there is little chance of pollen drift or production of viable seed in the commercial fields, since commercial cultivars either do not flower or can be prevented from flowering during production. Even if viable seed were produced, it would not become mixed with seed supplies because commercial fields are planted with stem pieces or cuttings, not true seed. The food commodity derived from sugarcane, sucrose, is sold as a crystalline product that is essentially free of protein. Thus, in the unlikely event that sugarcane producing a recombinant protein was mixed into the food supply, the food prod-

uct (refined sucrose) would remain unaffected.

New technologies currently under development hold great promise for increased efficiency in producing fuels from biomass. Even under very optimistic assumptions however, these new energy sources will probably be economically marginal. In addition, producing high-value recombinant proteins in a crop to be used for bioenergy production could greatly improve the economic feasibility of the integrated process. Additionally, producing pharmaceuticals, as an example of high-value proteins, in a biomass crop should eliminate the potential for accidental introduction into the food supply. There are several significant efforts worldwide underway to develop sugarcane as a biofactory system. Currently, Hawaii has excess capacity for growing sugarcane which could be simultaneously available for bioenergy and biofactory applications. Utilizing Hawaii's natural advantages and resources to produce high value proteins provides a great economic opportunity for the state and to meet critical human health needs with minimum risk to human food supplies or the environment.

Previously, we demonstrated the feasibility of producing an active pharmaceutical in sugarcane, and also showed that higher levels of protein accumulation need to be obtained before the approach could be economically feasible. One of the major limiting factors for recombinant protein accumulation in sugarcane is transgene silencing.

We are testing the utility of viral suppressors of gene silencing on recombinant protein production in transgenic sugarcane plants. However, since RNA silencing can be critical to several important plant functions including regulation of endogenous genes via the miRNA pathway, expression of viral suppressors may produce unacceptable side effects in transgenic plants. To explore these possibilities, we produced sugarcane transgenic lines which constitutively express one of four viral PTGS suppressors: P0 from *Sugarcane yellow leaf virus* (SCYLV), HC-Pro from *Sorghum mosaic virus* (SrMV), p25 from *Potato virus X* (PVX), and p38 from *Turnip crinkle virus* (TCV).

Expression data from P0 and HC-Pro transgenic plants showed that those lines with the highest mRNA accumulation for an *npt*II transgene also had high levels of the suppressor mRNA. Expression of five sugarcane genes predicted to be regulated by miRNAs (Jones-Rhoades and Bartel, 2004) was tested by qRT-PCR. For all five of these genes there were no consistent differences in HcPro and P0 transgenic lines as compared to WT sugarcane. Small RNA northern blots indicated that HcPro and P0 had no obvious effect on the accumulation of five different miRNAs. Morphologically, some suppressor lines are abnormal, but the frequency of these changes is similar for other (not expressing suppressors) sugarcane regenerated from tissue culture. We are currently analyzing the effect of P38 and P25 on transgene and endogenous gene expression, miRNA accumulation, and morphology in the transgenic sugarcane plants.

Jones-Rhoades, M. W. and D. P. Bartel (2004). "Computational Identification of Plant MicroRNAs and Their Targets, Including a Stress-Induced miRNA." *Molecular Cell 14*(6): 787-799.

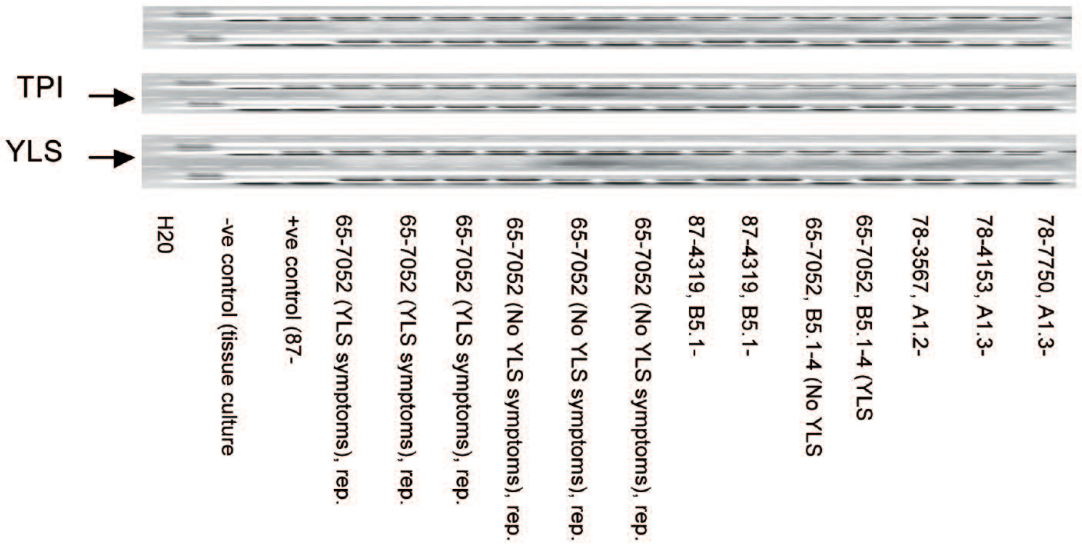
- S. Ancheta, W. Borth (UH), J. Hu (UH), T.E. Mirkov (Texas A&M) and M-L. Wang

Real-Time PCR-Based Quantification of Sugarcane Yellow Leaf Virus (SCYLV) in Hawaiian Sugarcane Cultivars

Sugarcane yellow leaf virus (SCYLV) has been reported on sugarcane worldwide and has caused significant sugar yield losses, especially when the crop is under stress. The current detection method using tissue blot immunoassay (TBIA) is not quantitative. Since information on plant virus titre was needed, we developed a real-time reverse transcriptase (RT)-polymerase chain reaction (PCR) assay for the detection and relative quantification of SCYLV in sugarcane cultivars in Hawaii. In addition, the relationship between virus titer and yellowing symptoms was examined in variety H65-7052, which frequently shows yellowing symptoms.

Sugarcane midribs (75 mg) were ground to a fine powder in liquid nitrogen and RNA was extracted using the RNeasy kit (Qiagen) manufacturer's protocol. Semi-quantitative RT-PCR was performed using 50 µg of total RNA treated with DNAase. Reverse transcription was performed using 5 µg of total RNA. For each PCR reaction, the cycle number was optimized in order to remain in the exponential phase of the PCR reaction. Primers were designed based on the SCYLV P0 reading frame sequence and triphosphate isomerase (TPI) primers were used as internal control. All primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). Each 50 µL PCR reaction contained 1 µL of cDNA with a final primer concentration of 400 nM. Cycling conditions were as follows: 95° C for 30 s; 35 amplification cycles of 95° C for 30 s, 55° C for 30 s, 72° C for 45 s; 72° C for 45 s for both primers. PCR products were visualized on a 2% agarose gel with ethidium bromide.

The results provided below show that the virus titers in the sugarcane cultivar H65-7052 are higher in infected plants with symptoms and lower in the infected plants without symptoms, indicating that the plant yellowing is positively correlated with virus titer.



Quantitative real-time PCR was performed using platinum SYBR Green qPCR SuperMix UDG (Invitrogen) in a DNA Engine Opticon 2 (MJ Research, Inc). The results of qRT-PCR confirmed that virus titer was lowest in the Hawaiian cultivars which usually gave negative

results in the TBIA. These cultivars (H78-7750, H78-3567, and H78-4153) were previously thought to be resistant to infection. Therefore, the RT-PCR method proved to be more sensitive than TBIA. The highest titres were observed in cultivars that always give a high percentage positive tissue blots. The only plants giving negative reactions with qRT-PCR were meristem tip cultured plants or plants derived from somatic embryos. Sugarcane varieties that supported only very low concentrations of virus apparently have some resistance to virus multiplication or to systemic movement of the virus and their resistant mechanisms are being studied.

The qRT-PCR method provided quantitative measurements of virus titre which will be studied in relationship to the starch and sugar contents in these lines to determine how the virus impacts on starch and sucrose accumulation.

- S. Lim, S. Schenck and Y.J. Zhu

Sugarcane with yellow leaf virus symptoms

Tropical Fruit Research

Molecular Biology Approaches to Disease Control

Tropical fruits are important crops in tropical regions, providing revenue to local economies. They are among the most valuable agricultural commodities grown and sold in the tropics. In addition, they have significant nutritional value and can play integral roles in the daily lives of consumers. Understanding and controlling diseases in tropical crops are one of our main objectives in research and development at Hawaii Agriculture Research Center.

Papaya (*Carica papaya* L.), Hawaii's fourth most important agricultural crop following sugarcane, pineapple, and macadamia nuts, is susceptible to more than a dozen fungal pathogens. The black spot fungus, *Asperisporium caricae,* is the most recent introduction of papaya disease species into Hawaii. This fungus is prevalent in the Caribbean, Central and South America, South Africa, Brazil, Australia, Taiwan, Texas, and Florida. It was first reported on the Island of Maui in February 2001, and shortly thereafter on islands of Hawaii, Oahu, and eventually on Kauai by August 2001 (Nishijima 2002). The other major fungal pests of this crop already established in Hawaii are, *Phytophthora palmivora* root and fruit rot, anthracnose (*Colletotrichum gloeosporioides*), and powdery mildew (*Oidium caricae*). These fungal diseases are serious constraints to papaya production, causing significant losses for most growers. A review (November 2001 by USDA, ARS) revealed approximately 171 different fungi reported on papaya throughout the world. Of those, only 34 (20%) have been reported in Hawaii. In addition, six of eight bacteria, five of eight viruses, three phytoplasmas, and one rikettsia known to cause papaya diseases are not yet present in Hawaii (Nishijima 2002). Those diseases could come into Hawaii and, together with established fungal diseases, could cause major problems.

At Hawaii Agriculture Research Center, we have several projects on tropical fruits, including papaya and pineapple. With a papaya genome project, our research team aims to find all genes or proteins related to disease resistance and further understanding the roles of these genes and proteins in the interaction of plant and pathogens. For several reasons, papaya can serve as a model for other tree fruits for the study of plant/pathogen interactions. Papaya is a diploid plant with nine pairs of chromosomes and a small genome size of 372 Mbp (Arumuganathana and Earle 1991; Storey 1941). Papaya can easily be genetically transformed, can easily make genetic crosses, has a short reproductive cycle, produces large numbers of offspring, and has continuous flowering through the year. It has, therefore, become an attractive model plant for fruit tree genetic, genomic, and proteomic research. Over the last few years, there have been several major advancements in papaya genetic, genomic and proteomic research.

Carica papaya L. is susceptible to many diseases, including those caused by virus, fungi, and oomycete pathogens. It is highly susceptible to *P. palmivora* (Nishijima 1994). Heavy yield losses associated with *P. palmivora* fruit and root rot can cause severe decline or death of papaya trees, particularly in poorly drained areas, during the cool and rainy season of winter (Nishijima 1994). Innovative control methods are needed to decrease dependence on fungicides, increase crop productivity, and improve pre- and post-harvest fruit quality. Bioengineering has provided partial resistance to *P. palmivora* (Erwin and Ribeiro 1996). Papaya expressing the grape stilbene synthase (*Vst1*) gene driven by its native promoter showed increased levels of resveratrol glucoside and resistance to *P. palmivora* as compared to untransformed controls (Zhu et al. 2004a). Likewise, papaya transformed with *DmAMP1*, under the control of the *CaMV35S* promoter, accumulated significant levels of *DmAMP1*, a defensin, providing increased resistance (Zhu et al. 2007).

Methods were developed for transforming papaya with the papaya ringspot virus (PRSV) coat protein gene, making a papaya variety that is resistant to the virus. Release of these transformed varieties saved Hawaii's papaya industry (Fitch et al. 1992; Gonsalves 2002).

Studies have been carried out on the systemic acquired resistance pathway in papaya (Zhu et al. 2004b). The key regulatory gene NPR1 has been cloned and the over-expression of NPR1 gene in papaya to activate the SAR is completed. Several pathogenesis related protein (PR genes) have also been cloned and characterized.

Good progress has been made in our lab and several other labs toward isolating tissue-specific and pathogen inducible promoters, which can be used to transform candidate genes for improved disease resistance or fruit quality.

A related wild type species of *Carica papaya,* named *Goudontiana* in the *Vasconcellea* genus was determined to be resistant. Molecular and biochemical characterization will be carried out to find its resistance mechanism and its equivalent in *Carica papaya*.

A good BAC library (Ming et al. 2001) and several cDNA libraries including a cDNA library from papaya root inoculated with *Phytophthora palmivora* were developed as resources for molecular research.

A high-density genetic map of papaya was constructed and important genes for controlling sex determination and fruit flesh color were tagged (Ma et al. 2004). It will be essential for cloning specific genes of interest such as the sex determination gene and disease resistant genes as well as for the integration of papaya's genetic and physical maps.

The genome sequencing of papaya has been completed. HARC researchers are collaborating with University of Hawaii, USDA, and University of Illinois to perform bioinformatics analyses, including data mining of genes and proteins involved in disease resistance and signal transduction involved in the interaction of plant and pathogens.

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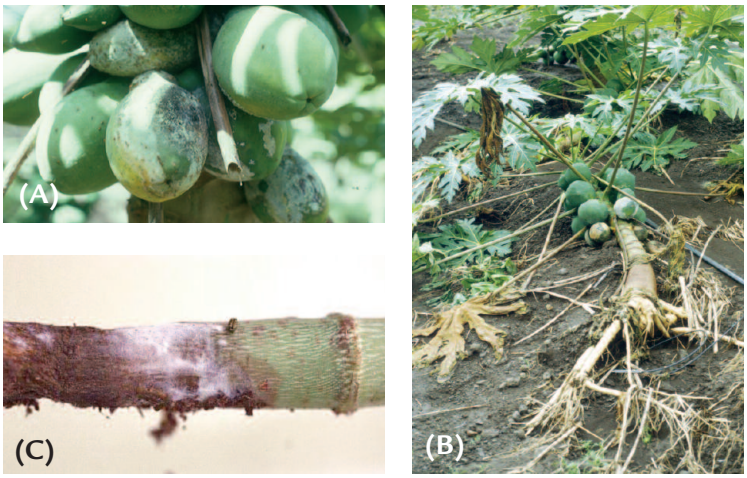
- Y.J. Zhu



Papaya Diseases
(A) *Fruit – rot,*
(B) *Root – rot,*
(C) *Stem – rot in Papaya caused by Phytophthora palmivora*

Pictures were taken from http://www2.hawaii.edu/~snelson/papaya/

Elicitor-Induced Plant Defense and Pathogenesis-Related Gene Expression and Accumulation in Carica papaya L.

Systemic acquired resistance (SAR) is a general defense response in plants that is characterized by the accumulation of salicylic acid (SA) and expression of pathogenesis-related (PR) genes. The basic elements of papaya SAR were reported to resemble the pathway described in *Arabidopsis thaliana*, and an *NPR*1 (non-expresser of pathogenesis-related genes) homolog (Cp*NPR*1) was isolated and characterized.

We produced transgenic papaya plants over-expressing the *CpNPR*1 and expressing the *Arabidopsis NPR*1 gene (At*NPR*1). Western blot analyses using an *NPR*1 antibody on non-induced samples showed that the *NPR*1 protein was more abundant in the Cp*NPR*1 and AtNPR1 lines compared to the non-transformed control. These results agree with previous studies that the *NPR*1 protein is constitutively expressed in the cytoplasm and that the higher protein expression is due to increased *NPR*1 gene expression.

Transgenic plants were challenged with a *Phytophthora*-derived elicitor, Pep-13 benzothiadiazole (BTH), a functional analog of salicylic acid and also with *P. palmivora* zoospores; and dH2O. Treated samples were collected at different times after inoculation. Since *NPR*1 regulates the expression of PR genes after pathogen induction, changes in the level of *PR*1 gene expression were determined using Northern and quantitative RT-PCR. Results showed that PR1 gene expression was faster and higher in the transgenic lines on samples treated with Pep-13 and BTH. On the other hand, *P. palmivora* zoospores inoculations on Cp*NPR*1 lines produced a higher *PR*1 gene expression compared to At*NPR*1 lines. This supports our hypothesis that over-expression of the *NPR*1 gene in papaya leads to a stronger defense response after pathogen infection.

The amount of pathogen present in inoculated non-transformed control and transgenic lines showed that the transgenic lines were more tolerant to *P. palmivora*. This was also determined using a DAS ELISA for *Phytophthora*. Preliminary ELISA results transformed control.

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

Developing an Agrobacterium rhizogenes Protocol for Papaya Transformation

Agrobacterium rhizogenes is a gram-negative bacterium related to the better known *Agrobacterium tumefaciens*. Unlike *A. tumefaciens*, *A. rhizogenes* causes adventitious hairy roots to form in infected plants instead of crown gall tumors. Using *A. rhizogenes*-mediated transformation may be an effective tool in studying root pathogen and plant interactions. This system can be further utilized to induce the formation of transgenic roots, and subsequently regenerated plants, in a short amount of time compared to the time required for root formation via particle bombardment or Agro-infiltration methods of transformation. This advantage will facilitate studying transgenes in the root and their molecular and pathological effects on disease resistance.

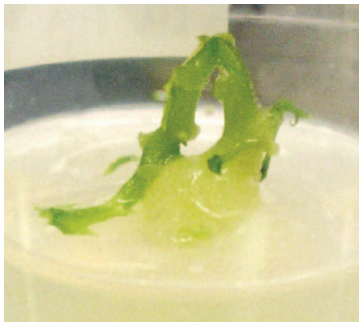
This protocol is being developed particularly for studies between *C. papaya* and of the pathogen *Phytophthora palmivora*, which is a fungal-like Oomycete that causes root, stem, and fruit rot. *P. palmivora* is particularly devastating to papaya growers in Hawaii and other

tropical regions because it flourishes well during rainy seasons where soils are saturated and chemical fungicides are washed away and ineffective. Our future objective is to use this transformation protocol to study and improve *C. papaya* resistance to *P. palmivora*.

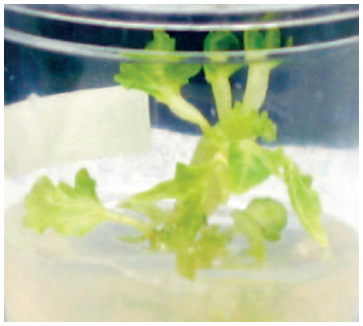
Preliminary transformations using *A. rhizogenes* to infect micropropagated and soil-grown *C. papaya* plants were successful. Roots emerged from the sites of infection typically 1-3 weeks after inoculation with the bacteria. In the micropropagated plants, callus formation occurs at the base of the hairy roots soon after the adventitious roots emerge. From there the callus and roots are excised and placed on media to promote plant regeneration. Improvements to the procedure are being developed for more prolific hairy root formation and efficient regeneration of plants from the roots.

Micropropagated papaya tissue culture shows adventitious root formation after inoculation with A. rhizogenes.

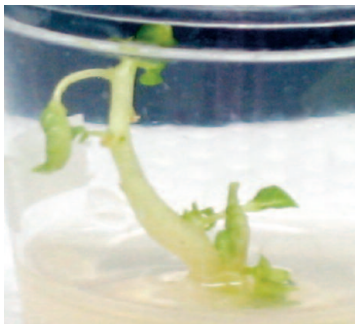
Root emerging from site of infection in a soil-grown young papaya plant.



(A) *Kp, non-transformed*



(B) *CpNPR1*



(C) *AtNPR1*

Tissue culture plants after P. palmivora zoospore inoculation. **(A)** *Kp, non-transformed control;* **(B)** *CpNPR1, transgenic line transformed with NPR1 homolog from papaya;* **(C)** *AtNPR1, transgenic line transformed with Arabidopsis thaliana NPR1 gene.*

- K. Noorda-Nguyen and Y.J. Zhu

Papaya Propagation by Micropropagation, Cuttings and Air Layering

Seed of "Rainbow" papaya and other hybrid cultivars is often not available to farmers; and in addition, several seeds must be sown in pots in a nursery or directly in the field to ensure that there will be at least one hermaphrodite plant in each planting position. Plants are grown until flowering to determine which are the desirable hermaphrodites, a process which leads to tall, weak plants . Micropropagation of selected high-yielding papaya hybrids was developed to provide a means of vegetative propagation. Micropropagation must be done in a lab and, with current methods, is an expensive means of propagation. Our project is directed toward increasing the number of plants available to the farmers who prefer to vegetatively propagate papaya and to enable the farmer to produce his own planting material from micropropagated stock plants.

Vegetative propagation enables the farmer to plant one tree per hole, reduces the amount of fertilizer, and eliminates the need for planting multiple seeds (3 – 9 per hole) or seedlings. Thinning is eliminated resulting in strong individual plants at each planting site. Vegetatively propagated Rainbow papayas in Keaau were shown to bear fruit 3 months earlier, 12 inches lower on the trunk, and produced 25,000 lbs/acre more fruit in the first year compared to thinned seedlings (Fitch et al., 2005a, b).

Micropropagated Rainbow papaya plants were produced in the lab in Aiea and shipped in glass jars to Hilo. They were transplanted into pots and used as stock plants for cutting material. The cuttings produced were made available for sale.

The objectives of the project were to:

1. Increase plant production in the nursery by doubling or tripling the number of plants obtained from a single micro-propagated plant and to establish disease free stock plants in the greenhouse.
2. Educate farmers on methods for rooting papaya cuttings from greenhouse-grown stock plants and field cuttings.
3. Encourage farmers to vegetatively propagate papaya.
4. Investigate different methods of propagation which might be more effcient.
5. Reduce waste incurred by over-planting of seed, and make use of the side shoots which are normally knocked off the plants in the field and discarded.
6. Experiment with crop protection chemicals for decontamination of the field-grown cuttings prior to rooting. Many of the survival problems associated with this tissue type were the result of rotting. Other problems are insects, fungi, mites, and scales.
7. Encourage farmers to practice good pest control in their fields to ensure clean propagative material.
8. Determine if age of cutting stems (young, green, or old corky stems), diameter, season, potting mixture, and irrigation regime have an impact on rooting success.
9. Produce and make available for sale rooted cuttings of hermaphrodites and to accomplish technology transfer of both the cuttings and micropropagation protocols to growers and tissue culture businesses on the Big Island.
10. Supplement the ongoing 'Rainbow' and 'Laie Gold' papaya micropro-agation projects in order to increase increase our output of plants to keep up with farmer demand.

From 947 papaya micropropagules shipped to Hilo, 915 survived transplanting in the nursery. Of those, 137 were potted for stock plants; 258 stock plants were still in the nursery at the end of December 2006, and 1,490 plants were sold.

We experimented with air layering of the greenhouse plants, but found that the papaya grew too fast indoors. Outside the greenhouse the air layer media and the bag broke before roots formed.

Additional experiments are being made using tops and internode sections from smaller micropropapagated plants in rose pots. We are encouraged that where earlier losses from field stock cuttings were more than 60%, loss from rot using greenhouse-grown micropropagated material has ranged from 0 to 7 percent. Tops of side shoots have the highest success rate for propagation, and the tops of the younger microprops root easily and uniformly. Tops from the mother plants in larger pots take much longer to root and tend to vary in size.

Funding for the project was from the Hawaii County Research and Development (HCRD) and Hawaii Agriculture Research Center (HARC), Hawaii Farm Bureau Federation (HFBF) and Hawaii Department of Agriculture (HDOA).

- A. Yeh and M. Fitch (USDA ARS)

Micropropagated papaya plants on arrival

Micropropagated plants 3 weeks after transplanting

Drip irrigation.

Organ-Specific Promoter in Papaya

The goal of this project is to identify papaya organ-specific genes using cDNA-AFLP (complementary DNA-amplified fragment length polymorphism) and then to isolate a set of gene promoters corresponding to these differentially expressed genes. Organ-specific promoters can direct transgene expression specifically in those tissues where the expression is required. Tissue-specific expression will limit novel protein production and thus could reduce impact on normal plant growth and development. These new promoters will aid current and future basic research and enable engineering of papaya and other dicotyledonous plants for economically important traits such as disease-resistance, enhanced production, and increased shelf life.

We compared the gene expression profiles of 15 different tissues/developmental stages of *Carica papaya* L. "Sun Up." More than 800 putative organ-specific cDNA-AFLP bands were identified from 264 AFLP primer pairs. We have cloned and sequenced 53 clones out of the approximately 150 bands isolated. The expression pattern of four clones were confirmed using quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

One of the clones, FH-74-400, is expressed at least 100-fold higher in hermaphrodite flowers than in any other organs. The translated peptide sequence of FH-74-400 shows homology with a polygalacturonase (PG) isolated from pollen of *Medicago sativa* (accession number: U20431). Parts of the

FH-74-400 sequence matches to papaya genome sequence supercontig_8.

The expression of FB-73-230, in fruit was 35-fold higher than all other organs. It was highest in the earlier stages of fruit ripening and decreased as the fruit ripened. There was no significant match to the sequence of FB-73-230 found in GenBank but it does match to papaya genome supercontig_74.

The sequence of a root-specific band, RY-6A-462, has homology to a nodulin-like protein in arabidopsis (AK176079). It is only expressed in the roots of young papaya seedlings, not in the roots of mature field-grown plants. RY-6A-462 matches to supercontig_0 of papaya genome.

SC-66-280 is expressed strongly in the seeds, but can also be detected in the fruit. It is expressed strongly in the late stage of development. The sequence is homologous to those of cytochrome P450 genes, with the best match to a gene from avocado (M32885). SC-66-280 matches to the papaya genome contig_37856.

We are continuing to sequence more putative organ-specific cDNA-AFLP bands and verify their expression patterns. The promoter regions of those cDNA fragments with desired expression patterns will be isolated with the help of the papaya genome sequence generated by the Hawaii Papaya Genome Consortium.

- E. Ahrendt, N. Limthong, R. Martin, Q. Yu, R. Ming (UIUC), P. H. Moore (USDA ARS), H. Albert (USDA ARS), R. Paull (UH) and M-L. Wang

Irradiation-Derived Sex Reversal Mutants for Cloning Sex Determination Genes in Papaya

Unlike most animals, most flowering plants are hermaphrodites possessing both male and female organs. Papaya (*Carica papaya* L.) is one of the few plant species that produce male, female, and hermaphrodite flowers on separate individuals. These variations have distinctive morphologies and sexual functions in a single species and serve as a useful model for investigating the genomics of sex determination in plants. Sex determination in papaya is controlled by a primitive Y chromosome, containing functional and degenerated genes that regulate sex organ development, inflorescence morphology, and embryo abortion. We are engaged in a project to fully sequence the male-specific region of papaya's Y chromosome (MSY) and its corresponding region on the X chromosome for obtaining sequence information necessary to characterize and clone genes controlling sex-determination. We used Co^{60} γ-irradiation to mutate pollen within flower buds of the gynodioecious variety 'SunUp' and the dioecious variety AU9 to produce Y deletion lines. Three mutants were found among nearly 5,000 plants derived from irradiated pollen. All three mutants were from the 5 krad treatment and had the long, pendulous, many flowered cymose inflorescence typical of male plants, but the flowers borne on the inflorescence were not the slender, anther bearing male flowers. One of the mutants exhibited typical female flowers with large functional pistils and no stamens, while the other two mutants showed bisexu-

al hermaphrodite flowers. A total of 392 pairs of primers were designed from five completely sequenced MSY BACs to cover about 580 kb of the MSY region and used to amplify the genomic DNA from the mutant plants. The sequenced PCR products confirmed that all three mutants had the male Y chromosome sequences except for a few single nucleotide deletions. Analysis of the deletion lines is concurrent with sequencing of the MSY to focus attention to regions with candidate genes for sex-determination.

- Q. Yu, P. H. Moore (USDA ARS), R. L. Skelton, A. Blas (UH) and R. Ming (UIUC)

Biocontrol of Nematodes on Pineapple

The aim of this project was to control reniform (*Rotylenchulus reniformis*) nematodes on pineapple using products that are environmentally safe, sustainable, and cost effective. Pineapple in Hawaii is severely affected by nematodes on every island. Without the use of preplant and postplant nematicides, no commercial production would be possible. Fallowing between crop cycles, intercropping with certain covercrops, and favorable cultural practices have been tried and gave some improvement, but have not proven to be cost effective for economic plantation production. There has been very little success in breeding for resistance since the more resistant wild-type *Ananas* species have undesirable agronomic and fruit characteristics. The trial was installed in a commercial pineapple field known to have high populations of reniform nematodes.

LCF (ABR Liquid Compost Factor) is a bionutrient product that improves the growth of plants. LCF is a fermentation product of an edible fungus and a mixture of pineapple, papaya juices, and sugarcane molasses. In addition to its fertilizer-like properties, LCF appears to aid plants in recovery from disease infection. LCF has induced nematode tolerance in some susceptible plants.

Paecilomyces lilacinus is a naturally-occurring soil fungus that parasitizes nematode eggs, larvae, and female adults. A commercial formulation of *P. lilacinus* strain 251 (Prophyta Biologischer Pflanzenschutz GmbH) was used in this study. Both products were applied through drip irrigation to test plots located within a commercial pineapple field and maintained by Dole Pineapple Co. All plots received Telone soil fumigant preplant. Treatments were: 1) Telone preplant, 2) Telone preplant plus Nemacur applied through drip irrigation at 24 weeks postplant, 3) Telone preplant plus Paecilomyces (0.2 g/plant) at 12, 16, 20, and 24 weeks postplant, and 4) Telone preplant plus LCF (2 qt/acre) at planting, 12, 24, and 36 weeks postplant. The trial was installed on July 27, 2005. Plot size was 25 ft by 3 beds (6 lines). Plant to plant spacing was 10 1/2 inches with a 3 ft unplanted border between each plot. There were 6 replicate plots per treatment.

Counts of reniform nematodes were taken in the plots on November 16, 2006. Counts for the 6 plots of each treatment were averaged and analysis of variance performed.

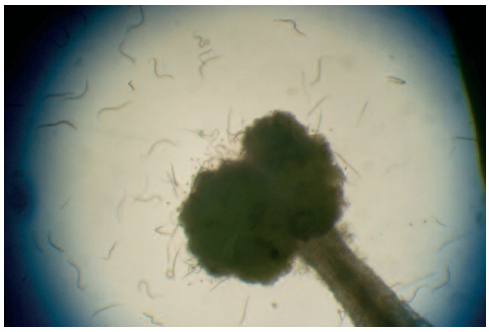
Pineapple harvest took place on October 30 through November 8, 2006. Results are reported as average fruit weight in grams of total fruit averaged for the 6 replicate plots per treatment.

Treatment	Average Count
1. Telone	6488 a
2. Telone, Nemacur	4421 ab
3. Telone, Paecilomyces	3737 ab
4. Telone, LCF	2035 b

Treatment	Average Fruit Weight
1. Telone	1456.6 b
2. Telone, Nemacur	1515.4 ab
3. Telone, Paecilomyces	1645.4 a
4. Telone, LCF	1632.7 a

The results of the trial have shown that biocontrol methods are as effective as postplant treatments of the soluble nematicide, Nemacur for reducing reniform nematode populations and maintaining fruit production of pineapple. All of the postplant treatments gave improved yields over preplant fumigant alone.

- S. Schenck and B. Sipes (UH)



Nematodes with root tissue in sterile culture.

Beverage Crop Research

Both coffee and cacao have existed in the Hawaiian islands since the 1800s. They face the same problems today as they did then: competition on the world market, prevention of the introduction of foreign pests and diseases, and maintenance of high quality. However, we now have better techniques at our disposal. As shown in the following articles, HARC scientists and their collaborators are engaged in breeding and selection programs, improvement of disease and nematode resistance, and quality enhancement aided by molecular genetics and biotechnology

Selection and Propagation of New Coffee Hybrids

Development of new coffee cultivars with uniquely Hawaiian adaptability and quality is the target of our breeding and selection program. Cultivars are being developed by traditional breeding and selection along with biotechnology techniques, such as propagation via tissue culture and DNA markers, which will shorten the time to accomplish our goals.

We initiated a field trial of 13 selected hybrid coffee families in a commercial field at Kauai Coffee (Eleele, Kauai) in 2004 to identify and select superior families and individual trees (HARC Annual Report 2005 p.24-25). During the first two years of the trial, growth and morphological characters were measured including tree height. Wind damage necessitated replanting about 30% of the field. Segregation in tree morphology was observed in some families, but was slight in other families.

The first harvest from the Kauai trial was conducted in the fall of 2006. Cherries were harvested from four replicated plots. Since it was the first year of harvest, yields from single trees were less than the yield potential of each tree. A total of 225 lbs of cherries was harvested and 42 lbs of green beans were produced.

Cherries were wet processed for evaluation of bean characteristics and cupping quality. Multiple quality evaluation was conducted by the Kauai Coffee Team and Hawaii Coffee Growers' Association (HCGA) members. Several Red Catuai, mokka and SL28 hybrids were selected as promising families when compared to coffee of known high quality. Additional evaluations will be conducted by professional cuppers outside of Hawaii.

Seeds from selected hybrid trees were distributed to HCGA growers for further field trials on other islands with different growing environments. It is expected that promising hybrid families and trees for each grower will vary due to interaction of genotypes and environment in their micro-environments.

To scale up the production for genetic research and clonal propagation, HARC developed a protocol for production of somatic embryos (SE) (HARC Annual Report 1988). The "Rita System"was developed by CIRAD in France. All of the 13 hybrids in the Kauai trial were selected for propagation. Leaves from vertical branches of the selected trees of hybrid families were used for explants and several different media were tested to improve initiation of primary callus. Initial conditions for somatic embryo multiplication and plant regeneration were tested using the control cultivar, 'Red Catuai'. We are continuing the research on optimization of the culture media and conditions for efficient SE production.

Funding for the field and laboratory work on the development of coffee cultivars was provided by the Hawaii Coffee Growers' Association and the State Department of Agriculture.

- C. Nagai, R. Heinig, D. Adamski, J. Buenafe and G. Williams (Kauai Coffee Co.)

Cloning and Characterization of Gene(s) Related to Organ Size in Coffee

Farm revenue from coffee in Hawaii has increased over the years due to its nationally and internationally recognized premium quality. In order to maintain this high quality coffee, the development of Hawaiian coffee cultivars with new and better flavor profiles along with high yields is required. One of the genetic resources for high quality is the variety 'Mokka', which has superior flavor, but low yields due to the small bean size. It produces the smallest beans among all *C. arabica* varieties. Two recessive mutations (*laurina, lr*, and *mokka*, *mo*) in 'Mokka' caused pleiotropic effects throughout the plant, including significantly decreased fruit and leaf size and whole plant stature. In contrast, variety 'Typica' is high yielding and one of the most commonly grown varieties in the world. It is grown in Kona as well as in Jamaica as the world-renowned Blue Mountain coffee. Several 'Mokka'-'Typica' hybrid cultivars are present in our coffee breeding germplasm collection. One of them, MA2, appears to be very similar to 'Kona-Typica' (KO32) based on amplified fragment length polymorphism (AFLP) marker analysis (Ray Ming, personal communication), but retains the small organ characteristics.

The goal of this research is to identify genes related to organ size in coffee by comparing gene expression patterns in 'Mokka Hybrid' MA2 and 'Typica' KO32. We intend to identify a set of genes with altered expression patterns caused by the *mo* and *lr* mutations. This represents the first step towards understanding the genetic control of organ size in coffee. The gene(s) identified in this project can be utilized in the coffee breeding program as marker(s) to maintain coffee yields while breeding for new improved flavor characteristics. Furthermore, the new knowledge on genetic control of organ size may be applied to other crops to improve productivity. We will also determine if molecular tools developed for other plants, potato (*Solanum tuberosum*), can be used to study relatively less characterized plant systems, such as coffee.

Microarrays are widely used to investigate global patterns of gene expression. Due to the unavailability of coffee microarrays, we used a potato microarray as a cross species platform to investigate global gene expression in MA2 and KO34. There are ~35000 spots on the potato microarray covering a total of ~12500 genes. Data from at least eight microarray hybridizations are needed to perform statistical analyses to identify differentially expressed genes. Messenger RNAs from the shoot tips of MA2 and KO34 were converted into cDNAs, labeled with Alexa 555 and Alexa 647 fluorescent dyes and hybridized with potato microarrays. After post-hybridization washings, microarray slides were scanned using a laser scanner to record the fluorescence intensity for both dyes at every spot. Data were normalized for intensity level using the software Genespring. All genes showing a differential expression ratio of less than two were removed from the trial. After statistical analyses (ANOVA), we obtained a list of 45 genes that were significantly differentially expressed between the two cultivars. Among these 45 genes, we found 27 homologous coffee sequences in the published databases. We have designed primers for these 27 genes and are currently verifying the expression patterns using qRT-PCR.

- R. Singh (UH), C. Nagai, M. Kumagai (UH), R. Paull (UH), H. Albert (USDA ARS) and M-L. Wang

Engineering Arabica Coffee for Nematode Resistance Using Cysteine and Serine Proteinase Inhibitors

Root-knot nematodes cause significant economic loss in coffee plantations throughout the world. In Kona, Hawaii, *Meloidogyne konaensis*, the Kona coffee root-knot nematode, has been shown to damage roots of the typica cultivar of Arabica coffee. Research into control of root-knot nematodes on Kona typica coffee was undertaken using biotechnology techniques. Cystatin, a modified cysteine proteinase inhibitor from rice, alone or in combination with a cowpea trypsin inhibitor was inserted into coffee somatic embryos using either *Agrobacterium tumefaciens* or particle bombardment. Over 2,500 viable lines remained after a seven-month selection period on antibiotic media. Plants were regenerated selected somatic embryos of 71 of these lines (HARC Annual Report 2003 p. 22-23).

A nematode biological assay was conducted on transformed plants in a secured laboratory in clay pots containing a soil-sand mixture. Ninety-one of the transformed plants, representing 41 lines, were challenged with *M. konaensis*. Depending upon their size, the plants received 4,000, 2,000, or 1,000 root-knot nematode eggs. Wild-type *C. arabica* from tissue culture and *C. arabica* transformed with the GUS gene were used as controls. After 347 days, plant growth and root weight were recorded. The root system was rated for galls and overall health. The nematode eggs and juveniles (J2s) were collected and counted to obtain the reproduction factor (Rf). Results observed in the nematode bioassay showed that transgenic plants varied from resistant to average to susceptible. The greatest number of resistant plants was obtained through particle bombardment of 35S-OcI-ΔD86/GO/CpTi gene construct. No resistant plants resulted from *A. tumefaciens*-mediated transformation of Tubulin-OcI-ΔD86 gene construct, although particle bombardment of the tubulin construct did give plants with resistance. Susceptible plants were observed with both constructs and both transformation methods.

Although traditional breeding methods have been used to produce resistant root-stocks in coffee for nematode resistance, we demonstrated that genetic modification can be used to control plant-parasitic nematodes in coffee. Transformation of *C. arabica* with plant proteinase inhibitor genes is an alternate method of developing resistance to *M. konaensis*.

Coffee transformed for resistance to root knot nematode located in a secure laboratory.

- R. Myers-Cabos (UH), C. Nagai, B. Sipes (UH), D. Schmitt (UH) and H. Atkinson (Leeds Univ.)

Construction of an Improved Genetic Map and a BAC Library for Arabica Coffee

Hawaii coffee farms have a long-standing reputation for producing high quality coffee, but breeding programs to improve available cultivars are needed to continue producing a superior product. Construction of an Arabica genetic linkage map is the first step towards marker assisted selection for traits such as high yield, large bean size, and superior cupping quality. A cross was made between 'Mokka hybrid' (MA2-7) and 'Catimor' (T 5171-7-1) to develop a segregating mapping population. These varieties are divergent in their cupping quality as well as tree and bean morphology. Two F1 plants (00-20-25 and 00-20-41) from the progeny were used to produce a true F2 population which was planted at Kunia, Oahu in 2005. Phenotypic data for tree height and width, branch angle, leaf characteristics, cherry weight, and green bean weight has been collected for two consecutive years from this population, and a preliminary linkage map has been generated from 61 F1 (pseudo-F2) individuals.

Amplified fragment length polymorphism (AFLP) markers were used to produce the genetic map from 61 individuals of the 00-20-25 true F2 population. In total, 1,035 polymorphic markers were generated, and from these, 830 markers were linked to 15 major linkage groups and 26 smaller ones. Eleven of the major linkage groups show recombination between 'Mokka hybrid' and 'Catimor' dominant markers, while the remaining four are segregated into two 'Mokka' dominant groups and two 'Catimor' dominant groups. There is no recombination between two parental genomes in the F1 (pseudo F2) population, because the recombination events mapped were the results of previous generation of self fertilization within each parental genome. The recombination events between parental genomes are mapped in the true F2 population and this map will be informative for mapping source and sink traits. Work is ongoing to add several hundred more markers to the final linkage map. These markers will be helpful in mapping the quantitative trait loci and tracking them in future populations.

A second project was undertaken for construction of a Bacterial Artificial Chromosome (BAC) library to be used in combination with the genetic linkage map. BAC libraries are an extremely useful tool for plant genetics. These libraries can be used for genome sequencing, cloning genes of interest, chromosome walking towards a target gene, and identifying underlining genes of Quantitative Trait Loci (QTLs). It should be possible to identify loci that influence cupping quality, tree and bean morphology, and disease resistance. A BAC library was constructed using genomic DNA from the small bean, high cupping quality, Arabica variety 'Tall Mokka'. Arabica coffee (*C. Arabica*) was chosen as the best candidate for the library because it is the most commercially important species in the genus, accounting for 70% of the world's coffee production, and 'Tall Mokka' is one of the parents of our mapping population.

The genomic DNA was partially digested with the restriction enzyme Hind III and ligated into the pIndigo BAC vector. The final library consists of 52,416 clones with an average insert size of 94 kb which represents 4x coffee genome equivalents. High-density filters are being made for further characterization of this BAC library using plastid specific probes and coffee genes. This Arabica BAC library will be used for cloning genes of interest to be utilized for coffee improvement. In addition to utility in applied research, this large insert genomic library will be essential for basic research such as understanding the basis for evolution of this allotetraploid genome from its two progenitor diploid genomes of *C. canephora* and *C. eugenoides*.

- A. Byers, M.R. Jones, R. Ostroff, Q. Yu, C. Nagai and R. Ming (UIUC)

Genetic Survey of Cacao in the Hawaiian Islands

High tonnage and superior quality are required to make cacao production profitable in Hawaii. Currently, however, Hawaiian cacao plantings are highly variable in both quality and yield and are not necessarily adapted to the islands' growing conditions. The genotypes of these trees are unknown and growers are not able to identify the types of cacao trees on their farms. Through the use of DNA marker techniques we are now able to assist cacao farmers in selection of superior cultivars based on their parentages/ pedigrees.

Cacao was introduced to Hawaii in the 1850's by various groups of researchers and growers; genetic types were mostly not known. Cacao trees planted in the field of Dole Food Co. Hawaii were identified by molecular analysis by microsatellite (SSR) markers (Schnell et al. 2005) as Upper Amazon Forestaro (UAF) x Trinitario types. The Cacao Chapter of the Hawaii Tropical Fruit Growers (HTFG) sent 15 leaf samples to the USDA ARS/SHRS for genetic fingerprinting to identify genotypes of trees on their farms. Although most of the samples were Trinitario or hybrids of UAF Forastero-Trinitario, six were identified as Criollo, which are currently grown only in limited areas in the world due to their susceptibility to diseases.

A broader, more inclusive survey of cacao trees throughout Hawaii was initiated to determine genetic diversity of cacao existing in Hawaii. Over 100 leaf samples were collected from cacao trees at various locations, including four botanical gardens on Oahu and Kauai, 11 farms on Oahu and Hawaii, three home gardens on Kauai and Oahu, and the University of Hawaii's Waimanalo Research Station. There was wide morphological variation among the trees in seed color and pod shape, texture, and color. Genomic DNA was extracted from leaf samples and sent to Dr. R. Schnell's lab (USDA ARS/SHRS) for SSR analysis.

SSR analysis was performed using 11 primer pairs that had been used to identify genotypes of cacao. It was found that 77 of the samples were genetically unique, indicating a high level of variability within the trees in Hawaii. These results suggest that Hawaii has adequate genetic diversity available for selection of superior types. Genetic diversity analysis of SSR markers is now under way. The genetic information from this study may be used to ascertain when and from where cacao was introduced to the islands. It may also help growers to predict and select their superior cultivars based on genotypes as well as morphology and yield performance at their growing environment. It would also be ideal if promising cultivars could be identified and developed from genetic resources already in Hawaii. No major diseases of cacao exist in Hawaii and importations pose the risk of disease introductions.

- R. Heinig, C. Nagai, G. Choobua (HTFG) and R.J. Schnell (USDA ARS)

Cacao pods in selection trial

Forestry Research

Investigating Koa Wilt in Hawaii: Examining Acacia koa Seeds and Seedpods for Fusarium Species

Fusarium may either elicit disease symptoms or be capable of reducing koa tree growth. Therefore, it is important to know which *Fusarium* spp. routinely colonize koa seeds, their relative abundance, and how they might affect spread of potential pathogens. We sampled *Acacia koa* (Fabaceae) seeds and seedpods from four of the Hawaiian Islands (Hawai'i, Kaua'i, O'ahu, and Maui) for colonization by, and contamination with *Fusarium* spp. (Hyphomycetes). Seeds were sampled from either bulk storage or from collections made from planted or natural *Acacia koa* trees displaying wilt/dieback disease symptoms.

Seeds were aseptically placed directly on a *Fusarium* selective agar medium. They were not surface sterilized because one of the goals of the evaluation was to observe the extent of surface contamination by *Fusarium* spp. Usually 10 seeds were placed on each plate of selective medium. Seedpods were aseptically dissected into pieces about 5 to 8 mm in length and width. Randomly selected pieces were surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite; 1 part standard household bleach in 10 parts water), rinsed in sterile, distilled water, and placed on the selective medium. All plates were incubated under diurnal cycles of cool, fluorescent light at about 24° C (75° F) for 7 to 10 days. Single spores of selected isolates were inoculated onto potato dextrose agar and carnation leaf agar for identification using the taxonomy of Nelson and others. Percentages of sampled seeds and seedpod pieces colonized with each *Fusarium* spp. were calculated.

Koa selections trial

The vast majority of healthy-appearing seeds from storage were not colonized by *Fusarium*. Stored seeds with superficial fungal mycelium, however, were extensively contaminated. Nearly 80% of the sampled seeds from forest trees with koa wilt disease symptoms had evidence of insect predation and more than 70% of the insect-predated seeds were contaminated by *Fusarium*. About 60% of healthy-appearing seeds from diseased forest trees were also contaminated.

Seedpods were commonly colonized by the same *Fusarium* species that contaminated seeds. Thirteen different *Fusarium* spp. were isolated from koa seeds and seedpods, mostly at low levels. Although *F. semitectum*, *F. subglutinans*, and *F. solani* were frequently isolated, *F. oxysporum*, the putative cause of koa wilt/dieback disease, was isolated very rarely from either seeds or seed coats. The ecological significance and potential disease roles of *Fusarium* contaminating koa seeds need to be determined.

- R.L. James (USDA), N.S. Dudley and A. Yeh

Investigating Koa Wilt in Hawaii: Pathogenicity of four Fusarium species on Acacia koa seedlings

Acacia koa A. Gray (Fabaceae) is a dominant endemic canopy tree in many Hawaiian forest ecosystems. Koa is present on the main Hawaiian Islands, growing in moist habits at elevations from 90 to 2,100 m. Koa has always been an important part of Hawaiian culture; koa wood was used for building many original Hawaiian structures, as well as being prized for sea-faring canoes. This important tree species is currently of primary importance in an expanding Hawaiian wood industry, being used for producing furniture, musical instruments, bowls, surf boards, and handicrafts.

The major factor limiting establishment and maintenance of koa is a wilt/decline disease that adversely affects tree survival and growth. High tree mortality following koa plantings due to this disease has restricted koa establishment, particularly in certain areas where disease severity is high. Koa wilt/dieback is putatively caused by the fungus *Fusarium oxysporum* f.sp. *koae* which was subsequently investigated for its disease impacts and biology. Limited genetic analysis of several Hawaiian isolates of *F. oxysporum* indicated potentially low genetic diversity, possibly due to recent introductions of pathogenic strains of this fungus into the state. If pathogenic strains were recently introduced and non-native to Hawaii, the severe disease impacts currently occurring may be a reflection of an invasive, well-adapted pathogen. Improved techniques for detection and management of this disease are urgently needed to limit pathogen spread and reduce disease impacts. Several other *Fusarium* species, particularly *F. solani*, are routinely isolated from diseased koa seedlings and trees. In addition, 13 different *Fusarium* spp. were recently isolated from koa seeds and seedpods. Some of these organisms may be involved in disease etiology and epidemiology and require testing for their ability to induce disease.

The present work was designed to evaluate pathogenic potential of selected *Fusarium* isolates associated with diseased koa plants and nearby soil in order to help clarify their potential roles in disease etiology. Tests were conducted under controlled conditions on koa seedlings within a greenhouse.

We tested ten *Fusarium* isolates, comprising four different species, for their pathogenic potential on *Acacia koa* seedlings under greenhouse conditions. Tested isolates were obtained in Hawaii from either diseased *Acacia koa* seedlings, soil adjacent to seedling roots, or seeds/seedpods. All tested *Fusarium* isolates completely colonized seedling root systems and became systemic, spreading to above-ground plant tissues (stems, branches, and leaves). Virulence was quantified on the basis of production of disease symptoms (mortality, wilting, foliar chlorosis or necrosis) and effects on seedling height, diameter, and root volume. Of the five tested *F. oxysporum* isolates, one exhibited high virulence, another was non-pathogenic, and the other three were moderately virulent. One tested *F. solani* isolate was quite virulent, whereas the other was only slightly virulent. One isolate of *F. subglutinans* was non-pathogenic and the other tested isolate was moderately virulent on inoculated seedlings. The one tested isolate of *F. semitectum* displayed moderate virulence. Pathogenic screening of many more isolates, particularly those classified within the *F. oxysporum* species complex, will be necessary to identify pathogens that can be effectively used to screen families of *Acacia koa* for potential resistance to the wilt/dieback disease that is seriously impacting this important Hawaiian tree species.

We were able to identify at least one candidate *Fusarium* isolate (0431B) that may have some potential for screening *Acacia koa* families for resistance to the wilt/dieback disease. Our inoculation study indicated that *Fusarium* isolates obtained from diseased koa seedlings, adjacent soil, and seeds/seedpods are not necessarily pathogens. Five tested isolates of *F. oxysporum* showed a wide range of virulence on seedlings in our test. This would indicate that both pathogenic and nonpathogenic strains of this species, the putative cause of koa decline/wilt disease, commonly colonize koa plants. Therefore, all isolates of *F. oxysporum* obtained from diseased koa plants are not necessarily the pathogen capable of eliciting koa disease. Isolates will have to be genetically analyzed to locate potential markers associated with pathogenicity. Such markers may be effective to quickly and easily locate pathogenic strains from within fungal populations. Further pathogenicity tests, particularly with isolates of *F. oxysporum*, are planned to obtain additional isolates that can be effectively used for resistance screening. When mixtures of pathogenic strains are available, we should be successful in screening for disease resistance.

Fusarium strain test on Acacia koa

- N.S. Dudley, R.L. James (USDA), R.A. Sniezko (USDA) and A. Yeh

Miscellaneous Crops

Exploring the Potential for Biodiesel Crops in Hawaii

HARC initiated research into oil crops during 2006. In early 2006, HARC was contracted by the Hawaii Department of Agriculture to author a report on the potential for biodiesel production from agricultural sources. The resulting report, 'Biodiesel Crop Implementation in Hawaii', outlined potential crops and their characteristics, land areas zoned for agricultural use on each island, by-products from processing these crops into biodiesel, and production schemes that could be implemented for widespread adoption of the recommended crops. The report was submitted to the State in October 2006.

Flowering Jatropha curcas

During the writing and after the submission of this report, HARC was expanding its research into potential biodiesel crops to field settings. After receiving a grant from the Hawaii Farm Bureau Federation to conduct field research on one of the potential crops outlined in the report for the State of Hawaii, HARC began field trials in late 2006. The test was begun with seeds of *Jatropha curcas* imported from Madagascar. This initial trial is evaluating planting density, irrigation requirements, seed yield, and other agronomic indicators such as insect pests and herbicide effects. *J. curcas* is a plant of Central American origin that can thrive on marginal lands, requires minimal irrigation and fertilization, and has oil contents ranging from 28-32%.

By the end of 2006, HARC was well-positioned to play a critical part in biodiesel research in the State of Hawaii. Relationships with UH-Manoa CTAHR personnel, UH-Hilo CAFNRM personnel, Pacific Biodiesel, and other groups interested in pursuing oil crops research have enabled HARC to expand its interest into other potential sources of vegetable oils for biodiesel. As concerns over fuel imports grow in coming years.

- M. Poteet

Biocontrol of Nematodes in Turfgrass

At least 20 species of parasitic nematodes can infest turfgrass and are a major problem for golf courses and lawns. Nematodes feed internally or superficially on plant roots and damage can range from slight chlorosis and water stress to severe necrosis with dead patches over the turf surface. Nematode multiplication and feeding are favored by warm temperatures of 68°F to 86°F which makes Hawaii suitable for nematodes all year. There are no species of grass known that are very resistant to nematodes. Cultural practices that promote vigorous root growth will make the grass more tolerant of damage. Chemical nematicides are toxic and not recommended except as a preplant soil fumigant. It is dangerous to apply toxic nematicides in areas open to the public and there are very few chemical nematicides cleared for use on turfgrass.

Biocontrol methods can be effectively used to manage parasitic nematode populations. These methods can serve to increase plant resistance to damage and reduce nematode numbers in soil. This project was designed to test ABR's Maui LCF syrup, a highly concentrated compost tea. It will also test MeloCon, a formulation of a strain of the nematode-destroying fungus *Paecilomyces lilacinus*. These treatments will be tested on Tifway Bermudagrass (*Cynodon dactylon*) which is the most common fairway grass used in Hawaii.

Root-knot nematodes, *Meloidogyne incognita*, were raised on kenaf in outdoor plots in HARC's Kunia substation. They were tested for their parasitism and multiplication on Tifway Bermudagrass in clay pots. The Bermudagrass was first seeded on netting and allowed to establish a root mass. The trial was designed with seven different treatments, each with six replicates. The replicate plots were 10 in X 10 in turfgrass sod placed on a potting soil: sand mixture in flats. Treatments were as follows: 1) untreated, with added nematodes, 2) Maui LCF 1% solution postplant and at 2 week intervals for two months, with nematodes, 3) Maui LCF 2% solution applied as above, with nematodes, 4) MeloCon 0.2 g/meter^2 at planting and at two months, with nematodes, 5) untreated, without nematodes, 6) Maui LCF 1%, without nematodes, and 7) Maui LCF 2%, without nematodes.

Meloidogyne nematodes were collected from the kenaf plots by digging up roots showing conspicuous galling, rinsing off surface soil, and bringing them to the lab for egg extraction. Roots were macerated in a blender with dilute hypochlorite in water and the suspension poured through a series of nested sieves. Most of the plant tissues were held in the coarser sieves and nematode eggs collected in a 500 mesh sieve. They were eluted into centrifuge tubes and centrifuged to bring eggs and particulate matter to the bottom. The pellet was then resuspended in a concentrated solution of sucrose and centrifuged again. The supernatant was poured through a 500 mesh sieve, rinsed several times with water and numbers of eggs per milliliter suspension were estimated.

At test installation, squares of sod were cut, placed in the flats and placed on greenhouse benches. The nematode eggs were applied as a drench at the rate of 20,000 eggs per replicate flat. MeloCon and Maui LCF treatments were applied over the turf as drenches. Continued applications are being made and data will be taken at the end of the trial. Nematode counts, root and plant mass weights per replicate flat will be reported.

- S. Schenck

Improvement of Anthurium Pest and Disease Resistance Through Plant Transformation

In Hawaii, anthurium growers suffer crop losses due mainly to bacterial blight, caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* and also the burrowing nematode, *Radopholus similis.* Generally, commercial anthurium cultivars are not tolerant to blight and nematode attack. Researchers from HARC, USDA, and growers with the Hawaii Anthurium Industry Association initiated this project to try to overcome these problems, by producing anthurium plants with improved resistance to these two organisms.

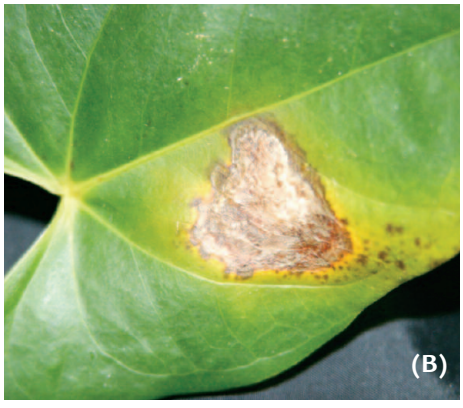
Two cultivars which are favored by the industry as parents for crossing purposes were chosen. 'Marian Seefurth' has a uniform, medium, pink spathe, while 'Midori' is a uniform, medium, green with a "vase-life" several weeks long. Both are susceptible to damage from blight infection and nematodes.

First, an efficient system for engineering anthuriums was developed. Stems, leaves, roots, and embryogenic calli generated from these tissues were co-cultivated with two strains of *Agrobacterium* carrying constructs with the candidate genes. A number of the genes to be incorporated into the anthurium plants have already been used successfully in other transformation projects. For example, in apple and banana, use of two of the constructs was previously shown to improve resistance to blight and nematode feeding respectively.

In this project, over 600 lines incorporating a number of different candidate genes have been produced. Molecular analysis of putative transformed lines is ongoing. The presence of the transgenes has been demonstrated using Polymerase Chain Reaction (PCR) assays. Reverse transcriptase PCR assays and western blotting indicate that the recombinant proteins are being expressed. For plants expressing antibacterial proteins, it has been shown that these recombinant proteins are biologically active as leaf extracts have been shown to have antibacterial activity when compared to controls.

Transformed lines are being multiplied for laboratory bioassays which will test for any improvement in their resistance. Also, plants are being potted at Green Point Nursery on the Big Island of Hawaii and these plants will be used in a large scale field trial in shade houses.

- M. Fitch (USDA ARS), Y.J. Zhu, T. Leong, H.R.K. McCafferty, S. Schenck, D. Gonsalves (USDA ARS) and P.H. Moore (USDA ARS)



a) Anthurium plants removed from tissue culture, hardened off and potted.
b) Anthurium leaf showing a lesion caused by the bacterium Xanthomonas campestris pv. *dieffenbachiae.*

Conservation

HARC Works with USDA-NRCS to Reduce Soil Erosion and Runoff

HARC is participating in the USDA-NRCS's Environmental Quality Incentives Program (EQIP) to reduce the amount of soil erosion that occurs at its Kunia and Maunawili Substations. The USDA-NRCS EQIP program is designed to give farmers and landowners support in projects that alleviate erosion hazards by developing specially designed conservation plans. This USDA-NRCS program is supported nationally through the Farm Bill. The USDA-NRCS helps offset costs associated with implementing these soil conservation plans through cost-sharing.

HARC currently has three EQIP contracts signed with USDA-NRCS. Two of these contracts are for the Kunia Substation located in Central Oahu. These two plans are part of a broader conservation plan for the Kunia district that incorporates plans for landowners located in an area above HARC's facilities. These plans are utilizing diversions, grassed waterways, and sediment basins. HARC is also using a drought-tolerant, sterile grass known as vetiver to create natural diversions when it is planted across slopes with erosion potential. USDA-NRCS design engineers created an innovative grassed waterway using rock dams, vetiver strips, and sediment basins that has greatly reduced soil loss from the substation's upper fields.

The third EQIP contract is for HARC's Maunawili Sugarcane Breeding and Forestry Substation. This contract is primarily focused on re-grading and re-routing the main field roads that have suffered extensive erosion during the last decade. By re-routing the roads along contours, the maintenance of and erosion from these areas will be decreased. The road work will also include the installation of water-bars to move water off the roadway into vegetated areas.

HARC will continue to work with USDA-NRCS on conservation projects in the future. The Kunia and Maunawili Substations continue to provide support for USDA-NRCS by installing potential planting materials for other conservation projects. Using HARC's facilities for testing and demonstration of new windbreak materials, erosion-control measures, and engineering designs, has created a collaborative relationship with this federal agency.

- M. Poteet

Services

Pathology Services to Member Companies

Monthly seed farm inspections were carried out and any problems reported to plantations. Due to a recent outbreak of leafscald disease, caused by the pathogenic bacterium, *Xanthomonas albilineans,* all current commercial varieties were re-screened for susceptibility. The results showed no significant changes in LS ratings, indicating that no new race of the pathogen had appeared in Hawaii.

Screening of new varieties for eyespot disease susceptibility was carried out at Maunawili breeding station. All of the 47 varieties checked using *Bipolaris sacchari* toxin were highly resistant except for H98-8281 (E5), H97-1734 (E5), H97-0186 (E4), and H99-3316 (E5). The appearance of smut whips in a plantation field of resistant variety H87-4319 prompted retesting for a possible new smut race on Maui. Disease-free seed was cut at Maunawili. Half of the seed pieces were inoculated with smut spores collected on Maui and the other half with spores from Kunia, Oahu. After six months, there are still no smut whips in the test plots indicating that there is no new smut race in Hawaii.

Four new foreign clones to be used in our breeding program were imported from USDA Sugarcane Quarantine, Beltsville, MD. They are: MPTH 97-194, MPTH 97-221, MPTH 98-283, and Q 157. They are currently planted in a HARC Maunawili quarantine field and have so far not shown any disease symptoms. They will be released in July 2007. Seed of several Hawaiian *Saccharum. officinarum* clones were sent to USDA, Beltsville, MD as requested by a researcher.

- S. Schenck

Kunia Substation Report

HARC's Kunia Substation conducted projects in a diversified mix of crops including papaya, coffee, cacao, sugarcane, jatropha and corn in 2006. Kunia personnel irrigated, fertilized, controlled weeds, insects and diseases, collected data and harvested the trials.

Papaya was grown for three HARC research projects and for 'UH Rainbow' hybrid seed production for Hawaii's papaya industry. A presentation was made at the annual papaya industry conference on Rainbow seed production at HARC. 'Rainbow' seed production will cease in 2007 because the 'Kapoho' variety used as the male to cross with the transgenic 'SunUp' was being infected and killed by the papaya ring-spot virus.

Four acres of coffee trees were maintained and additional areas were planted for selection and development of superior cultivars. Coffee trees in Field M1 were stumped to a height of 18 inches, and re-growth was thinned to two shoots per stump. Calcium amendment was applied to all of the coffee orchards in 2006. Nitrogen and potassium were drip-applied at monthly intervals based on estimated coffee demand. Kunia personnel also maintained the coffee at the Maunawili Substation. Two weed control trials in coffee were conducted at Kunia with 2,4-D and oxyfluorfen (over coffee) to control broadleaf weeds, especially Aiea morningglory. We are seeking registration of both herbicides in coffee via IR-4 program for minor crops.

An early Hawaii research planting (1985) of cacao is being maintained at Kunia. These trees are being used for selection of superior cultivars. Kunia personnel also provided assistance in collecting data for cacao harvested at the Dole farm in Waialua.

Sugarcane cultivars were screened for smut disease at Kunia, with 820 plots planted in 2006 with inoculated new and industry standard cultivars. The plots were evaluated, ratooned then the ratoon cane evaluated again for smut-infected stalks. Sugarcane was also grown to provide material for use in other research projects.

Jatropha curcas was planted at Kunia as a potential crop for biodiesel. Its agronomic requirements and productivity will be evaluated.

Corn was planted for seed production, isolation plots and winter nurseries. We had clients from Nebraska, Iowa and Minnesota. The seed production was both for UH Supersweet No. 10A sweet corn seed and for mainland clients. We produced 1,680 lb of premium 10A seed and marketed these seeds to local farmers at $15 per pound. Some of the isolation plots required staggered planting and detasselling. The winter nurseries required bagging of tassels and ears, and hand pollinating; some plants were selfed and others crossed.

A new weather station was installed in early 2006 to measure radiation, air temperature, rainfall, wind speed and relative humidity. The new site is located over Bermuda turfgrass and in an open area representative of our farm. We also maintain a Class A pan as well as a 5 ft elevated pan for evaporation measurements. This weather station is being shared and maintained by HARC and a neighboring farm.

Several soil conservation structures in cooperation with NRCS were installed at the substation to reduce soil erosion

– L. Santo

Facility Administration

The Experiment Station at Aiea was sold to obtain funds to purchase 108 acres at Kunia, which it had been leasing from Campbell Estates for the last 40 years. The mandated dissolution of the Estate provided HARC with the opportunity to rejoin its field and lab activities at the same location. HARC transitioned from landlord to tenant at Aiea, where it will remain in that capacity until a new facility is built.

- B. Vance

Computer System Administration

Six new computers and a printer were added to the local area network. New users were provided basic training and all users were required to pass an on-line information technology (IT) security test. To reduce our vulnerability to external abuse, peer-to-peer software was removed from users' computers. The IT policy was revised and unused IP addresses blocked. The business office's accounting software was upgraded.

- B. Vance

Quality Assurance Unit (QAU)

HARC's QAU conducted a field-phase inspection of a pesticide application to banana. A follow-up audit of the resulting reports was completed as well as for another Good Laboratory Practice study of a pesticide for sugarcane. These studies will become part of a larger submission package that their respective sponsors will submit to the Environmental Protection Agency in fulfillment of the Federal Insecticides, Fungicides and Rodenticides Act.

- B. Vance

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Blake Vance, Vice President/Facilities Administrator
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Florida Chow, Human Resources
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Ladislao Gonzalez, Watchman, Maintenance
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Roland Fernandez, Experimentalist
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Roger Styan, Experimentalist, Supervisor

Maui Substation

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Teodoro Bonilla, Field Worker
Romeo Cachola, Field Worker
Luis Dela Cruz, Weighing Machine Operator
Wilson Galiza, Foreman
Domingo Vallecera, Field Worker

Kauai Substation

Fernando Garcia, Field Worker
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Consultants

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Kuo-Kao Wu, Sugarcane Breeding

Sugar Company 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**
2005			
Gay & Robinson, Inc.	5,096	59,612	11.70
Hawaiian Commercial & Sugar Co.	16,639	192,730	11.58
Totals & average	21,735	252,342	11.61**
2006			
Gay & Robinson, Inc.	3,463	39,635	11.45
Hawaiian Commercial & Sugar Co.	16,950	173,550	10.24
Totals & average	20,413	213,185	10.44**

* Includes Kekaha salvage cane
** Weighted average

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