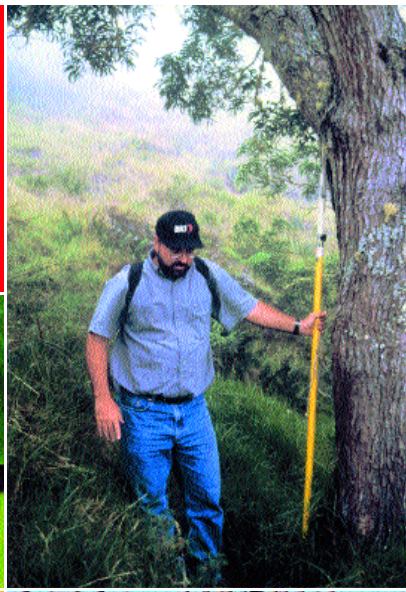
HARC

2000 Annual Report



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On The Inside

Front cover images clockwise from bottom left corner:

1. New low-acid pineapple *(Ananas comosus)* fruits from Hawaii are transforming the fresh fruit market.
2. Hawaii produces specialty coffees *(Coffea arabica)* on the four major islands.
3. Awapuhi *(Zingiber zerumbet)* ready for harvest for upscale shampoo products.
4. Koa *(Acacia koa)* tree ready for transformation into high-value wood products.
5. Two-year old sugarcane *(Saccharum spp. hybrids)* ready for harvest and milling.
6. Wheat *(Triticum aestivum)* represents the rapidly growing seed service business in Hawaii.

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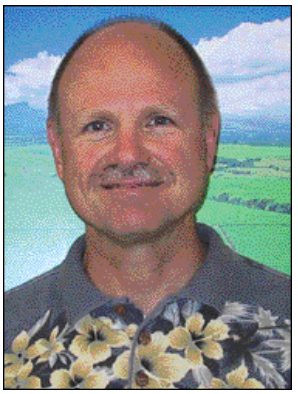

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Message From The Director...

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

We present the 106th Annual Report of the Hawaii Agriculture Research Center

Continuing a recent trend, there was an additional reduction in sugarcane acreage in 2000. Since 1990, 11 sugar plantations have ceased operations vacating over 100,000 acres of Hawaii's prime agricultural land. Our sugar industry is now comprised of two large farms utilizing 62,000 acres. Located on their own land in excellent production areas with high sunlight and well-developed water sources, the surviving plantations are committed to achieving sustainable operations, but are realistic about the changes that will be required in operations, including the need for crop and product diversification plans.

While the rapid reduction of acreage in the sugarcane industry has provided numerous opportunities for diversified crops, these new agribusinesses must now develop the infrastructure necessary for successful agricultural operations. There seems to have been an unrealistic expectation that a varied agricultural industry including import replacement and export crops, should have quickly developed on the newly available land. Although there are instances of successful agricultural operations on the former sugar acreage, for the most part the land is vacant or in low return pasture operations. It is clear that only a small portion of the land is needed for import replacement; thus the main emphasis needs to be on export crops or services. Over 100 years ago, the sugar planters were in a similar position. They recognized the difficulty of delivering a competitive agricultural commodity, sugar, to a distant marketplace. They also faced the usual challenges of land, water, capital, internal road and railroad systems, processing facilities, threats to production including procurement of nutrients and the control of pests. The individual sugar companies (there were 51 in 1900) developed the infrastructure necessary for sustainability at the farm level; however, in the broader matters of labor procurement, product marketing, transportation, refining and research, the sugar planters banded together and pooled resources. Vertical integration of the industry from the field to the retail store was achieved by the Hawaiian sugar industry long before the concept was considered by others. Cooperation was the key to success for the high-volume, low-value commodity being produced at great distance from the market.

Diversification of Hawaii's agriculture has been strongly advocated since the 1970s, but what is the vision? Obviously, an increase in both import replacement and export crops is desired. We believe the production of raw commodity crops is no longer feasible for Hawaii; although there is certainly opportunity for the development of commodity-based specialty products and high-value by-products. Two examples from Hawaii's largest agricultural industries are washed, large crystal raw sugars (Maui Brand Sugar) and the new low-acid pineapples produced by Hawaii's pineapple companies. Another example of export success is the ornamental flower and potted plant industry which, when taken as a group, is the third largest agricultural industry. Import replacement, especially in the vegetable and fruit crops,

Stephanie Whalen joined HARC in 1973 as an analytical chemist. She was named President of the 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.

HARC

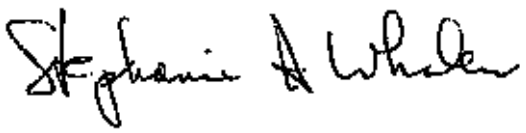


has proven to be successful and it is estimated that another $100,000,000 of import replacement is realistic. The diversified crops industry faces challenges of finding affordable land, water, labor, road infrastructure and maintenance, capital, transportation and marketing. Certainly, a spirit of cooperation is needed within and among the diversified crop industries, especially those exporting crops.

The three featured articles in this report emphasize HARC's diversification and the focus on the development of a modern, technology-based agriculture in Hawaii. Improving the understanding of fungal disease resistance is especially important for expanding the tropical fruit industry. With the shift of research emphasis toward modern biotechnology and away from chemicals, solutions to the serious fungus diseases in fruits such as papaya are now on the horizon. Another area of emphasis is the development of high-value products such as pharmaceuticals using sugarcane and other crops as the production system. Our isolation from major crop production makes us an ideal location for this highly regulated activity.

Breeding, selection and the identification of promising new cultivars continues to be the primary activity of our sugarcane team. The leading sugarcane cultivar at the end of 2000 was H78-7750 occupying 32 percent of the area in cane. The coffee breeding program initiated in 1997 is beginning to yield progeny for evaluation. Amplified fragment length polymorphism (AFLP) analysis of the genetic diversity in our coffee germplasm collection was completed and submitted for publication. We look forward to developing linkage maps for coffee and using the newly developed tools in a marker-assisted breeding program. Our work on forest trees, papaya, pineapple, taro and selected botanicals continued with emphasis on improving yield potential through improved agronomic practices, breeding and selection using traditional tools as well as modern biotechnology. Contract services primarily to the seed industry continued to diversify our funding base, allowing the maintenance of a diverse, multi-disciplinary staff to assist our members with their research needs. We continued to help raise the level of understanding and recognition of the economic value of the agricultural sector to the State.

I want to extend our appreciation to our member companies for their support and commitment to research, to the State and Federal legislators for their support, to partners in the agricultural community and to our many clients.



Building erected in 1904 to house the Agriculture and Entomology Departments

100 years ago - 1900

The turn of the twentieth century was an exciting time for the Hawaii sugar industry and its Experiment Station. Sugar production had reached 289,544 tons on 51 farms, the largest crop ever produced to that time. The yield of sugar was 4.53 tons per acre.

Walter Maxwell, the first Experiment Station Director resigned to take a position in Australia similar to the one he held in Hawaii. He was succeeded by Dr. R. E. Blouin. At the 20th annual meeting of the Hawaiian Sugar Planters' Association, (forerunner of HARC) president C. M. Cooke announced that the Experiment Station laboratory would be moved from its location at the Robinson Block to the Makiki St. property of the Experiment Station. "A suitable building was erected on the grounds of the Experiment Station and thoroughly equipped under the direction of Mr. R. E. Blouin for the execution of all kinds of chemical work."

The first attempt to produce cane seedlings was made under the direction of Dr. Blouin, but no germination was recorded.

In 1900, Dr. R. C. L. Perkins, working for the British Museum, observed and captured a leafhopper, which was later identified as a new species. The leafhopper became a serious pest of sugarcane and threatened the sugar industry with extinction.

The Young brothers initiated their launch service when Herb and Bill Young took the Billy (a 22-foot boat powered by a Union 4-horsepower gas engine) to Pearl Harbor through the then undredged channel.

Twenty-five Okinawan laborers arrived for work in the Ewa cane fields on Oahu. Puerto Ricans landed for the same purpose in Lahaina, Maui.

Improving Fungal Disease Resistance In Papaya Using A Chitinase Gene Transformation System

Y. Judy Zhu, Maureen Fitch (USDA/ARS), Stephen Ferreira (University of Hawaii), Terryl Leong, Leslie Akashi and Paul Moore (USDA/ARS)

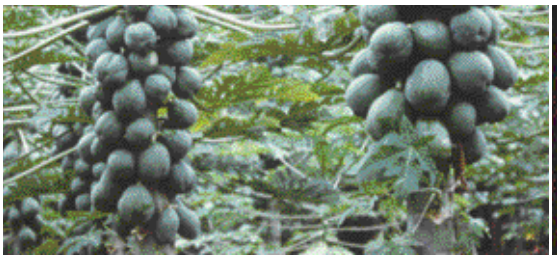
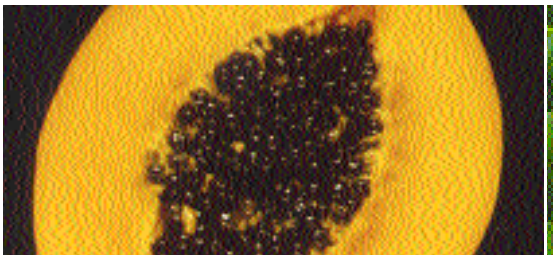
In Hawaii, fungal pathogens of papaya frequently cause significant losses for growers. Losses generally range from 10-30% annually on most farms.

The purpose of this project centers on developing and evaluating the rice chitinase transgenes in papaya to confer broad-spectrum resistance to fungal pathogens. Recent literature suggests that chitinase activity is associated with plant disease resistance for a wide range of fungal pathogens. This notion is supported by the absence of chitin in higher plants and by the observation that chitinase has anti-fungal activity *in vitro*. The introduction of chitinase genes into plants under the control of a constitutive promoter increased plant fungal resistance in greenhouse and field studies.

Chitinases catalyze the hydrolysis of chitin, an important structural component of the exoskeleton in many insects and the cell walls of most fungi, such as *Colletotricum gloeosporioides* (anthracnose) and *Oidium caricae* (powdery mildew) in papaya, with the notable exception of the pythiaceous fungi such as *Phytophthora* sp. and *Pythium* sp. Although the cell walls of pythiaceous fungi do not contain chitin, zoospore germination is inhibited by chitinases.

Phytophthora rot of Papaya fruit caused by the fungal pathogen, *Phytophthora palmivora*

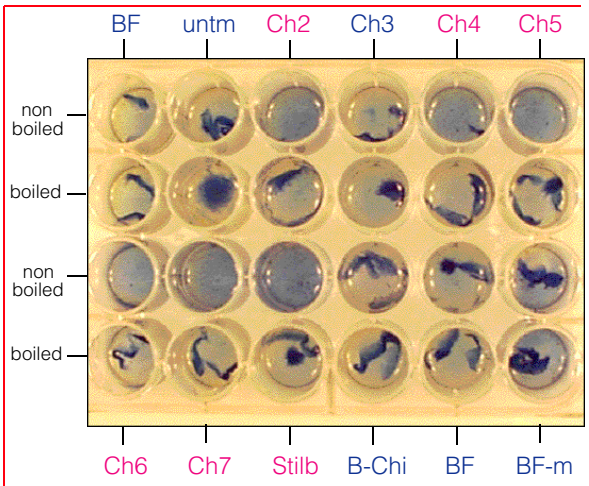
To obtain fungal resistance in papaya cultivars, the original construct of a rice chitinase gene (with hygromycin selection marker (hpt) was inserted into papaya. Embryogenic calli generated from the Kamiya cultivar were bombarded with gold particles coated with a rice chitinase gene construct. Six independent transgenic lines have been selected through hygromycin antibiotic selection and confirmed by Polymerase Chain Reaction (PCR), Western and Southern blots for the presence of chitinase transgene. In order to select transgenic plants more efficiently using the antibiotic, the rice chitinase gene was sub-cloned into a transformation vector with kanamycin resistance gene. The new transformation


construct was bombarded into embryogenic calli generated from the Kapoho cultivar in June and again in October 2000. About twenty callus lines have been selected after a three-month kanamycin selection period. Fifteen of these callus lines have been confirmed with GUS staining assays and plants are being regenerated from the GUS-positive callus. The process of enzyme-linked immunosorbent assay (ELISA) testing is underway to confirm the presence of the kanamycin resistance gene, NPTII. PCR or Southern blot analysis will be performed on these lines to further confirm the integration of the transgene into the papaya genome.

The Kamiya transgenic plants are in the process of rooting and micropropagation for greenhouse and field evaluation. *In vitro* pathogen incubation assays were performed to test antifungal activity of rice chitinase transgenic lines. The boiled and unboiled crude protein extracts from Kamiya transgenic lines, were filter-sterilized and incubated with zoospores of *Phytophthora palmivora* at 24°C for 48 hours. Fungal mycelia which developed from the zoospores were stained with blue cotton dye. We found that non-boiled protein extracts from transformed plants reduced spore germination and germ tube elongation of *Phytophthora palmivora*. Inhibition of zoospore germination and mycelial growth of *Phytophthora* by crude enzyme extracts from six different transgenic papaya plants transformed with rice Chitinase. In the boiled samples, antifungal activity was abolished indicating that chitinase proteins did enhance the antifungal activity in the transformed plants. Neither untransformed Kamiya papaya plant extracts nor the extraction buffer itself had any effect against *Phytophthora palmivora*.

We are encouraged by the initial results obtained in this project and look forward to advancing the work in greenhouse and field studies.



In vitro pathogen assay against P. palmivora mycelia

Production of Recombinant Protein in Tropical Plants

Ming-Li Wang, Cindy Goldstein and Henrik Albert (USDA/ARS)

The world market for pharmaceutical proteins is currently many billions of dollars per year and is projected to continue increasing significantly for the foreseeable future.

Sugarcane fields may serve as the biofactories of the future

Currently these proteins are produced in mammalian or microbial cell-culture "fermenters". Maintaining large-scale cell-cultures under sterile conditions requires very expensive equipment, facilities and highly skilled staff resulting in high production costs. Crop plants engineered to express such recombinant proteins have been demonstrated as an alternate production system. Farmland and tractors may prove to be a much more economical production system than high-tech cell-culture facilities. This would give agricultural producers some very high value alternate crops that could be grown with existing methods and equipment.

To assess whether tropical plants, such as sugarcane and rice, are good “biofactory” systems to produce high-value proteins, we used a human hormone, granulocyte macrophage colony-stimulating factor (GMCSF), as the model recombinant protein. Our major achievement during the past year has been the construction of recombinant gene vectors for plant transformation. A series of multifunctional vectors were constructed for plant transformation via *Agrobacterium*. These vectors will be used for transformation of rice, where we will try to direct GMCSF expression specifically in the seed. These vectors contain a convenient insertion site for the gene of interest and three gene markers for transgenic plant selection and screening. These three markers can be removed after the transformation has been confirmed. Rice transformation using *Agrobacterium* is currently underway.

Rice was chosen as one of our test plants for three reasons. First, while rice is currently not grown commercially in Hawaii, in the past it was widely grown, and in fact was a major export crop. From this historical experience and recent experience at the HARC Kunia substation, we know that rice can be grown in Hawaii's environments. Secondly, our previous research with *Agrobacterium* transformation of rice convinced us that transgene silencing occurs much less frequently in this system than in our transgenic sugarcane. Lastly, producing recombinant proteins in



seeds offers significant stability and storage advantages over whole plant production. Work in other labs has shown that recombinant proteins can be extracted efficiently from rice seeds after months of ambient temperature storage. Particularly, proteins directed to accumulate in specialized vacuoles called protein bodies have been shown to remain stable and to have native biological activity after months of seed storage. Whereas plants producing a recombinant protein throughout the plant body would likely require processing immediately after harvest to prevent protein degradation, seed-specific production would allow storage and shipment to a distant processing plant over a long time frame.

For sugarcane, we have constructed five GMCSF gene-expression constructs. Some of the features included in this series of constructs are the use of three different gene promoters, the use of subcellular targeting signals for improving yield and the addition of a peptide tag to allow efficient affinity purification. These gene constructs were transferred into sugarcane via particle-gun bombardment. Some small plants have now regenerated from the earliest bombardments.

The feasibility of using sugarcane as the recombinant protein production system was also assessed using transgenic plants produced by previous, unrelated projects. Although most of the transgenic sugarcane lines tested accumulated significant amounts of their particular recombinant protein while still in callus tissue, the plants regenerated from these callus lines have undergone transgene silencing at a high frequency. This resulted in the recombinant protein accumulation being very low or undetectable. This phenomenon, while very useful for certain applications, is a major barrier to developing sugarcane as an efficient biofactory system. In another project, the mechanisms of transgene silencing are being studied and a possible future project will be the testing of transgene constructs designed to avoid the problem.

Sugarcane was chosen as one of our test plants because Hawaii's climate is very favorable for sugarcane, because of the expertise with this crop at HARC and at the plantations, and because the existing mills can potentially be used for initial extraction steps. If sugarcane can be used to produce high-value products in addition to sugar, it would be possible to utilize our growing conditions, expertise and mills for a far greater return. Possibilities for using sugarcane for co-production of recombinant proteins together with fiber or other products also exist.

Recent results from our lab indicated that all seven sugarcane plantlets tested from the earliest round of GMCSF transformation are accumulating GMCSF protein. A great deal of time and work are still required to determine how many of these plants will reach maturity without undergoing transgene silencing and if so, whether GMCSF levels accumulated under field conditions can reach economic levels. Nevertheless, these results are very encouraging. Given the large number of transgenic GMCSF plants we now have in the pipeline, we are confident that this project will provide good data on the suitability of sugarcane and rice for biofactory use.