On the Inside

Front cover images, clockwise, from top right corner:

(1) Lance Santo, HARC Agronomist, inspecting morning glory problem in sugarcane field
(2) Koa (*Acacia koa*) in flower
(3) Taro (*Colocasia esculenta*) leaf
(4) Rice *(Oryza sativa)*
(5) Coffee (*Coffea arabica*) flowers
(6) Pineapple (*Ananas comosus*)

Background Image:

Varieties of Hawaiian sugarcanes (*Saccharum spp.*) — photo courtesy of Maui Nui Botanical Gardens, Kahalui, Maui

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Robert Osgood · Susan Schenck · Blake Vance

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Jim Carr · Nick Dudley · Axel Lehrer · Heather McCafferty · Ray Ming · Chifumi Nagai · Robert Osgood · Lance Santo

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To our Board of Directors, Members, Advisory Council and Friends,

Over the past two years the sugar industry in Hawaii was reduced to two operating companies, Hawaiian Commercial & Sugar Co. and Gay & Robinson, Inc. Combined area for both companies at the end of 2002 was 42,767 acres. Sugar production totaled 246,204 tons in 2001 and 270,081 tons in 2002. A portion of the State land leased at Kekaha previously farmed by AMFAC Sugar Kauai is now farmed by Gay & Robinson. The remaining lands at Kekaha are used for other agricultural activities including aquaculture.

Currently, H78-7750 and H77-4643 dominate the acreage planted on Maui and Kauai, respectively. Other varieties widely planted are H78-3657, H78-4153 and H65-7502. Field-scale block testing is recommended for H83-7061, which has outperformed standard varieties in Leeward makai and middle mauka zones on Maui. H83-7061 is a seedling of H62-4671, a variety that still holds all time Hawaii yield records in excess of 21 TSA. Training at the plantations was concentrated on weed control and variety identification. Work continues to improve on the genetic transformation of sugarcane with emphasis on incorporation of high value proteins.

Two-year-old coffee progeny from 160 crosses and selfs were evaluated in 2002 with wide variation in plant morphology and fruit characteristics. Cupping of the progeny will commence in early 2003. Family selections and outstanding individuals will be moved to the next level of selection. A cross between distant Arabica coffee cultivars resulted in an F1 generation of 120 individuals for which over 450 molecular markers have been identified and a linkage map developed. The majority of coffee cultivars growing in Hawaii were DNA fingerprinted in 2001.

Stephanie Whalen joined HARC in 1973 as an analytical chemist. She was named president of Center and Director of the Experiment Station in 1994. Ms. Whalen also serves on National Pesticide and Air Quality Committees. Her goal is to see environmental responsibility and a thriving technology lead agricultural development in Hawaii.

Excellent progress has been made on the molecular mapping of papaya concentrating on sex expression. Papaya has been transformed with several genes expected to provide fungus and insect resistance. In collaboration with PBARC, papaya breeding continued with emphasis on back crossing UHRainbow papaya with its Kapoho parent to produce a market-sized fruit with yellow flesh for the export market. Backcrosses of Laie Gold papaya with its Kamiya parent were made to produce a fruit suitable for the Oahu market. We continue to supply the papaya industry with hybrid, virus-resistant seed.

Emphasis in the forestry program has shifted from fast-growing introduced

eucalyptus to the endemic Hawaiian koa tree (*Acacia koa*), on which the Hawaii woodcraft industry is based. Primary interest is in the selection of koa adapted to low elevation culture as a sustainably harvested forest product.

Taro cultivars were micropropagated and field tested to determine propagation rates and agronomic practices for mechanized drip irrigated production.

Work was completed on a survey of the movement of aster yellows disease and its associated leafhopper beyond the initial sightings in watercress fields on Oahu. No movement was detected.

The analytical laboratory developed methods for determining sugars, and selected organic acids and chlorogenic acids in coffee in an attempt to link coffee chemistry to organoleptic traits in the cup. Cooperative studies on stevia and *`awa* (*kawa*) continued.

Good progress was made on identifying fungi for the bioremediation of chlorinated hydrocarbons in soil. We surveyed a wide range of plants for the ability to bioremediate soils contaminated with lead and PCBs.

HARC actively participated in the legislative and regulatory arenas with respect to environmental issues of concern to agriculture. For example, water quality and quantity, and runoff into streams and the ocean continue to be important topics in Hawaii and the rest of the nation. Staff worked with the U.S. Fish and Wildlife Service to ensure that habitats critical to endangered species would be designated in appropriate locations throughout the islands. Assistance in environmental regulatory compliance continues to be available to the agricultural community.

Stephanie Whalen
President and Director

100 Years Ago: 1901 - 1902

The sugar industry was booming in Hawaii in 1901 with production reaching 350,000 tons valued at $28,000,000. Sugar had become the predominant business in Hawaii although prices for sugar had declined somewhat owing to expanded Cuban production destined for the United States and to the expansion of the European sugarbeet industry. It was noted by the HSPA board of Trustees that "the expense of maintaining the Experiment Station is considerable but there seems no question as to the value of the experiments made and work done". Mr. C. F. Eckart replaced Mr. R. E. Blouin as director of the Experiment Station; Blouin resigned after only one year of service owing to problems with his health. Mr. Eckart was a chemist who was first employed by the Paauhau Sugar Company after his graduation from the University of California. In 1902, research under the direction of Mr. Eckart included variety, fertilizer and irrigation trials. Great concern was expressed by the sugar planters over the poor condition of Hawaii's forests due primarily to overgrazing. The condition of the Hamakua forest was noted in the Planters' Monthly a year after a destructive fire in 1901 did catastrophic damage to stands of native koa and ohia.

Immigration of "Porto Ricans" for cane field labor commenced in December 1900 and continued for nearly a year. About 500 people immigrated and were situated on the plantations.

The Hawaiian Pineapple Co. was organized on December 4, 1901 and homesteaders began planting pineapple at Wahiawa.

The first beer was brewed in Honolulu in 1901 by brewmaster E. J. Waterman of the Honolulu Brewing Company. Electric streetcar service was started on August 31, 1901 by the Honolulu Rapid Transit and Land Co. Waldron Co. began operations. Mail delivery commenced on August 22, 1901 and radio telegraph service began between the islands.

— R. V. Osgood

Dr. W. Maxwell
1895 - 1900

R. E. Blouin
1900 - 1901

C. F. Eckart
1901 - 1913

Purification of SCYLV Particles for Antiserum Production

The polerovirus (family: *Luteoviridae*) Sugarcane Yellow Leaf Virus (SCYLV) is present in sugarcane, even before showing the characteristic yellowing that gave it its name. Therefore testing of cultivars for virus presence is important to determine a certain cultivar's susceptibility (for breeding purposes). If susceptible cultivars are planted, the percentage of infected plants in the field should also be monitored. Virus detection with a RT-PCR method (or the electron microscope) have both proven to be impractical in the large scale application. The tissue blot immunoassay that was developed using a polyclonal rabbit anti-SCYLV antiserum is now the standard test method in Hawaii and most parts of the world. Since the supply of the antiserum is nearly depleted, efforts were made to produce a new polyclonal antiserum with an equal or greater specificity.

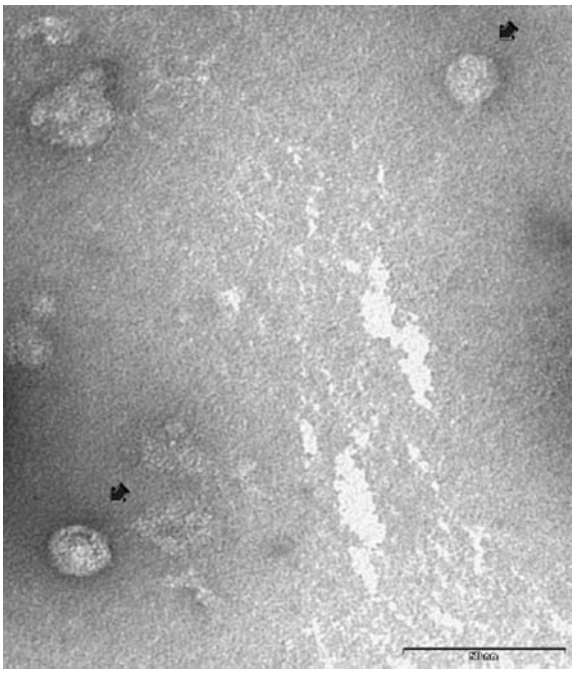

Figure 1 – Virus particles were observed under the transmission electron microscope using negative staining (size standard within electron micrograph: 50 nm).

Virus particles of luteoviruses are known to be present in plants only in very small quantities and therefore the highly susceptible cultivars H 73-6110 and H 87-4094 were chosen for virus extraction. Luteoviruses are phloem-limited, so the plant tissue had to be disintegrated completely under mild conditions in order to avoid losing large amounts of the virus. The tissue was therefore digested with a mixture of pectinase and cellulose in cold phosphate buffer. The particles were then purified from solution using selective precipitation and several ultracentrifugation steps. Seed pieces of infected plants were germinated in wet vermiculite and roots harvested after four weeks. Roots always showed strong reaction in the immunoassay so they were also chosen as source material. The virus yields from leaf and root tissue are shown in table 1.

All virus extracts reacted strongly in a dot-blot using the rabbit anti-SCYLV antiserum. Antigenic properties of virus particles were nearly completely lost when extracts were denatured (incubated for 90 minutes at 65°C in the presence of SDS). Icosahedral virus particles about 20 nm in length were observed in the extracts with the transmission electron microscope (see figure 1).

— A. T. Lehrer and S. Schenck

***Table 1** – Estimated yield of virus particles from 500-g tissue each, based on absorption measurements at 260 and 280 nm. The ratio of A_{260}/A_{280} was around 1 (as expected for luteovirus particles).*

Tissue used for extraction	Yield per 500 g fresh weight	Quality as antigen	Loses antigenicity when denatured
H 87-4094 (leaves)	163 µg (~ 3.2 µg / g FW)	+++	Yes
H 73-6110 (leaves)	325 µg (~ 6.5 µg / g FW)	+++	Yes
H 73-6110 and H 87-4094 (primary roots)	809 µg (~ 16.2 µg / g FW)	+++	Yes

How Healthy Is a Healthy Looking Sugarcane Plant?

Lessons learned from the study of diseased plants in Hawaii carrying the Sugarcane Yellowleaf Virus (SCYLV)

When, in the late 1980s, yellowing symptoms were first observed in sugarcane fields on the islands of Hawaii and Oahu, nutrient deficiencies, chloride or herbicide toxicities were first suspected to be the cause. The symptoms started as a yellowing of the leaf midrib in contrast to other yellowing phenomena caused by drought or malnutrition. When these symptoms could not be relieved by fertilizer application or a changed watering schedule, it became obvious that it may be a plant disease which was then called Sugarcane Yellowleaf Syndrome (YLS). Eventually, a positive strand RNA virus, called Sugarcane Yellowleaf Virus (SCYLV), was discovered in diseased plants. It belongs to the family of *Luteoviridae* and is today considered to be a *Polerovirus.* An antibody-based tissue-print test was developed which allowed rapid screening of leaf samples for SCYLV infection. Many of the commercial cane varieties in Hawaii were completely infected in all fields. These susceptible cultivars did not always show YLS symptoms. Some cultivars always tested virus-free and were therefore tentatively called resistant. The questions that remained unanswered were: How healthy are those dark green, but virus-infected plants? How do they perform with respect to growth, sugar yield and other pathogens? How does the viral infection spread and how can it be controlled? Unfortunately there were no susceptible, virus-free plants that could be used for infection experiments or for growth and yield comparison. So USDA/HARC personnel generated virus-free, susceptible plants of cultivar H 87-4094. This cultivar served as a test-system for all following experiments.

In order to observe virus movement within the sugarcane plant, virus-free plants of H 87-4094 were inoculated via one source leaf using the vector-aphid *Melanaphis sacchari* F. Three weeks later, SCYLV could first be detected in the youngest leaves and roots. After 11 weeks all parts of the plants were infected with the pathogen (see figure 1). Movement of the phloem-limited virus occurred as expected from source to sink tissues along with the assimilate stream.

SCYLV is phloem limited, but its multiplication is only possible in tissues that have active nucleic acid and protein synthesis. The active cells surrounding the phloem are therefore the logical tissues to have high concentrations of viral particles. This was shown to be true by electron microscopy of SCYLV-infected tissue. As a phloem-limited virus, it must also be able to reach the sieve tubes after assembly in order to be transmitted via phloem-feeding insects. Since carbohydrate movement depends on having intact phloem cells with control of plasmodesmal pore size, a massive accumulation and possibly aggregation of SCYLV particles could slow down assimilate transport drastically. It was observed that sucrose concentration in leaves of virus-infected plants was permanently elevated, especially in the morning (figure 3). Starch levels in infected leaves were also elevated, another sign of decreased carbohydrate transport rate (figure 3). This indicates that sucrose translocation is slowed down by virus infection, but direct damage to the tissue caused by the virus could not be observed (no mottling, spots or any other classic disease symptoms, and no leaf yellowing). The stress caused by elevated sugars in the leaf results in a lower photosynthesis rate (figure 4) and ultimately also to a change in chlorophyll type in the green leaves. This was documented by a shift in the chlorophyll a:b ratio from 3.1 ± 0.2 to 2.6 ± 0.2 in potted plants of H 87-4094.

Fields of SCYLV-infected cane usually remain symptomless. Nonetheless, effects of the infection can be measured. In a direct side-by-side experiment comparing infected and virus-free H87-4094 plants, the germination in a 100-m cane line was reduced by 30.6%. A side-by-side comparison of infected and virus-free plants revealed the harmful effects of the SCYLV infection. Infected plants had a reduced assimilate translocation rate with the eventual consequence of producing other changes in their physiology. Therefore any severe stress (by any environmental factor) can eventually trigger development of the symptomatic stage. Drought stress seems to be one of the important elicitors of the disease symptoms.

Diseased plants in this study showed reduced growth (stunting) and also a shorter individual leaf life expectancy from emergence to shedding. Due to the shortened growth period of leaves (rapid leaf change) and the insufficient recycling of nutrients, stalk ultimately died prematurely. These were the effects seen in Maui fields in the mid-1990s. Selection of resistant cultivars appears to be the best.

— A. T. Lehrer (Universität Bayreuth and HARC), E. Komor (Universität Bayreuth) and S. Schenck (HARC)

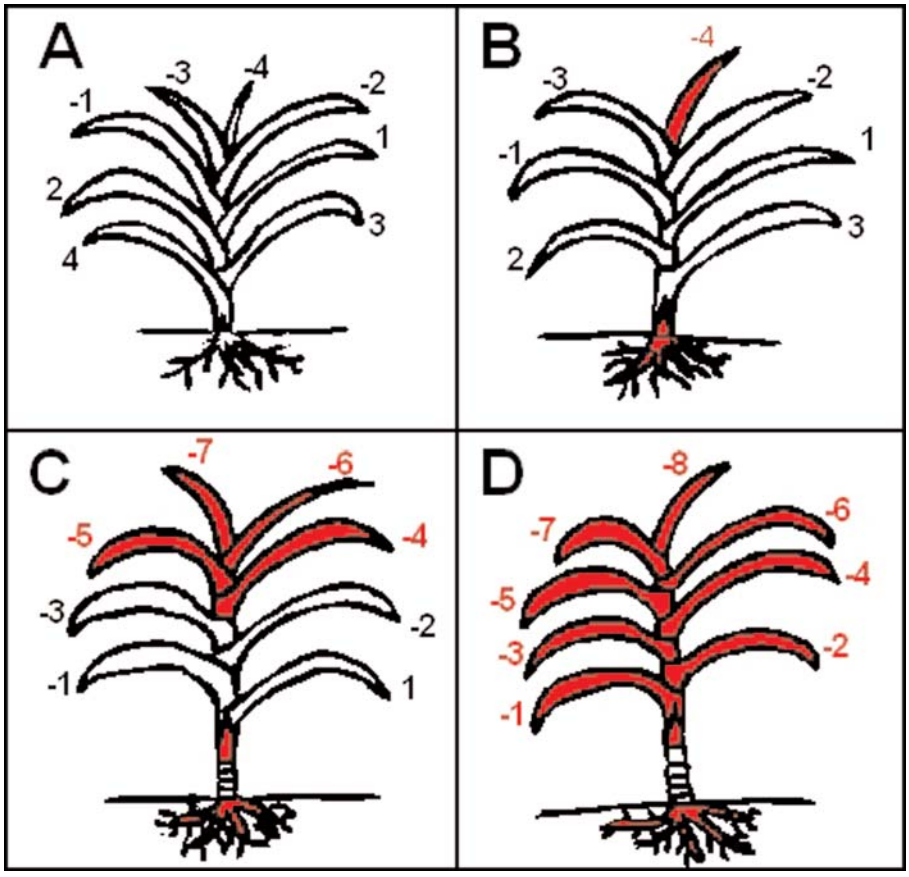

Figure 1: *Progression of SCYLV-infection (indicated in red color) in a sugarcane plant, which had been experimentally infected with viruliferous aphids at leaf #1 (top-visible dewlap). A: 1 week, B: 3 weeks, C: 7 weeks, D: 11 weeks after infection. The counting of leaves in the figure was "frozen" after the infection, i.e. leaf #1 was called #1 throughout, although with time other leaves above successively contained the top-visible dewlap.*

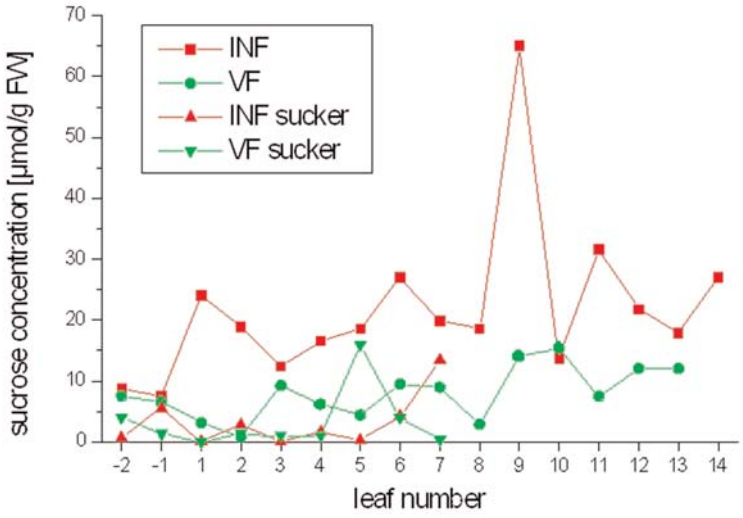

Figure 2: *Sucrose concentration of each individual leaf of virus-free and with SCYLV infected plants of H 87-4094. INF and VF are primary shoots (6 months old) and "suckers" were young shoots, about 2 months old (also virus-free (VF) and infected (INF) with SCYLV). Samples were taken at sunrise.*

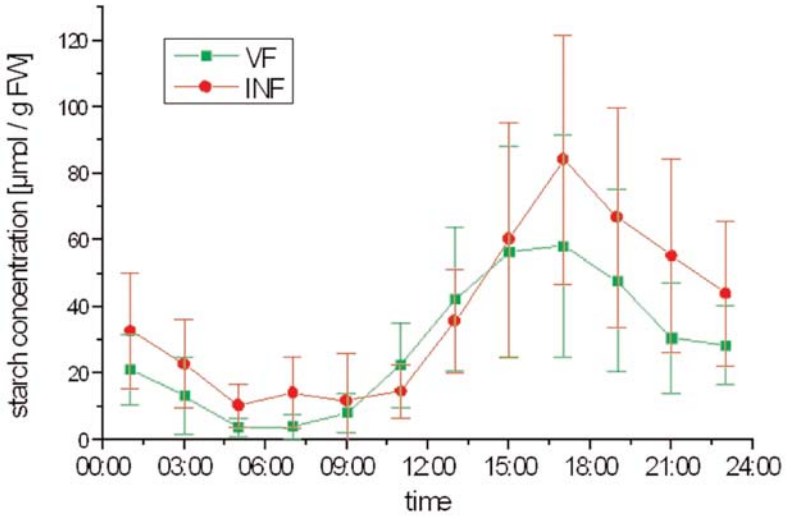

Figure 3: *Starch concentration (expressed as glucose) in TVD-leaves of H 87-4094 over the course of a day. Each data point represents the mean of six individual plants (with SD).*

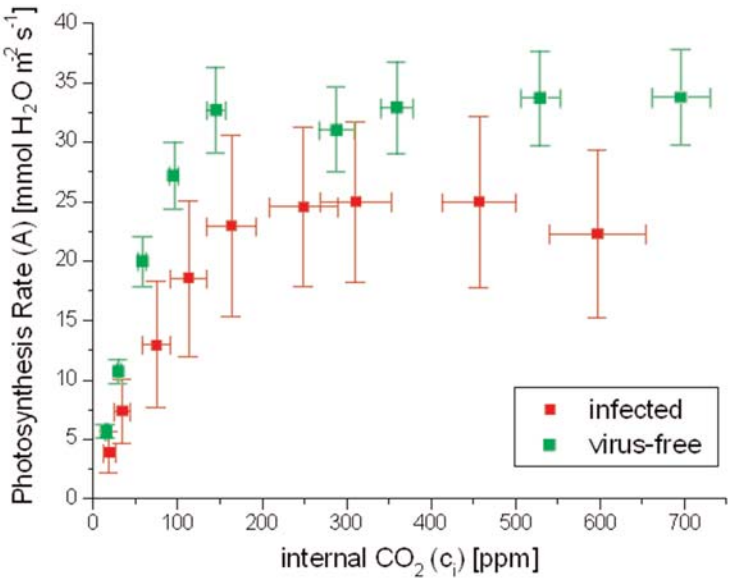

Figure 4: *Photosynthetic rate of 3-month-old infected and virus-free plants in relation to their internal CO_2-concentrations (with SD), achieved by applying different external CO_2-concentrations.*

Sugarcane Research

Analysis of Native Hawaiian Sugarcanes Genetic Relationships

Sugarcane was being cultivated by Hawaiians when Captain James Cook first arrived in the islands (Cook, 1785). These *Saccharum officinarum* cultivars were too soft for milling and susceptible to numerous diseases. Since they were thought to be infertile, other *S. officinarum* and *S. spontaneum* clones formed the basis of the original Hawaiian breeding program. However, it is uncertain whether or not any of the native Hawaiian germplasm did in fact enter the commercial gene pool. If not, the Hawaiian canes should remain a distinct gene pool. It is also not known how much diversity exists within the Hawaiian canes. A research project is currently underway using RFLP analysis that is expected to answer these questions.

Moir (1932) arranged the native Hawaiian varieties into groups and families based purely on their morphological characteristics. Mangelsdorf (1956) surmised that they might all be selections of somaclonal mutants of a single *S. officinarum* introduction. Our results to date have shown them to be mostly within a group with 0.85% similarity and differing from the early Hawaiian commercials. However, they are clearly not all somaclonal mutants of one original clone. There are at least six separate groups within the larger group. Most of Moir's surmised groupings do appear to be valid genetically. Results confirmed Moir's statements that some canes were imported from foreign countries for breeding at an early date and were given Hawaiian names. The cultivars Lahaina, Moana, Kokea, and Lehu appear to fall into that category. Lahaina is reported to be the parent of H-109, one of the first Hawaiian-bred commercials and our study does show a close genetic relationship between the two. Several of the Hawaiian cultivars in the collection that were thought to be the accessions of the same clone did not appear to be the same genetically. Possibly look-alikes were given the same name although not really related. To date, we have analyzed over 50 different samples using 190 different markers. Additional marker analyses should further clarify the results, although much of the history of these ancient cultivars will, no doubt, always remain a mystery.

— S. Schenck, M. Crepeau and R. Ming

References:

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Mangelsdorf, A. J., 1956. Sugarcane breeding: in retrospect and in prospect. Proc. IX Congr. ISSCT: 560-575.

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

Inheritance of Resistance to Yellowleaf Syndrome

(Presented as a poster at the 24th Congress ISSCT, 17-24 September, 2001)

Sugarcane yellowleaf syndrome virus (SCYLV) is widespread throughout Hawaii's sugarcane crops. Tests determined that many symptomless plants were infected with SCYLV and that the virus was more widespread than had been thought. The older Hawaiian cultivars still in existence were tested for SCYLV infection and this information was compared with the breeding records. Infected cultivars were more common than uninfected ones. In the cases where both parents were known, crosses between susceptible varieties produced susceptible offspring. Crosses between uninfected cultivars produced both resistant and susceptible progeny. The unknown male parents in many of the records and the fact that many of the old Hawaiian varieties have been lost meant that additional data needed to be obtained from

continued crossing studies.

Dr. Susan Schenck, HARC Plant Pathologist

Some of the current commercial cultivars consistently gave positive reactions for SCYLV in the field. Examples of these are: H73-6110, H78-3606, H83-7206 and H87-4094. Others have never given positive reactions in dozens of tests in many locations: H78-4153, H78-7750, H87-4319 and H82-3569. There are in addition, certain cultivars that give variable reactions. Since SCYLV has existed in Hawaii at least since the 1980s, all of the older commercial cultivars have probably been exposed to infection. The fact that some still remain virus-free indicated that they are indeed resistant. In order to test this hypothesis, the cultivars remaining virus-free were inoculated with viruliferous aphids. None of the resistant plants listed above subsequently tested positive. Virus-free plants of the susceptible cultivar H87-4094 became reinfected when inoculated. This indicated that varietal differences in virus resistance and symptom expression exist and that breeding for resistance or tolerance might therefore be possible.

Luteoviruses, including SCYLV, are not mechanically transmissible, but are transmitted naturally by aphid vectors which are each specific for one or a few viruses. The most common vector of SCYLV on sugarcane in Hawaii is the sugarcane aphid, *Melanaphis sacchari.* Colonies of this species were maintained on SCYLV-infected sugarcane plants in pots. Virus-free sugarcane plants were inoculated by transferring aphids from the infected host plant using a small watercolor brush. After allowing the aphids to feed for 48 hours, the aphids were removed from the inoculated plant. Virus could usually be detected in susceptible plants within three weeks. There was no aphid feeding preference between different cultivars. Inoculations with SCYLV of progeny among crosses of Hawaiian cultivars is ongoing, but some information has been obtained to date. A self of the resistant variety H78-4135 (H78-4135 x H78-4135) produced seed that yielded 17 progeny plants. When these were inoculated with aphids, only two of the plants eventually tested positive for SCYLV. The self of susceptible variety H73-6110 (H73-6110 x H73-6110) yielded 23 progeny plants. Although the parents were infected with SCYLV, none of the progeny were initially infected indicating that SCYLV is not seed transmitted. However, after inoculation, 22 of the 23 progeny became infected. Subsequently, crosses were made between susceptible and resistant cultivars and the progeny were inoculated. Preliminary results of inoculations of these progeny showed 27% of them to be infected. These have now been planted in field plots where they will be exposed to natural infection.

All of the progeny of the LA Purple (susceptible) by MOL 5829 (resistant) have been tested for SCYLV infection at least three times in their field plots. Although LA Purple gives strong positive reactions, very few of

the progeny gave unequivocal positive results. Of the first 50 progeny assayed, those that tested negative were planted in pots and inoculated with aphid vectors. Only four of these eventually tested positive and the results were variable. Of these 50 clones, 23 had at least one positive reaction out of a total of ten tissue blot assays on five different dates. RFLP analysis of these clones is being carried out in an effort to find a marker or markers associated with SCYLV resistance.

Consistent differences were observed between Hawaiian sugarcane cultivars. Infected cultivars vary in frequency and severity of symptom expression. It may be that the variable reactions seen with H65-7052 and H78-3567 are an indication that these support a low or variable virus titre that is sometimes below the level of detection by the tissue blot immunoassay technique. In any case, the results of several years of testing indicate that SCYLV titre varies between sugarcane cultivars. Whether the lack of SCYLV infection in some Hawaiian cultivars is due to their resistance to infection or because they do not support multiplication of virus to a high titre, this characteristic appears to be heritable. Studies of crosses of susceptible cultivars resulted in progeny of which a large percentage subsequently become infected with SCYLV. The opposite is true for progeny of crosses of resistant varieties. Crossing studies are still underway and to date there is insufficient data to estimate the ratio or pattern of inheritance. It is unlikely that a single gene is involved in resistance or that breeding resistant parents will result in 100% of the progeny being resistant.

— S. Schenck and A. T. Lehrer

Production of Recombinant Protein in Sugarcane and Rice

Use of a crop plant as an efficient biofactory requires both an efficient transformation system for the particular species, and the ability to produce and accumulate high levels of the engineered high-value protein. We have been able to increase the efficiency of the sugarcane transformation system primarily by generating larger numbers of plants from transgenic cultures. A more stringent procedure for selection of transgenic plants has also led to increased numbers of plants positive for the gene of interest, improving efficiency by minimizing the percentage of plants that are carried through the tissue culture and regeneration process, without containing the gene of interest. Hundreds of transgenic sugarcane plants have been generated, some carrying a gene for a high-value human therapeutic protein (GMCSF) and some with a reporter gene, GUS, which permits convenient measurement of expression levels.

We have identified several sugarcane varieties, including Hawaiian commercial cultivars, that respond well to the tissue culture system and give rise to many transgenic plants. As we work with new genes for high-value protein production, we now have an efficient method for using particle gun bombardment to insert genes of interest and produce large numbers of plants.

Approximately 400 plants, independently transformed with GMCSF, were taken from sterile tissue culture conditions and transplanted to soil. Over 300 of the plants survived transplanting and were tested for expression of the GMCSF protein. Analysis of leaf samples showed low levels of GMCSF accumulation, with the highest lines at 0.03% of total soluble protein. In previous experiments (HARC 2000 Annual Report, p. 14) with smaller numbers of sugarcane lines expressing the GUS gene, that transgene silencing occurred in all of the lines after plant regeneration. It had been our hope, that even with our existing gene introduction system, by producing larger numbers of transgenic lines, we would be able to obtain occasional lines which were not silenced,

and would accumulate high amounts of the desired protein.

Experiments are now underway to obtain plants with higher levels of gene expression. High copy numbers of transgenes and complicated integration patterns, which commonly occur as a result of gene gun bombardment, often correlate with high frequencies of transgene silencing. Three approaches are currently under evaluation to reduce transgene copy numbers, simplify integration patterns, and ultimately elevate gene expression levels. One of the new approaches to improve expression is based on introduction of smaller pieces of DNA. Another approach utilizes a site-specific recombination system, Cre-lox, to resolve multiple copies down to a single integrated transgene copy. In the third method, a transposable element from maize, Ac/Ds, is incorporated in the gene constructs. Sugarcane calli transformed with the GMCSF gene using the first two approaches are under selection. The third methods will be evaluated using transient expression of the reporter gene, GUS.

We are also evaluating the expression of GMCSF gene constructs in rice. Although rice is not currently a commercial crop in Hawaii, we know it can be grown here. Historical records and recent experience at HARC's Kunia substation show rice is suitable for production in Hawaii. From our previous research with *Agrobacterium* transformation of rice, we are convinced that transgene silencing occurs at much lower frequencies than in sugarcane. Furthermore, work in other labs has shown that recombinant proteins can be stable in rice seeds stored or shipped at room temperature. We have produced several transgenic rice plants carrying the GMCSF gene under the control of a seed-specific promoter. Accumulation of GMCSF in the seeds of the small number of rice lines obtained to date is also rather low. In at least one of these lines, mRNA encoding GMCSF does accumulate at high levels, indicating that the gene is not silenced. That even this line does not accumulate high levels of GMCSF protein, suggests the protein itself may be unstable, and is rapidly turned over in the cell. If this proves correct, we may have produced some non-silenced sugarcane lines, but our assays which measured only GMCSF protein did not detect these lines due to the protein's short half-life. Experiments are currently underway to resolve these possibilities.

New methods for selecting transgenic sugarcane plants and improving expression levels have been incorporated into current experiments. A system to separate transgenic from nontransgenic sugarcane plants was tested using the sugar mannose as the basis of the selection process. Although this has been shown to be somewhat effective for some crops, it has not been as clear-cut or efficient as our current procedures. Evaluation of the mannose selection system was carried out with several sugarcane varieties to ensure thorough testing of this method.

— C. Goldstein, M-L. Wang and H. Albert (USDA/ARS)

New Race of Sugarcane Smut Fungus, Ustilago scitaminea

Sugarcane smut caused by *Ustilago scitaminea* was first reported in Hawaii in 1971. In susceptible cultivars it can spread rapidly, cause significant yield losses and reduce cane stands to unmillable grassy stalks. In Hawaii, the disease is controlled by continued monitoring of seed fields, hot water treatment of seed and breeding for resistance, all of which add to the cost of production. In 2001, some seed fields of the completely resistant cultivar H78-7750 became infected. Up to 20% of stools were observed to have smut whips.

Smut whips were collected from Maui and

from Oahu and spore suspensions of each of these were used to inoculate uninfected seed pieces of H78-7750. The seed was then planted on Oahu. Seven months after planting, whips appeared in the cane inoculated with smut from Maui. At eight months after planting, the H78-7750 with smut from Maui had about 40% of the stools with whips giving it a susceptibility rating of about S7 on a rating scale of 1 to 10 (Ladd *et al.* 1973). The cane inoculated with smut from Oahu remained free of whips. The line was ratooned and the Oahu smut-inoculated canes are still free of whips after six months.

A trial was installed on Maui testing the susceptibility of the current commercial varieties to the Maui smut. The results, shown below, indicate that some varieties that were previously smut resistant are susceptible to the new smut, while others have the same rating as before. Based on this preliminary evidence, we conclude that a new race of *Ustilago scitaminea* has appeared in Hawaii. A similar case occurred in 1976 when the resistant variety H50-7209 suddenly became infected (Comstock and Heinz 1977). For several years the two smut races could be differentiated on the basis of host susceptibility, but eventually the races could no longer be distinguished. Although the occurrence of new, more virulent races of *Ustilago scitaminea* is relatively rare, the Hawaii plantations now give all seed, including resistant varieties, a short hot-water treatment before planting.

— *S. Schenck*

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Ladd, S. L., D. J Heinz, H. K. Meyer, G. W. Steiner, R. S. Byther 1973. Hawaiian Sugar Planters' Association, Experiment Station, Pathology Report 34.

Breeding and Selection

Sugarcane crossing began on November 15, 2000, and was completed within six weeks. We used 2411 flowering stalks from 308 parents to make 706 polycrosses. In 2001, we evaluated 359 clones in 23 FT7 yield tests with 6.7% yielding more sugar per acre than standard checks. These clones will be tested again in FT7s at different locations.

This year we have revised our selection procedures. Bunch planting FT1 and regular FT2 tests were eliminated. In January, we transplanted about 45,000 single spaced seedlings in our FT1 test and the plants were ratooned mechanically in September. The ratoon FT1 plants will be selected nest year (2002) directly for FT4. This change may have the following advantages:

Variety	Rating to Existing Smut Race	Preliminary Rating to New Smut Race
H65-7052	(S2)	(S5)
H77-4643	(S5)	(S3)
H78-3567	(S5)	(S0)
H78-4153	(S5)	(S3)
H78-7750	(S0)	(S3)(S7 in first trial)
H83-7061	(S0)	(S6)
H87-4319	(S0)	(S0)
H87-5794	(S0)	(S0)
H88-2953	(S2)	(S5)
H90-7492	(S0)	(S0)

1. Saving time — the time spent for FT1 selection and FT2 planting is saved.
2. Ratooning ability can be evaluated in early stage of selection, which was not possible in the past.
3. Smut infection rate in the ratoon crop is always higher than in the plant crop through natural infection. This eliminated smut spore inoculation in FT2.
4. FT1 selection rate can be used for family fuzz selection. FT1 selection rate was not used in the past because seedlings were in bunch planting.

At the end of 2001, 1,200 clones were selected from FT4 and advanced to FT5, and 23 FT7 tests were installed.

The top 4 cultivars by the end 2001 were H78-7750, H77-4643, H78-3567 and H78-4153. H78-7750 continued to be the leading cultivar for the second year. It occupied a total of 18,816 acres or 39.8% of total cane area. H77-4643 ranked second, occupied 8,565 acres or 18.1% of total cane area. It was the leading variety on Kauai. H78-3567 moved up to the third-ranked cultivar this year. It occupied 6,408 acres or 13.5% of total cane area. Its acreage increased to 270 acres on Kauai. H78-4153 continued to decrease in acreage and dropped to the fourth-ranked cultivar. It occupied 5,494 acres or about 11.5% of the total cane area.

New clones with commercial potentials are 91-4392, 93-4398 and 93-6002 for Makaweli soil on Kauai; 79-6503, 90-5555 and 90-7453 for rocky and dry area on Maui; 82-3569, 88-6401, 95-0171 and 95-1446 for sandy area on Maui; 82-3569, 85-4501, 85-1605, 86-3792, 87-4394 and 88-6401 for Windward area Maui. 87-5794 had the best FT7 records in Leeward region and H87-4319 is the best clone for mill water irrigated field.

— K.K. Wu

Comparison of Glyphosate Additives as Substitutes for Ammonia Sulfate

Ammonium sulfate (AMS) is known to increase the uptake of glyphosate and improve weed control, but the addition of a solid into the spray mix requires adequate agitation. Three liquid additives intended to replace ammonium sulfate were compared in a field trial at HARC's Maunawili Substation. The additives were Magnify (ammonium salts, alkyl polyglucoside and dimethylpolysiloxane), Request (ammonium polyacrylates, hydroxy carboxylates and sulfates) and Embrace Plus (ammonium sulfate and polyacrylamide polymer). The additives were applied at 0.50, 0.31 and 3.75 percent by volume, respectively, with 0.5 percent by volume of glyphosate product (Roundup Ultra). The Roundup Ultra rate of 0.75 lb ai per acre and the low concentration were expected to be sublethal to mature weeds. The sublethal rate was necessary to provide only partial control of weeds to enable the comparison of possible treatment differences. A nonionic surfactant (Latron AG-98) at 0.12 percent by volume

Lance Santo, HARC Agronomist, inspects a morning glory problem in sugarcane field.

was added to all treatments to mask possible surfactant effects of the active and inert ingredients of the three additives. Three additional treatments were ammonia sulfate at 1.5 percent by weight with Roundup Ultra plus additional surfactant, Roundup Ultra with additional surfactant but no other additive, and an untreated control for relative comparisons.

The weed control with additive was not significantly different among treatments but significantly different from the no additive treatment and the untreated control. Ammonium sulfate and Embrace Plus consistently provided the best control of broadleaf and grass weeds. Broadleaf control (Japanese tea and horseweed) was between 80 to 100 percent with additives and 65 percent without additives. Grass control (crabgrass and swollen fingergrass) was between 75 to 85 percent with additives and 60 percent without additives. Magnify, Request and Embrace Plus improved the activity of Roundup Ultra and are effective as ammonium sulfate replacements.

— L. Santo

Tropical Fruits

Genetic Diversity of Carica papaya L. as Revealed by Amplified Fragment Length Polymorphism

Genetic relationships among *Carica papaya* cultivars, breeding lines, unimproved germplasm and related species were established using amplified fragment length polymorphism (AFLP) markers. Seventy-one papaya accessions and related species were analyzed with nine *Eco*R I – *Mse* I primer combinations. A total of 186 informative AFLP markers was generated and analyzed. Cluster analysis suggested limited genetic variation in papaya, with an average genetic similarity among 63 papaya accessions of 0.880. Genetic diversity among cultivars derived from the same or similar gene pools was smaller, such as Hawaiian Solo hermaphrodite cultivars and Australian dioecious cultivars with genetic similarity at 0.921 and 0.912, respectively. The results indicated that self-pollinated hermaphrodite cultivars were as variable as open-pollinated dioecious cultivars. Genetic diversity between *C. papaya* and six other *Carica* species was also evaluated. *C. papaya* shared the least genetic similarity with those six species with an average genetic similarity of 0.432, whereas the average genetic similarity among the other six species was 0.729. The results from AFLP markers provided detailed estimates of the genetic variation within and among papaya cultivars, and supported the notion that *C. papaya* diverged from the rest of *Carica* species early in the evolution of this genus.

— M. S. Kim (UH), P. H. Moore (USDA/ARS), F. Zee (USDA/ARS), M. M. M. Fitch (USDA/ARS), D. L. Steiger, R. M. Manshardt (UH), R. E. Paull (UH), R. A. Drew (Nathan Campus Griffith University), T. Sekioka (UH) and R. Ming

Linkage Mapping Revealed Suppression of Recombination at the Sex Determination Locus in Papaya

There have been growing interests in comparative analysis of papaya (*Carica papaya* L.) and *Arabidopsis* genomes because of their close taxonomic relationship, small genomes and the availability of genomic tools. A papaya BAC library was constructed and used for gene isolation, physical mapping and comparative genome analysis. However, genetic mapping of the papaya genome has lagged behind because of the low level of polymorphism among existing papaya lines. A high throughput AFLP marker system using Li-Cor automatic sequencers was used to construct a genetic linkage map of papaya with 54 F2 plants derived from the two cultivars Kapoho and SunUp. A total of 507

UH Rainbow Papaya

AFLP markers were generated from 339 *Eco*R I/*Mse* I and *Pst* I/*Mse* I primer sets. The genetic distribution of these markers and two morphological traits were evaluated using the MAPMAKER program. Although 89% of the markers fit expected Mendelian segregation ratios, segregation distortion was observed as has been reported in other plant species. Of the 509 markers, 379 were mapped to 9 linkage groups corresponding to the nine chromosomes with an average distance of 4.1 cM between markers. Notably, a total of 58 (more than 15%) of the mapped markers were found to co-segregate with the sex determination locus. This suggests severe suppression of recombination in this region and strongly supports the previously proposed hypotheses that genetic recombination is suppressed in the region where the papaya sex determination gene is located.

— H. Ma (UH), P. H. Moore (USDA/ARS), Z. Liu, M. S. Kim (UH), Q. Yu (UH), M. M. M. Fitch (USDA/ARS), A. H. Paterson (Univ. GA) and R. Ming

Preliminary Molecular Characterization of the Sex Determination Locus in Papaya

Sex determination in papaya (*Carica papaya* L.) is controlled by a major gene with three alleles (M_1, M_2 and m). Because all combinations of dominant alleles (M_1M_1, M_1M_2 and M_2M_2) are lethal to the zygotes, males (M_1m) and hermaphrodites (M_2m) are enforced sex heterozygotes controlled by the different dominant alleles. Females are homozygous recessive (mm) at the sex determination locus. The homozygous dominate lethal

Hand-Pollinated female papaya used for F1 Hybrid seed production

factor is responsible for a skewed 2:1 (hermaphrodite:female or male:female) segregation for sex types in all F2 populations using female as a parent. We used an F2 mapping population composed of 54 individuals, including 34 hermaphrodite and 20 female plants, derived from the cultivars Kapoho and SunUp. The methylation status of the papaya sex locus was evaluated by comparing AFLPs using the cytosine methylation sensitive enzyme, *Pst* I, and the non-sensitive enzyme, *Eco*R I, as the rare cutter. A total of 1771 AFLP markers were generated from 781 *Eco*R I/*Mse* I and *Pst* I/*Mse* I primer sets. The high-resolution genetic map revealed that 225 AFLP markers co-segregated with sex. The large number of markers without recombination suggests severe suppression of recombination in the genomic region containing the sex locus. Among the 1479 *Eco*R I /*Mse* I AFLP markers, 14.2% co-segregated with sex, whereas only 5.9% of the 292 *Pst* I/*Mse* I AFLP markers co-segregated with sex. This difference indicates that the cytosine in this genomic region is highly methylated.

— H. Ma, P. H. Moore (USDA/ARS), Z. Liu, M. S. Kim (UH), M. M. M. Fitch (USDA/ARS), T. Sekioka (UH), A. H. Paterson (Univ. GA) and R. Ming

Physical Mapping of Sex Determination Gene in Papaya

Carica papaya is a polygamous plant species with female, male and hermaphrodite sexes. Sexual polymorphism makes papaya a model plant for studying sex determination and differentiation. Classical genetic analyses led to the conclusion that sex determination in papaya is the result of a single gene with three alleles. We are using three hermaphrodite sex-linked markers, W11, T12 and CPBE55 for fine mapping of the sex locus on 991 F_2 and 755 F_3 individuals derived from the two cultivars Kapoho and SunUp. No recombination was found among these three markers in either the F_2 or F_3 populations. BAC end cloning and chromosome walking have allowed construction of a 900 kb BAC DNA contig containing the W11, CPBE55 and T12 markers. The physical distance between W11 and CPBE55, CPBE55 and T12, and W11 and T12 is 500 kb, 400 bp and 900 kb, respectively. The large distance spanned suggests severe suppression of recombination in the genomic region that harbors the sex determination gene. We also cloned and sequenced 62 hermaphrodite sex-linked AFLP fragments based on a mapping population of 54 F2 plants. Forty-three SCAR markers were developed and 27 of them were used to screen a papaya BAC library. Six hermaphrodite sex-related BAC contigs have been constructed with genome coverage of 200 to 900 kb each. The overall coverage by all sex related BAC contigs currently extends to more than 2 Mbp. Southern hybridization of three markers on BAC contigs reveal three duplicated segments in this genomic region. Southern hybridization of selected markers to total genomic DNA of the three sex types indicates that these markers are present only in male and hermaphrodite plants but not in female plants. Chromosome *in situ* hybridization is under way to determine the physical location of the sex determination gene.

— Z. Liu, P. H. Moore (USDA/ARS), H. Ma (UH), M. S. Kim (UH), Q. Yu (UH), M. M. M. Fitch (USDA/ARS), A. H. Paterson (Univ. GA) and R. Ming

Cloning and Preliminary Characterization of PFL, the Papaya Homolog of FLORICAULA/LEAFY Genes

The instability of papaya flowers, which results in malformation of fruit that is unmarketable, can be observed in any papaya field. Understanding the flowering process in papaya is the first step towards developing

strategies to control carpellody and sex reversal of papaya flowers. The homologous genes *FLORICAULA (FLO)* in *Antirrhinum* and *LEAFY (LFY)* in *Arabidopsis* initiate flowering in these two distantly related plant species. Using an *Arabidopsis* cDNA clone of the *LFY* gene as a probe, two papaya BAC (bacterial artificial chromosome) clones were identified as containing the *LFY* homolog. The smaller positive BAC clone (60 kb) was digested with *Hind* III and ligated to a plasmid vector pPCR-Script. The sub-clones were screened with the *LFY* probe and the positive sub-clone was sequenced from both directions. Direct sequencing of the BAC clone was carried out at the 3' end of the sub-clone where the third open reading frame of the *LFY* homolog is interrupted. This papaya *LFY* homolog, *PFL*, shares 65% identity with the *Arabidopsis LFY* gene and encodes a protein sharing 71% identity with the *LFY* homologs of two woody tree species: California sycamore (*Platanus racemosa*) and black cottonwood (*Populus trichocarpal*). Despite extensive sequence similarity in two conserved regions, the proline-rich and acidic motifs differ between *PFL* and its counterparts in other plant species. This difference may not affect the gene function as demonstrated by the *Pinus radiata LFY* homolog *Needly*. Genomic and BAC Southern analyses indicated only one copy of *PFL* in the papaya genome. *In situ* hybridization using anti-sense RNA transcribed from *PFL* cDNA clone as a probe demonstrated that *PFL* is expressed strongly in young flower primordia. These results suggest that *PFL* could function as a flower meristem-identity gene in papaya.

— Q. Yu (UH), P. H. Moore (USDA/ARS), H. H. Albert (USDA/ARS), M. M. M. Fitch (USDA/ARS), M. W. Crepeau and R. Ming

Molecular Cloning and Characterization of Genes Involved in Papaya Systemic Acquired Resistance (SAR) and Engineering of Papaya to Enhance SAR

Plant disease is the most serious problem for the Hawaii papaya industry and development of disease resistant papaya is critical for maintaining its competitiveness. Systemic acquired resistance (SAR) is recognized as a plant response to pathogen attack in many plant species and can provide broad-spectrum resistance against a variety of diseases. In the SAR response, a set of defense-related genes are turned on when a pathogen such as *Phytophthora palmivora* attacks papaya plants. In the plant disease resistance response a protein, NPR1 (*NONEXPRESSER OF PR-1*), plays an important role in signal sensing and subsequent activation of SAR. Overexpression of NPR1 in *Arabidopsis* and rice leads to increased resistance against diseases for both plants. We have completed the cloning of an NPR1 gene homolog from papaya. The gene product

Terryl Leong, Special Projects Assistant, breeding to incorporate the papaya ringspot virus resistance into papaya lines.

shares 71% and 67% amino acid similarity with the rice and *Arabidopsis* NPR1 proteins, respectively. Two expression vectors containing the papaya NPR1 gene were constructed. We are using them to transform papaya for overexpression of NPR1 in order to enhance disease resistance.

In order to develop a molecular tool to screen large numbers of transgenic lines, four pathogenesis-related (PR-1) - like genes were isolated from papaya. One of them (designated PR-1 A) is inducible by benzothiadiazole (BTH) root drenching. Within hours of treatment, a significantly higher level of defense-related gene activity can be detected. Increased PR-1A gene expression was detected following BTH treatment. Previous work showed that BTH drench treatment increased ß-1,3-glucanase and chitinase enzyme activities, activated SAR, and reduced blight and root rot symptoms of seedlings inoculated with *Phytophthora palmivora* fungal pathogen. The expression of PR-1 A can be used as a molecular marker to evaluate SAR in individual transgenic papaya plants.

— X. Qiu (UH), M-L. Wang, Y. J. Zhu, R. Ming, P. H. Moore (USDA/ARS) and H. H. Albert (USDA/ARS)

Transformation of papaya for improved insect resistance

Papaya (*Carica papaya* L.) can be affected by a number of pests and diseases in its growing cycle. Of interest here is a leafhopper, *Empoasca stevensi*. This insect, belongs to the genus *Empoasca* and family Cicadellidae. Nymphs and adults suck sap from the phloem, usually from the surface of the leaves. Secretions from the insect during feeding cause a condition called hopperburn. Symptoms in older plants include tip burn, wrinkling and cupping of leaves and marginal burning, whereas younger plants show stunted growth. In severe cases defoliation of the affected plant can occur. Added to this, leafhoppers of the genus *Empoasca* are also vectors of plant pathogens. Examples include transmission of *Xanthomonas* bacteria and banana bunchy top virus. In January 2001, a new disease carried by a leafhopper was identified in the Pearl City area of Oahu. This disease, carried by the Aster leafhopper (*Macrosteles fascifrons*), was discovered on watercress. However, the insect has a wide host range including vegetables, grasses and fruits, and includes papaya. At present, leafhopper populations in papaya are controlled by spraying. This is neither a cheap nor environmentally friendly system. The aim of this project is to find an alternative method to control leafhoppers by engineering resistance into papaya.

The intention is to insert a gene with known insecticidal properties under the control of a phloem/leaf-specific promoter into papaya plants. The insecticidal gene is a lectin, GNA, from snowdrop (*Galanthus nivalis*). Use of a specific promoter rather than a constitutive one should allow higher levels of expression in the phloem and reduce exposure of non-target insects and other consumers of the plant material to GNA. Previous studies on brown leafhoppers show that GNA has an antifeedant effect and causes insect mortality. The promoter to be used in this study is from tomato. It is a Rubisco small subunit gene promoter, rbcS. This promoter has been used in other plant species and in apple, for example, it produced leaf- and stem-specific expression.

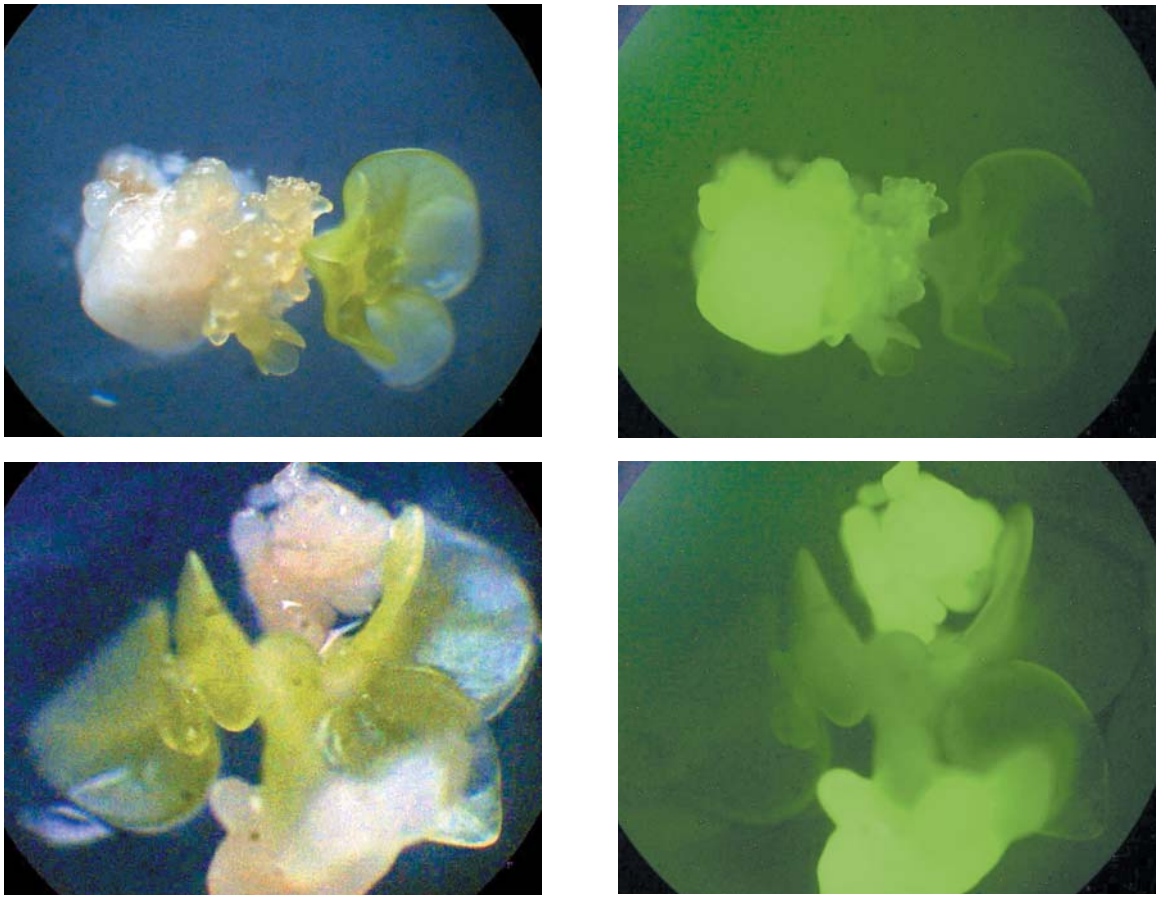
We are in the process of assembling constructs for particle gun bombardment. The rbcS promoter will be used to drive both GNA and GUS (beta-glucuronidase) expression. Analysis of transgenic plants will be carried out to screen for presence of the GNA gene and also to detect where expression occurs. It is hoped that this project will produce transgenic papaya with increased insect resistance.

— H. McCafferty and Y. J. Zhu

Evaluation of Green-Fluorescent Protein as a Selectable Marker for Genetically Engineering Papaya

Antibiotic resistance genes are commonly used as selectable markers during production of transgenic plants. But there is some concern that the gene might be transferred to pathogenic bacteria, which would then become resistant to the antibiotic and thus pose a danger to humans. To address this problem, other markers are now used, such as beta-glucoronidase (GUS), luciferase (LUC), and mannose selections. These reporter systems have been instrumental in many studies but neither allows for convenient non-invasive *in vivo* analysis. Green fluorescent protein (GFP), isolated from the jellyfish *Aequorea victoria*, has been widely used in transgenic plants. Plants in which the gene has been incorporated can be easily detected when illuminated with an ultraviolet light. The intrinsic fluorescence of GFP allows for non-invasive analysis that can be monitored without the destruction of the biological sample. This project will determine the effectiveness of GFP as a selectable marker in papaya and is expected to provide a useful tool for further research on papaya and on plant/fungal interactions.

We transformed papaya embryogenic callus with GFP constructs using biolistics. Two different GFP constructs were used in this experiment. The first was GFP plus an antibiotic selection marker, *npt*II. The second one was GFP in the pCambia vector without *npt*II. Visual selection was carried out once a week starting 15 days after bombardment. For the construct containing GFP and *npt*II genes, the calli were split into two parts. In the first callus batch, transformed cells were selected on antibiotic G418 selection medium. In the second part, selection was



GFP in the regenerating papaya embryogenic calli

based on the GFP expression. The transformation efficiency of the two selection systems will be compared. Selected GFP calli will be regenerated into plantlets for Polymerase Chain Reaction (PCR) and Southern blot analysis to confirm the transgene integration.

Calli containing green-fluorescent cells were isolated and cut into pieces of not less than 5 mm, since smaller calli may revert to non-regenerable forms. As plantlets emerged and developed significant amounts of chlorophyll, green GFP fluorescence was masked by red chlorophyll fluorescence and tissues appeared yellow. Mild mosaic symptoms were sometimes observed on leaves with strong GFP fluorescence. These leaves were analyzed with fluorescence microscopy and bright green fluorescent spots as well as some very faint spots were observed. There was also a variation in the level of GFP expression among different plants within a line or among different lines transformed with the same transformation vector.

To further test transgene integration, *npt*II ELISA and GUS assays were done to check for the presence of the *npt*II and GUS genes, respectively. Most of the plant lines that have been tested so far were both *npt*II positive and GUS positive. In comparison, the GFP signal is not amplified as it is with GUS because GFP fluorescence is not the result of an enzymatic reaction. More conclusive tests, such as PCR and Southern blot analysis, will also be done.

Visual selection of GFP-transformed cells is a possible means of avoiding conventional antibiotic selection. This study showed that the use of GFP as a non-toxic marker to identify transgenic cells after transformation is an effective procedure for discerning transgenic cells and removing untransformed or non-expressing tissue. Through the use of GFP in plants, fluorescent imaging microscopy can be used to track the expression and location of proteins and other microstructures within organisms as diverse as viruses, nematodes and fungi such as those with a destructive effect on agricultural crops.

— Y. J. Zhu, R. Agbayani, H. Albert (USDA/ARS) and P. Moore (USDA/ARS)

Genetic Diversity in Pineapple Assessed by AFLP Markers

Pineapple (*Ananas comosus* L.) cultivars are often derived from somatic mutations and propagated vegetatively. It was suspected and has been suggested by isozyme data that there was little genetic variation among Smooth Cayenne cultivars. A thorough investigation of the genetic variation within the cultivated species *Ananas comosus*, particularly among commercial cultivars, will provide critical information needed for crop improvement and varietal protection. One hundred fifty one accessions of *A. comosus* and 15 accessions of related species were evaluated with AFLP markers. The average genetic similarity of *A. comosus* was 0.74, ranging from 0.662 to 0.979, suggesting a high degree of genetic variation within this species. With over 100 AFLP markers, discrete DNA fingerprints were detected for each commercial cultivar, breeding line and intra-specific hybrid. Self–incompatibility, high levels of somatic mutation in vegetatively propagated plants and the sampling of intra-specific hybrids developed from a breeding program may account for this high degree of variation. However, major variety groups of pineapple, such as Cayenne, Spanish and Queen, could not be distinctively separated. These variety groups are based on morphological similarity; the similar appearance can be caused by a few mutations which occurred on different genetic backgrounds. Our results suggest that there is abundant genetic variation within the existing pineapple germplasm for use in selection. In addition, the discrete DNA fingerprinting patterns for

commercial cultivars can be detected for varietal protection.

— C. Y. Kato, C. Nagai, P. H. Moore (USDA/ARS), F. Zee (USDA/ARS), M. S. Kim (UH), D. L. Steiger and R. Ming

Genetic Transformation of Pineapple with Nematode Resistance

Pineapple growers in Hawaii have long been using pre-plant fumigation and post-plant nematicides to control nematodes. Some of these nematicides have been taken off the market and others are currently under EPA review and may not be available in the near future. Since pineapple plants do not have natural resistance to nematodes, genetic transformation for development of nematode resistance was chosen as the alternative. In collaboration with the University of Hawaii and Leeds University, HARC transformed UK pineapple plants by insertion of cystatin, a nematode resistance gene originally isolated from rice flowers.

During the period of 1998-2000, we produced putative transgenic pineapple plants (HARC 2000 Annual Report, p. 20). Over 300 of these plants were bioassayed in Dr. Brent Sipes' lab for nematode susceptibility. Twenty individual plants from a single transformation event (line #256) were compared with 20 non-transformed control plants for their ability to suppress nematode reproduction. Three out of the 20 transformed plants consistently supported lower nematode reproduction rates than the controls as measured by egg counts. Nonetheless, the amount of cystatin produced in transgenic pineapple line #256 was less than 10% of that produced in transgenic potato controls as estimated by Western blot analysis. We are continuing to evaluate plants with lower nematode reproduction by Western Blot, Southern blot and PCR methods.

We are continuing our effort to produce large numbers of new transgenic plants with cystatin gene. Four DNA constructs with 3 promoters, including sugarcane ubiquitin (pBI121/ubi9-D86 and pUC19/ubi9-D86), tubulin (pBI121/tub-D86), CaMV35S (pBI121/35s-D86) were inserted by particle gun bombardment. Gene constructs with pineapple root-specific promoter, CLCT, isolated at Dr. David Christopher's lab (UH) were also transformed into pineapple using both bombardment and *Agrobacterium*-mediated transformation. The goal is to develop transgenic pineapple which produce cystatin only in roots but not in fruits.

— C. Nagai, B. Sipes (UH), S. Tom, J. Buenafe, M-L. Wang and G. Uruu (UH)

Preplant Herbicide Application of Oxyfluorfen and Azafenidin in Pineapple

Oxyfluorfen (Goal 2XL) and azafenidin (Milestone) were found to control most weeds in pineapple with preplant broadcast applications in three trials conducted in 1998 and 1999. Efficacy data from these experiments and in other crops were adequate to determine the effective rates for both herbicides. In 2000 through 2001, both herbicides were further evaluated at four rates as possible alternatives to bromacil (Hyvar X) in pineapple. Both have the advantages of using lower rates and having less soil mobility compared to Hyvar X. The primary objective of the current trials was to evaluate pineapple tolerance to Goal and Milestone at rates of 0.5 to 2.0 and 0.375 to 1.25 lb a.i. per acre, respectively.

Pineapple was found to be very tolerant to preplant application of Goal at all growth stages. Goal treatments had no visual injury symptoms or any adverse effect on plant height, weight or fruit yield. Milestone had some significant adverse effects on appearance and height at 120 days after

Pineapple

application for only the high rates of 0.625 and 1.25 lb a.i. per acre. By 180 days, the plants affected by Milestone had recovered. Plant weights at 60, 120 and 180 days were not significantly different from the hand-weeded untreated control for all rates of Milestone. Milestone had no measurable adverse effect on the fruit yields. The average fruit size was larger with Goal and Milestone as compared to the hand-weeded untreated control but were only significant at 0.05 level with Goal at rates of 1 and 2 lb a.i. per acre at one of two sites.

— L. Santo, G. Ito

GLP Field Trials in Banana

In 2002, four field trials were installed and harvested for magnitude of residue samples for the registration of a new fungicide in banana. One of the trials was a residue decline study with several samplings after the last application. These trials were installed at Keaau and Pepeekeo on the Island of Hawaii. Ten applications were made at 7- to 10-day intervals which started after normal bagging or about two weeks after flowering and completed up to the commercial harvest stage (green fruit). Treatment consisted of bagged and unbagged treated bunches and bagged and unbagged untreated bunches. The Advantage 5.0 Electronic Field Notebook software was used to record the field data for these trials. Frequent communications and notebook updates were maintained to keep the study director well informed on the study's progress.

— L. Santo

Coffee Research

Development of New Coffee Cultivars for Hawaii

The coffee breeding and selection program to develop uniquely Hawaiian coffee was initiated in 1997 with the support of the Hawaii Coffee Growers' Association (HCGA). The objective of the program is to produce cultivars with desirable characteristics such as enhanced flavor, excellent bean and cupping quality, increased yield, and disease resistance adapted to specific growing conditions in Hawaii.

During the last 5 years, individual, potentially elite trees were selected from five coffee growing areas in Hawaii, and a field was established from seed and cuttings (clones) in a common field at Kunia, Oahu, (HARC 1997 Annual Report, p 21-22). A large scale coffee crossing program was undertaken (HARC 1999 Annual Report, p 19). About 1500 progeny trees resulting from these 165 crosses were in the field at Kunia in summer 2000 (HARC 2000 Annual Report, p 21). They were evaluated for their tree morphology (summer 2001) and fruit (cherry)/seed (bean) characteristics in fall 2002. Yield, cherry/bean characteristics, and cupping quality of the original selected trees were also evaluated in the fall of 2001.

New coffee hybrid H98-36 (mokka hybrid x mergogipe)

Out of 120 crosses made in 1999, twelve progeny families were selected as superior. Selection was based on tree morphology and height, cherry size and yield potential. They included larger-bean-size Mokka hybrids and higher yielding Margogipe hybrids. Seeds of these trees were collected and planted in a greenhouse for further field evaluation in 2003 at Kauai Coffee Co.

— C. Nagai, R. V. Osgood, K. Weaver, J. Clayton, C. Cavaletto (UH) and S. Bittenbender (UH)

Construction of a Genetic Map for Arabica Coffee Using AFLP Markers

Molecular marker linkage maps are being developed as scaffolds for phenotype mapping in many crop plants to assist directed germplasm improvement through marker-assisted technologies. We are using amplified fragment length polymorphisms (AFLPs) to construct a genetic linkage map on a pseudo F_2 population of arabica coffee (*Coffea arabica L.*) derived from a cross between the cultivars Mokka hybrid and Catimor. The Mokka hybrid, commercially grown in Hawaii, is known to produce high beverage quality and have small bean size and small narrow leaves. The Catimor cultivar originates from a hybrid between *C. arabica* and *C. canephora* backcrossed to *C. arabica*. Catimor does not produce high quality coffee but has large bean size and resistance to coffee rust *Hemileia vastatrix* Berk. The pseudo F_2 population of 130 individuals showed a large variation in tree morphology including plant height, width, branch angle, leaf characteristics and bean (seed) size. Sixty trees from this population were selected, based on plant height distribution, for linkage map construction. A total of 456 dominant markers and eight co-dominant markers were generated from 288 AFLP primer combinations. Segregation distortion was observed on 117 of the 456 markers. Linkage groups were constructed using Mapmaker v.3.0 resulting in 16 major linkage groups ranging from 4 to 21 markers and 15 small linkages consisting of two to three linked markers each. Total length of all mapped markers was 1796.54 cM with an average interval distance of 12.8 cM between adjacent markers.

— H. M. Pearl, C. Nagai, P. H. Moore (USDA/ARS), D. L. Steiger, R. V. Osgood and R. Ming

Morphological Evaluation of Arabica Coffee Mapping Population

A segregating mapping population of Arabica coffee (*Coffea arabica L.*) was developed from a cross between the cultivars Mokka Hybrid (MA2-7) and Catimor (T 5175-7-1). Catimor is a variety developed in Central America from a hybrid between *C. arabica* and *C. canephora* and backcrossed to *C. arabica.* Catimor trees typically have large, round leaves, resistance to rust, high cherry yield and large size beans, but are known to

have lower cupping quality. Mokka, a mutant of variety Bourbon, was developed in Brazil and has small, thin leaves, small beans, rust susceptibility, and excellent cupping quality. In June 2001, 135 seedlings of the population were planted at HARC's Kunia Substation together with selfed progenies of both parents. Morphological data for tree height and width, and leaf characteristics were collected in 2002. For plant height and width, the distribution of the data showed transgressive segregation exceeding parental values at both ends. For leaf length, width, ratio and area, the distributions of the progeny data were within the range of their parent values. This population was used for constructing the first Arabica genetic map with amplified fragment length polymorphism (AFLP) markers, and will be used for mapping quantitative trait loci (QTLs) controlling coffee yield and quality.

— K. Weaver, H. Pearl, R. Ming and C. Nagai

Chemical Profile Analysis of Coffee

Coffee is a complex mixture of many hundreds of compounds. It is not currently known which of these compounds significantly contribute to the overall taste of brewed coffee. Coffee quality and consequently pricing, is assessed by panels of trained specialists who "cup" coffee. This is a rating system based upon the taste they perceive, and is wholly subjective. Another issue affecting the world price of coffee is supply. At present, the coffee market has been flooded with lower quality coffee, and the world price has dropped as a result. One way to increase coffee prices is to increase quality. An objective measure of quality is needed, that can verify variety, origin and can link chemical composition to quality.

A project was initiated to chemically profile green coffees growing in Hawaii. The aim of the project was to attempt to determine the components in coffee that contribute to quality (a good coffee taste). Samples were harvested over 2000 and 2001 harvests and wet processed following commercially acceptable processes. Samples were then split and half were analyzed for simple organic acids, sugars and chlorogenic acids. In order to do this, analytical methods were developed to simultaneously determine the concentrations of simple organic acids and sugars. The other half were roasted and cupped by a trained cupping panel and rated with regard to a number of set descriptors. The data from both sets of determinations were statistically analyzed for correlations with chemical profiles alone, varieties and hybrids, locations, and year of harvest could be discriminated. Further funding will be sought to extended this study to cover coffees from different origins throughout the world.

— S. Steiman and M. C. Jackson

Genetic Transformation of Coffee for Nematode Resistance Using Cysteine and Serine Proteinase Inhibitors

Hawaii is free of the diseases found in many coffee growing regions around the world. The damage by nematodes, however, has been observed in over 80% of Kona coffee farms. *Meloidogyne konaensis*, the Kona coffee root-knot nematode, causes extensive root damage, decreased yields, and death of coffee trees in severely infected areas. Genetic engineering is being explored as a method of controlling this nematode (HARC 2000 Annual Report pp. 20-21). Proteinase inhibitors, cystatin from rice, and cowpea trypsin inhibitor (CpTI), were inserted into Coffea arabica cv. Typica 'Guatemala'.

Two methods of gene transformation were used to insert the cystatin gene into tissue cultured leaves and somatic embryos.

Agrobacterium tumefaciens-mediated transformation was performed on 5,413 leaf discs of Kona Typica coffee. *Agrobacterium*-infected leaf discs were placed on media containing geneticin sulfate (G418) for a 4–month period for selection. After another 7 months, embryogenic calli were produced from 3 of the leaf discs. Regenerated plants were obtained from two somatic embryo lines.

Somatic embryos, derived from leaf discs, were also used for transformation. *A. tumefaciens*-mediated transformation and particle gun bombardment were performed using 2 cystatin constructs with the promoters, 35S and Tubulin, as well as, with the dual proteinase inhibitor, cystatin and CpTI. Twenty-six lines of plants were regenerated from *Agrobacterium*-infected somatic embryos cultured for 7 months under selection with G418. A total of 60 plates of somatic embryos were transformed with gene bombardment. Bombarded somatic embryos were selected for 7 months. Seven lines of the bombarded embryos have regenerated 82 plants.

Leaf tissue will be analyzed by PCR for presence of the inserted gene. After root induction of the shoot regenerants, the plants will be tested for their effectiveness against nematodes in an *in vitro* assay.

— R. Myers (UH), C. Nagai, B. Sipes (UH) and D. Schmitt (UH)

Forestry Research

Biosystems Technology Program: Gorse Control Project

Gorse (*Ulex europaeus*) is a spiny legume shrub that can grow over 3 m tall, and has a life span of 20 to 30 years. Its natural range is western Europe, where it occurs singly or in small clumps, but where it has colonized new environments, it forms dense impenetrable thickets. Gorse first became naturalized in Hawaii in the 1920s. It can be found on 7000 acres of valuable range land on Humu'ula on the eastern flank of Mauna Kea on the island of Hawaii.

The gorse control project proposes a forestry-based strategy by shading out germinating seedlings until the seed bank is effectively exhausted, or by providing sufficient cash flow to justify intensive gorse control. The objective is to screen a range of tree species and provenance to identify which tree species are best adapted to the various environmental conditions and have the ability to out compete gorse. Trial planting of commercially important conifers and hardwoods as well as native forest trees, such as koa (*Acacia koa*) noted for its beautiful wood, and mamane (*Sophora crysophylla*) the primary food source for the endangered endemic bird palila, will be planted within the gorse containment area for native forest restoration evaluations.

Tree improvement

No significant new eucalyptus plantings were installed during this time period. However, previously installed clonal tests indicate that selected eucalyptus clones outperformed operational seedlings by over 25% in terms of annual volume accumulation at several test locations on the island of Hawaii. An additional koa test was installed on a former pineapple site with Maui Pineapple Company, Ltd. Growth measurements were continued for previously installed trials over a range of diverse sites across the state.

Silviculture

Timber stand improvement work is focused on developing silvicultural treatments for koa management, which will lead to better understanding of methods for promoting koa stand vigor and growth. Initial tree girdling treatments were imposed after

completing the environmental permitting process.

— *N. S. Dudley*

Miscellaneous Crops

Field Testing Induction of Systemic Acquired Resistance (SAR) in Taro

Taro Leaf Blight (TLB) can have a devastating effect on taro production. In previous greenhouse tests, a foliar application of a suspension of the chemical benzol (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) was found to reduce the diameters of lesions when leaves were inoculated with the fungus *Phytophthora colocasiae*, the causal agent of TLB.

This line of research was expanded to a field trial on Maui with two additional chemical inducers being added: Messenger, a harpin protein-containing material, and KeyPlex, a formulation of micronutrients. Both of these inducers have been reported to induce SAR in other crops. Thirteen completely randomized blocks of four treatments each were laid out on the western slope of Haleakala covering a total area of approximately 0.1 ha. Chemicals are applied at three-week intervals starting at the time of transplanting. Treatments were as follows: BTH 100 g ai/ha; Messenger 315.1 g/ha (9.45 g ai/ha); KeyPlex 5.2 l/ha; and a water control.

The three inducers are hypothesized to have different modes of action in the induction of SAR defenses. Whereas BTH mimics one of the normal components in the signal transduction pathway that activates SAR proteins, the harpin protein triggers an earlier response in the SAR pathway. Stimulation at this earlier point is thought to turn on two parallel pathways, SAR and Ethylene-Jasmonic Acid (wound response). Each pathway's end products are proteins that will help protect the plant against pathogenic attacks. KeyPlex's mode of action is not understood at this point, but its application has also been seen to increase production of the proteins theorized to belong to the SAR pathway.

Leaves from one of the blocks were collected at intervals from 1 hour to 20 days after an application. Analyses of these leaves for levels of marker enzymes for SAR induction are underway.

Fig. 1. Phytophthora colocasiae *lesions observed during prolonged cold rainy period*

Fig. 2. *Rose Beetle Damage*

During the early part of January 2002, the islands were subjected to a prolonged cold rainy period. During this period *Phytophthora colocasiae* lesions were observed in the test field and surrounding taro field. Data were collected and are being analyzed to determine if differences in rate or degree of infection can be seen between treatments.

The possibility has been broached that elevated levels of chitinase from the induction of the SAR pathway may have effects on insect pests attacking the leaves of the taro. Data on rose beetle damage among the treatments have been recorded. Even if one or all of these treatments substantially reduces damage from fungus infection or insect predation, and/or increases corm weight, the level of application of these inducers would appear to be too high to make them cost effective. The next step is to find the lowest level of application (amount or frequency) that offers significant protection.

— J. Carr (USDA/ARS), J. Zhu, G. Ito, R. Osgood and P. Moore (USDA/ARS)

Micropropagation of Taro

Micropropagation can be used to produce large numbers of taro propagules in a shorter period than conventional propagation methods. The objective of this project was to produce micropropagules of a wide range of taro cultivars for field evaluation. HARC's micropropagation protocol using stationary liquid culture (HSPA 1994 Annual Report pp 47-48) developed for 'Lehua Maoli' and 'Bun-Long' taro cultivars was applied to 13 new taro cultivars including five dasheen types (triploids). The taro cultivars used in this project were selected in a companion study by Dr. John Cho, UH, Kula Branch Station, Maui.

A total of 52,600 propagules were produced during 2001. An average of 55.5 ± 8.6 % of the original shoots produced propagules. Nine of the cultivars produced over 5,000 propagules per cultivar from 4 to 6 initial shoots within about 12 months. Speed and efficiency of multiplication varied among the cultivars. Cultivars 'TCE18' and 'Ngachete' multiplied fast, and produced 5,000 propagules in seven and nine months respectively, whereas four cultivars did not multiply efficiently, resulting in only 1,300-2,000 propagules during 12 months. Various culture medium modification experiments were performed to try to increase multiplication rates. No major effects were found in multiplication or growth rate by changing amounts of BAP (6-benzylaminopurine, 0-4 mg/L) in media. Genotype difference was the major factor affecting cultivar performance in culture.

Micropropagules of seven cultivars were planted in the field during 2001 and four of these are now at the second generation of propagule evaluation. We have not observed somaclonal variations (variations due to *in vitro* culture) among first-generation micropropagules in six of these cultivars. Cultivar TCE 46 exhibited color variations among 2,800 plants in one field trial. Random counts in one line in the field showed that 67% of the plants had mostly green stems and 33% had mostly purple stems. These micropropagules were derived from four shoots of TCE46 which did not show any color variation during micropropagation in the lab. We have not confirmed coloring of 'TCE46' mother plants, but we expect that purple and green variegation (color chimeras) also existed in the original plants. Two different color types might have been produced during micropropagation.

Micropropagation-derived plants often produced more side shoots than those from conventional propagules. For example, in Hawaiian sugarcane, significantly higher numbers of shoots were obtained in most varieties. The number of side shoots of

'Iliuaua' taro was compared between two propagation sources, micropropagation and conventional side-shoot (huli) propagation. No significant difference was found between huli-propagated (4.7 ± 1.0) and micropropagated (4.5 ± 0.6) Iliuaua.

The project confirmed that HARC's taro micropropagation protocol can be applied to a wide range of taro cultivars, including dasheen (triploid) types, without somaclonal variation in the field.

— *C. Nagai and J. Buenafe*

`Awa

Awa (kava kava) has been grown in the Hawaiian islands for about 1,000 years. There are approximately thirteen cultivars of Hawaiian `awa. Each has a unique morphology and anecdotally has a slightly different effect when the root is consumed as an extract beverage. This is thought to be due to the relative amount of the six main kavalactones found in the roots of these plants. A study is ongoing, in collaboration with the University of Hawaii College of Tropical Agriculture and Human Resources. The aim of the study is to determine the optimal light, fertilizer rate, amount of mounding and degree of pruning for Hawaiian cultivars. In addition, anecdotal evidence suggests that kavalactone content in healthy `awa plants reaches a maximum at eighteen months after planting. This study also intends to generate data to objectively define the plant age at which kavalactone content is maximized. A problem that Hawaii's `awa growers face is that many `awa buyers are only willing to buy `awa root based upon its kavalactone content. Pricing is based upon the percentage kavalactones. This means that currently, in order to determine kavalactone content, prior to reaching a negotiated price of purchase, a grower must dig up one or more of his `awa plants for analysis. If the kavalactone content is low, the grower does not have the option to replant the `awa, thus effectively sacrificing those plants that were harvested and the potential revenue from them. Therefore, this study will attempt to define a strategy to predict the kavalactone content in the root. Rather than study each individual Hawaiian cultivar, one (Green Mo`i) cultivar is being compared with a very different cultivar (Isa) from Papua New Guinea. The rationale is that based upon the scale of response of these two cultivars, the other Hawaiian cultivars are likely to respond somewhere in between. Plants were sampled at the time of planting in order to determine plant part chemical profiles (relative concentrations of the six different kavalactones). Plants were then sampled every six months and the chemical profile and overall kavalactone content were compared over time. The plants are now two years old and will be grown for a further six months. All data will be collated and subjected to statistical analysis in order to determine which treatments affect chemical composition and overall kavalactone content. It is expected that a set of cultural practice guidelines will emerge that maximizes the yield of kavalactones. This information will be relayed to Hawaii's `awa growers through information bulletins and presentations at growers' meetings.

In addition to this study, HARC's Analytical Chemistry Laboratory continues to provide analytical services for `awa growers and distributors in Hawaii and the rest of the South Pacific.

— *M. C. Jackson and S. Bittenbender (*UH)

Separation of Glycosides from Stevia

A grant from the the Agricultural Development in the American Pacific Land Grants Program was received to develop a method to purify the sugar-like molecules (glycosides)

from the stevia plant. The aim of the project was to purify the glycosides in much the same way as sucrose is purified from sugarcane. This was not successful, as the glycosides could not be crystallized from an aqueous solution even at extremely high concentration. The study therefore focused on developing a method to separate some of the very similar glycosides found in partially purified, commercial stevia preparations. Stevia contains a number of similar glycosides, however only two are found in any significant quantity: stevioside and rebaudioside A. Although stevioside is somewhat sweet, it also exhibits a bitter aftertaste and therefore is an undesirable component in commercial preparations. An extremely effective chemical means was found to separate rebaudioside A from stevioside, resulting in a very sweet preparation. The process was shown to be cost effective and could be scaled up to a commercial scale. Negotiations are currently underway to license the technology.

— *M. C. Jackson*

Potential Herbicides for Stevia

Weeds are a significant limiting factor for stevia production. The only herbicide currently registered for use in stevia is glyphosate, but it can be injurious to these plants. A field trial was conducted to identify herbicides that can be safely applied over the crop. Twenty-four herbicides were broadcast over stevia and plant injury and growth were evaluated. The herbicides tested were oxyfluorfen, imazethapyr, thiazopyr, fluazifop-p-butyl, imazapic, bentazon, clopyralid, clomazone, sethoxydim, azafenidin, diuron, terbacil, linuron, pendimethalin, flumioxazin, metribuzin, flumetsulam, propanil, prodiamine, norflurazon, carfentrazone-ethyl, halosulfuron-methyl, sulfentrazone and MSMA. The highest registered label rate for other crops was applied over mature stevia at 30 gallons per acre.

Stevia appears to be tolerant to the following herbicides: thiazopyr (1 lb a.i. per acre), flumioxazin (0.1 lb a.i. per acre), flumetsulam (1.5 lb a.i. per acre), pendimethalin (1.6 lb a.i. per acre), norflurazon (3.93 lb a.i. per acre), sulfentrazone (0.25 lb a.i. per acre) and fluazifop-p-butyl (0.37 lb a.i. per acre). Pendimethalin (Pentagon) and sulfentrazone (Authority) were further tested on stevia regrowth. The treatments were factorial combinations of 1 and 2 lb a.i. per acre of Pentagon and 0.25 and 0.50 lb a.i. per acre of Authority, with and without a sequential application at eight weeks, with and without plastic mulch, and untreated control. The initial application was made three days after the one-year old field was harvested. Pentagon had no adverse effect on stevia at both rates and with sequential application, whereas Authority reduced the growth and yield of stevia. Pentagon effectively controlled all grass weeds until harvest at 95 days with the lowest rate of 1 lb a.i. per acre. Broadleaf weeds were partially controlled with Pentagon at 1 lb a.i. per acre. Higher rate of 2 lb a.i. per acre or a sequential application of Pentagon improved the control of broadleaf weeds and increased yields. It is recommended that Pentagon be labeled for use in stevia.

— *L. Santo*

Improving Selectable Marker Gene Technology

Achieving successful genetic transformation of plants requires the use of selectable markers. However, to address some environmental concerns and to gain the public acceptance of genetically modified crops, it is desirable to remove these markers after being used for transformation. In order to do this, we have analyzed the use of inducible promoters. These are genetic

switches that inside an organism (plant) can be used to turn genes on and off. We have tested promoters that respond to induction by either dexamethasone or ethanol as candidates to control the expression of a recombinase inside the plant cells. In the presence of the recombinase, genes that are flagged by the right signals should eventually be removed.

The dexamethasone-inducible promoter was tested in tobacco plants as a model system. Using Luciferase under the control of the dexamethasone-inducible promoter, we were able to quantify the response to the induction, as well as to optimize time and concentrations of dexamethasone to use. We also found that Luciferase expression in this case was strictly controlled by the induction. By making crosses of plants carrying the dex-inducible recombinase expression with plants carrying appropriate flagged selectable markers, we should be able to remove the selectable markers after transformation.

We have also made genetic constructs to express the recombinase under an ethanol-inducible promoter in monocotyledoneous and dicotyledoneous plants. The entire system is being tested using tobacco plants transformed by means of *Agrobacterium.*

— R. S. Arias

Assessment of Genetic Diversity in Macadamia by AFLP Markers

Macadamia is a high-value nut crop native to Australia, which has become a significant crop in Hawaii. World production of macadamia nuts is based on two species, the smooth shell *M. integrifolia* Maiden and Betche and the rough shell *M. tetraphylla* L.A.S. Johnson. The smooth shell *M. integrifolia* produces higher quality nuts and all Hawaiian cultivars are of this species. AFLP markers were used to analyze 27

Macadamia raceme

macadamia accessions representing four species: *M. integrifolia, M. tetraphylla, M. ternifolia* and *M. hildebrandii* as well as a wild relative, *Hicksbeachia pinnatifolia*. Six primer pairs generated a total of 105 polymorphic markers. Pair-wise simple matching coefficients were used to construct a similarity matrix which revealed a higher level of diversity than expected. The average genetic similarity among the 19 *M. integrifolia* accessions was 0.840 and ranged from 0.697 to 0.985, whereas the average genetic similarity among the nine established *M. integrifolia* cultivars was 0.881 and ranged from 0.826 to 0.985. The average genetic similarity among all 26 accessions of the four macadamia species was 0.774. Cluster analysis revealed four main clusters with all

four macadamia species clustered independently. Within the major cluster of *M. integrifolia*, nine cultivars were separated into two distinctive sub-clusters, suggesting two diverse gene pools may have been major contributors to macadamia variety improvement programs. Our results show a higher level of genetic variation exists among the macadamia germplasm compared with that of other tropical tree crops that we have studied such as coffee and papaya.

— D. L. Steiger, P. H. Moore (USDA/ARS), F. Zee (USDA/ARS), Z. Liu and R. Ming

Molecular Diversity of Ralstonia solanacearum Isolated from Ginger in Hawaii

The genetic diversity of *Ralstonia solanacearum* isolates from ginger (*Zingiber officinale* Roscoe) growing on the island of Hawaii was determined by analysis of amplified fragment length polymorphisms (AFLPs). Initially 28 isolates of *R. solanacearum* collected from five host plant species worldwide were analyzed by AFLP. A second analysis was conducted on 55 *R. solanacearum* isolates collected from three ginger farms along the Hamakua Coast of the island of Hawaii, the principal area of ginger cultivation in the state. From the initial analysis, *R. solanacearum* from ginger in Hawaii showed a high degree of similarity at 0.853. In contrast, the average genetic similarity between *R. solanacearum* from heliconia and ginger was only 0.165, and isolates from ginger showed little similarity with strains from all other hosts. The second analysis of 55 isolates from ginger in different Hawaiian farms confirmed that isolates from ginger were distinct from race 1 strains of tomato, but isolates from ginger showed greater diversity among themselves. The greatest diversity occurred among isolates from a farm where ginger is frequently imported and maintained. Our results provide genetic evidence that *R. solanacearum* strains from ginger in Hawaii evolved separately from local strains in tomato (race 1) and heliconia (race 2) strains.

— Q. Yu (UH), A. M. Alvarez (UH), P. H. Moore (USDA/ARS), F. Zee (USDA/ARS) and R. Ming

Services

Assessment and Improvement Recommendations for the Molokai Irrigation System

The Hawaii Agriculture Research Center conducted an evaluation of the Molokai Irrigation System (MIS) for the State of Hawaii, Agribusiness Development Corporation to recommend changes to mitigate the current drought in central Molokai. The system in Waikolu valley (dams, wells and tunnel), Kualapuu (transmission pipeline and reservoir) and Hoolehua (distribution pipeline and users) was visited on August 15, 2001. Past reports, memoranda, meeting minutes and data relative to this task were reviewed at the State of Hawaii, Department of Agriculture (DOA), Agricultural Resource Management Division office in Honolulu. Relevant data were digitized for analysis. Weather, tunnel flows, pumping, reservoir depth and customer use data were obtained and utilized. Various individuals from the DOA, MIS Users Advisory Board, University of Hawaii Cooperative Extension agents, and interested community members were contacted to document the issues and concerns of the Molokai community.

The current water shortage is primarily the result of the most severe drought since the inception of the MIS. The drought started in 1998 and is continuing through 2001. Rainfall total of 7.97 inches in 1998 was a record low compared to the annual average

of 22.68 inches since 1970 at the Kualapuu reservoir in central Molokai. The rainfall in 1999 and 2000 were the second and sixth lowest totals at 9.22 and 11.84 inches, respectively. The dry weather has decreased water collection in Waikolu valley and increased water demand in central Molokai resulting in the Kualapuu reservoir depth dropping to 4 ft, the lowest level on record.

The findings indicate that improving the water collection in Waikolu valley, reducing system losses and developing new sources could result in obtaining additional water. New sources being considered are stream diversions of Waihanau, Kawela, Kaunakakai, Manawainui and use of some brackish wells. The additional water may be sufficient to increase the customer base from the current 2,931 acres to about 6,000 acres with a total of about 12 mgd. This assumes that more than 6 mgd can be gained by system improvements and from new sources. The 12 mgd is still not enough to support the 9,960 acres in the current service area of Hoolehua. Therefore, expansion of the MIS to Kalamaula homestead is not feasible unless more water can be obtained from the northeastern Molokai such as Pelekunu stream with an average flow of 17.2 mgd. Any development in the northeastern mountains will be costly and likely met with environmental and cultural opposition. The Kalamaula area could be served directly by diverting the water flow of 0.5 mgd from Waihanau stream to irrigate about 125 cultivated acres.

Recommendations for the development of new water sources are long-term courses of action. Environmental and cultural issues of the impact of water removal on the ecosystem, other water sources, the Public Trust Doctrine and Hawaiian rights require studies before any new water project can proceed. Four new sources are proposed: stream diversions on Kawela, Kaunakakai, Manawainui and development of brackish wells near the current MIS system.

Short-term actions are more feasible, and these emphasize the improvement of the efficiency of the water collection, transport, storage, distribution and customer use. These recommendations are divided into system and management improvements. It is roughly estimated that up to 20% more water can be gained by minimizing known system losses. Water use as measured by MIS customers' meters has never exceeded the west portal tunnel flow, the water collected in Waikolu. The west portal flow provides the best estimate of the total water available before transmission, storage and distribution losses. Evaporation loss alone is about 300 million gallons annually or about 15% of the total available water. Seepage loss from the reservoir could be higher than evaporation loss, but was not measured. The storage of water in Kualapuu reservoir is expected to be the difference of the west portal flow and the flows adjusted for evaporation loss and customer use. From 1990 through 1999, the expected cumulative water storage is expected be 2.540 billion gallons, which is more than the capacity of the 1.4 billion-gallon reservoir. Since the reservoir depths have steadily decreased, there are likely other major losses in addition to evaporation such as errors in the measurement of the west portal flow and the customer water usage. Twenty-seven of 30 recommendations are short-term actions to minimize losses, improve irrigation efficiencies and better manage the MIS.

— L. Santo

Crop Services at HARC's Kunia Substation

In 2001 and 2002, the Kunia Substation came into its own as a facility for winter nurseries, seed production, growout and research. During the 2001 and 2002 winter seasons, the entire substation's available land was in production. It was also necessary to utilize

land on a neighboring farm to complete an additional ten acres of winter nursery work. The substation provides an excellent facility for this type of work. It has a well trained, dedicated staff. Most temperate, subtropical and tropical crops can be grown year round on the approximately one hundred acre substation. The longest and shortest day lengths are 13.5 and 11 hours, respectively. During the winter period, supplemental lighting is successfully used to grow daylength-sensitive crops. The annual long-term rainfall is 27 inches, with most rainfall occurring during November through February. All fields are drip-irrigated.

During 2001 and 2002, the substation grew crops of barley, coffee, corn (field and sweet), papaya, potato, rice, sugarcane, sunflower and wheat. The land at Kunia is well utilized throughout the year. A large field block of the farm had a rotation of potato, field corn, sunflower and rice in 2001 and in 2002, some field blocks had a rotation of rice, corn and potatoes. Forty-five acres of potato for seed indexing were planted in 2001, double the acreage of the previous year. Potato clients were pleased with the quick growth of potatoes on the substation that provided them with early indexing information. In 2002, a new potato client was added and the acreage increased to sixty acres. A change in netting design and net size has allowed for a reduction in cost in controlling birds on cereal crops grown for seed, a major constraint to production in the past. Considerable success has also been established in the control of the nutritional requirements and weed control in rice grown at the station. Research done by the Kunia staff to evaluate various herbicides for rice during the summer 2001, proved to be very valuable in the 2001 and 2002 winter growing seasons. Herbicide trials in summer 2002 provided excellent information on various herbicide mixtures useful in growing potatoes. This information was utilized successfully in the 2002 growing season. The Kunia substation staff continues to strive to develop improved cost effective methods of growing crops and welcomes the opportunity to grow new crops, not previously grown on the station.

— L. Poland

Pathology

The Pathology Department provides services that include field inspections, disease diagnoses, pathogen isolations and control recommendations. Soil and root sampling for nematode population estimates, damage estimates and species identification are also provided. These services are available to the HARC Kunia Substation, HARC clients on a consulting basis, sugarcane producers, and HARC and USDA personnel in support of research projects. Sugarcane plantations routinely require seed farms inspections and laboratory screening of field cane for systemic diseases such as ratoon stunting disease, leafscald and yellow leaf virus. In addition to sugarcane, services were provided for wheat, barley, rice, tomato, potato, lettuce, taro, papaya, coffee and peppers during the years 2001-2002.

— S. Schenck

Environment

Natural resource protection is a critical component of farming since without good stewardship of the land, air and water, agriculture cannot be sustainable. Hawaii's unique island ecosystems present formidable challenges to conservation amidst an ever-growing human, animal and alien invasive species population. The coordinated efforts and financial support of both the public and private sector are necessary to adequately manage these special places. HARC's staff works closely with State lawmakers and regulatory agencies to promote environmental protection in scientifically

sound and economically feasible ways.

We keep abreast of the latest federal and state laws and rules in order to help farmers apply the laws correctly to their everyday workplace situations. We assist our members in understanding and complying with the many environmental laws and regulations that affect agriculture. HARC continues to provide training for members and others who need to know and understand this specialized information.

This year, we worked with the Hawaii state legislature, regulatory agencies and other stakeholders to develop appropriate environmental policies regarding surface and coastal water quality, wastewater reuse, used oil management and endangered species habitat, among others.

— J. Ashman

Quality Assurance Unit

HARC's participation in the pesticide registration process under the Environmental Protection Agency's Federal Insecticide Fungicide, and Rodenticide Act has tapered off markedly in the last 10 years. Nonetheless, an independent Quality Assurance Unit (QAU) is maintained to inspect and audit such studies. The QAU participated in a papaya and a banana magnitude of residue study. HARC's facilities were inspected for compliance with the EPA's Good Laboratory Practice standards by two of our sponsors, one in 2001 and the other in 2002.

— B. Vance

Computer System Administration

The local area network (LAN) at the Experiment Station is tightly integrated with the USDA/ARS. Technical support is provided to USDA/ARS users. HARC updated its inventory with the addition of 6 new computers including 4 laptops that help fulfill the mobile phase of our disaster recovery plan. New users to the LAN were provided with access to HARC's LAN and in some cases e-mail accounts; they were given an introduction to networking, network printing, and e-mail. Continued assistance was provided to users to facilitate the migration from Microsoft's Office suite to Sun Microsystem's StarOffice suite. Documentation of frequently asked questions for each of the StarOffice modules was enhanced. Standard Operating Procedures were revised to document the restarting of the file server, the e-mail gateway PC, and the telephone exchange. Memory was added to select printers and PCs. A firewall was added between the Internet and our local area network. Our telephone system was replaced with one that incorporated voice mail.

— B. Vance

Laboratory Services

Analytical Chemistry Services

Our contract laboratory provides analytical chemistry services to Hawaii's agricultural community, industries, government agencies and also to US mainland and international companies. We analyze for natural and synthetic compounds in plants, soil, water and air using gas chromatography and high-performance liquid chromatography instrumentation. Our analytical residue determinations range from quick screens for general information to detailed research studies including pesticide registration projects for the United States Environmental Protection Agency (USEPA).

HARC's work on papaya provided data to support the pending USEPA registration of

imidacloprid. This study is proposed to add the group of crops including papaya, star apple, black sapote, sapodilla, mango, canistel and mamey sapote to the imidacloprid registration. These crops will probably be added to the the registration labels of Provado® and Admire®. Both products protect crops against sucking insects such as leafhoppers and aphids and are very important to the papaya industry in Hawaii. Our study included the field trials and the determination of residues of imidacloprid and its four metabolites in papaya. The reports and raw-data packages were submitted to the Interregional Research Project No. 4 (IR-4), a national agricultural program which supports research on pest control agents for minor crop uses. IR-4 will prepare the final imidacloprid registration report for USEPA.

International projects included sample analyses for fourteen different residue trials from pineapple companies in the Philippines for their pesticide registration and import tolerance levels.

Other services including process development of agricultural crops into value-added products provide an advantage to Hawaii's businesses. Process method development and analytical services were provided by HARC's Chemistry Laboratory for processing stevia and decaffeinated coffee.

— *J. Pitz*

Entomology

Monthly visits were made to HC&S to assess percent damage caused by lesser cornstalk borer on young cane fields, and to assess percent parasitism of lesser cornstalk borer by *Horismenus elineatus*. Data were gathered less frequently from G&R.

It was decided to discontinue the colonies of lesser cornstalk borer and *Horismenus elineatus* at the HARC laboratory in Aiea. A summary of the lesser cornstalk borer program was written in collaboration with Asher Ota, and posted on the HARC website. Two training session in the identification and management of arthropod and rodent pests of sugarcane were given to field staff at HC&S.

Leafhopper monitoring

A grant was received from the Hawaii Department of Agriculture to monitor the movement of a newly-introduced leafhopper on watercress farms in the Aiea/Pearl City area. This work was carried out in collaboration with Crop Care Hawaii LLC. Yellow sticky cards were placed on the borders of five watercress farms in the Aiea/Pearl City area and in mixed vegetable farms in Ewa and Kunia to monitor the potential spread of *Macrosteles* sp. nr. *severini,* a newly-introduced leafhopper (Homoptera: Cicadellidae) that became established on watercress in 2000. Trapping started in late March 2002, and continued through late July 2002. Traps were changed about every 10 days. Traps were also placed on a lettuce farm in Haleiwa and a tomato farm in Waialua and changed every two weeks. Low numbers of *M.* sp. nr. *severini* were trapped on three of five watercress farms sampled in April, and also in low numbers on one watercress farm in June and July. *Macrosteles* sp. nr. *severini* was never found on yellow sticky cards placed in Ewa, Kunia, Haleiwa or Waialua. Data on different leafhopper species trapped at different sites during the study were summarized in a report to the Department of Agriculture.

Routine monitoring of pests on crops grown at the Kunia substation for HARC scientists and HARC clients was carried out in 2002. These crops include papaya, coffee, wheat, rice and potatoes.

Assistance was given to HARC scientists

testing for resistance of sugarcane to lesser cornstalk borer and resistance of papaya to mites.

— H. Smith

Apple Snail Control

Grants were received to study the effect of extracts from neem (*azadirachta indica*) on the golden apple snail (*pomacea canaliculata*). The golden apple snail is a major pest throughout the State of Hawaii, inhabiting taro lo`i and causing potentially severe loss of wetland taro. In southeast Asia, this snail is also a major pest in rice fields. Chemicals have been registered with the US Environmental Protection Agency, but have since been withdrawn due to their excessive toxicity and lack of specificity. Relatively more benign natural molluscicides have therefore been sought in this project.

Initially, neem leaves and fruit, and papaya fruit skin and leaves were tested for molluscicide activity. In aquaria, snails were exposed to these plant parts, and were either able to eat them or were prevented from eating them by placing them in a container with a mesh lid. The aim of the experiment was to discriminate between potentially water-soluble molluscicides leaching into the water and molluscicides only active by ingestion. In neither case was molluscicide activity observed. Extracts were therefore made and added to the aquaria as liquid concentrates. Pronounced molluscicidal behavior was observed for papaya fruit skin and neem whole fruit extracts. Due to the lack of significant stands of neem in the islands, only papaya extracts were pursued to determine the active compounds. Papaya fruit extracts were examined and the active ingredient identified. The purified compound was extremely active at low concentrations against golden apple snails, with 100% mortality within a few hours after application.

Funding is now being sought to undertake a field trial on the island of Kauai to determine efficacy, selectivity and potential phytotoxic effects. Funding is also being sought to expand field trials to southern China, in collaboration with researchers at the University of Beijing.

— M. C. Jackson

Bioremediation

There are a number of projects currently underway which are aimed at the problems related to the difficulty of remediating heavily chlorinated compounds from soils. These compounds include pesticides such as heptachlor, heptachlor epoxide, PCP, DDT, and other compounds such as polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs). These compounds remain in soils for decades and have the potential to contaminate crops grown in their presence. The projects are briefly described below:

1. A project is underway to try to address the problem of chlorinated pesticide contamination of agricultural soils. In the past, mainland USA and Hawaii have experienced potential public health problems related to residual toxic compounds found in produce, in some cases resulting from compounds not applied in more than twenty years. Microbial degradation of chlorinated pesticides is being studied in an effort to isolate a microbial species capable of completely degrading such compounds.

2. In another current project, Hawaiian sourced microbial organisms are being screened in liquid and soil based cultures for their ability to degrade PCBs and PAHs. Gas chromatography (GC) and and GC-mass spectrometry are being used to detect compounds and potential degradation products. Laboratory phase

studies are ongoing with tests on small amounts of soil.

3. A project was initiated in late 2002 to select and test an appropriate microbial species for degradation of heptachlor in a field site where very low levels of heptachlor have been found. The laboratory phase of this project is now underway.

— *M. C. Jackson and S. Schenck*

Proprietary Contracts

A number of contracts were secured to work for local and US mainland companies on proprietary projects. Many were related to botanical development and included crops such as `awa and stevia.

— *M. C. Jackson*

Wood Treating

Samples of treating solution from a local wood treating company were routinely analyzed for the presence to two wood preserving compounds, chlorpyrifos and iodopropynylbutyl carbamate (IPBC). These analyses are ongoing.

— *M. C. Jackson*

Administration & Support Staff

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Sandra Kunimoto, Dir. of Marketing & Bus. Dev.
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Kunia and Maunawili Substations

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Angel Galvez, Mechanical Operator
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Ernest Gamatero, Experimentalist
Richard Kinoshita, Breeding Station Supt.
Rogelio Pascua, Experimentalist
Leslie Poland, Kunia Farm Manager
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
Francisco Habon, Field Worker
Gael Ito, Experimentalist
Pacifico Padilla, Senior Field Worker
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Kauai Substation

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Shawn Steiman, University of Hawaii
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Sugar Company Production

Company	2001 Acres Harvested	Tons Raw Sugar (96°)	Tons Sugar Per Acre
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**

** Includes Kekaha salvage cane*
*** Weighted average*

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