TROPICAL AND SUBTROPICAL AGRICULTURAL RESEARCH

Under PL 89–106, Special Research Grants Program
Cooperative State Research Service, U. S. Department of Agriculture

Progress and Achievements
The Pacific Basin Administrative Group
1992

College of Tropical Agriculture
and Human Resources
University of Hawaii

College of Agriculture
and Life Sciences
University of Guam
On the Cover

A researcher samples guttation fluid from the surface of a cabbage seedling for assay by immunofluorescent colony staining, an extremely sensitive technique developed to detect the bacterial cells that cause black rot in crucifer crops. The technique aids researchers in monitoring the progress of infection nondestructively in symptomless plants, part of a project to control black rot with monoclonal antibodies. The project is described on page 14.
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ACKNOWLEDGMENT

This research was supported by the Special Grants Program for Tropical and Subtropical Agricultural Research, Cooperative State Research Service, U. S. Department of Agriculture, under PL 89-106.
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The current program in tropical and subtropical agricultural research had its legislative origin in Section 406 appended to PL 89-808. Section 406 was an outgrowth of the World Food Conference held in Rome, Italy, in November 1974. At that conference, U. S. representatives announced that the United States could not “feed the world,” but would contribute to the assistance of developing countries in efforts to strengthen their own food production capabilities. Section 406 authorized the U. S. Secretary of Agriculture to enter into research contracts and cooperative agreements with land-grant universities and other appropriate research institutions; conduct research on crop and food products and make the results available to friendly developing nations; increase cooperation with the Peace Corps, Agency for International Development, and foundations to accomplish the overall mission of the program; and authorize expenditures up to $33 million annually to accomplish the missions of the program.

The initial funding to USDA for research under this program occurred in the late 1970s, when an appropriation of under $400,000 was allocated to the Agricultural Research Service (ARS). During the first years of operation, research was conducted by ARS and cooperating universities. In 1982, the decision was made that for Fiscal Year 1983 most of the program would be transferred from ARS to the Cooperative State Research Service (CSRS) of the USDA. The budget was split, with $735,000 allocated to the ARS tropical agriculture research program and $2,980,000 allocated to a new CSRS tropical and subtropical special research grants program. CSRS administers the program through the Caribbean Basin Administrative Group (CBAG: University of Florida, University of Puerto Rico, and University of Virgin Islands) and Pacific Basin Administrative Group (PBAG: University of Hawaii and University of Guam). Small increases in the appropriation have been obtained since 1985 to the present (FY 1992) level of $3,320,000, which CSRS divides equally between the Caribbean and Pacific Basins. Research is conducted through Special Grants in Tropical and Subtropical Agricultural Research awarded by CSRS.

The program supports research on the organisms, environments, and processes of tropical agricultural systems of the American-affiliated Pacific area. Fifty-nine active projects now focus on critical agricultural problems in the Pacific region. The program provides assistance to domestic agriculture in many ways, including research on tropical and subtropical crops grown in the United States, control of threatening pests, enhancement of the genetic stocks for many major U. S. crops, protection and conservation of soil and water resources, and the development of technologies important to the small farmer. The program’s research projects deal with high-priority areas to solve critical problems as well as to develop new opportunities for agricultural production, information technology, and marketing competitiveness in the tropics and subtropics of the Pacific Basin and elsewhere.

The program’s objectives are to identify and fill knowledge gaps in tropical agricultural systems of the Pacific Basin and to facilitate the transfer and use of results. Priority research thrusts are developed by the PBAG and announced in the Call for Preproposals to be used in formulating objectives for project research plans. The Pacific Basin research thrust areas for 1991-92 are:

Development of management strategies for improving production while conserving resources and preserving environmental quality;

Improvement of postharvest processing and distribution technology for agricultural products of the tropics, including quality preservation;

Development of new crops, germplasm, and products based on demonstrated economic potential in Pacific Basin areas;

Application of biotechnology to agricultural systems to increase efficiency and quality of plant or livestock production;
Management of risk and uncertainty in tropical agricultural systems;
Improvement of human nutrition through studies of the composition of foods, food consumption practices, and nutrient requirements in the tropics;
Resolution of livestock production problems unique to tropical areas;
Studies of family resource management in tropical agricultural production and consumption systems.

New research projects are selected from grant proposals submitted each year by eligible faculty. The selection process includes an internal preproposal screening at the college level and a full proposal evaluation by external peer reviewers. The PBAG evaluates the review comments in selecting proposals to be funded. Project funding is usually for three years, with projects funded annually based on federal appropriations to the program. Current research grants are reviewed and evaluated for satisfactory progress each year by PBAG.

Individual project completion reports are submitted to USDA/CSRS for final approval. Technical results are commonly published in peer-reviewed journals and presented at discipline-based conferences and workshops. Summary highlights and importance to users are published in Progress and Achievement Reports such as this one approximately every two years.

This research program continues to make significant progress in knowledge-based development that is pertinent to real-world challenges facing agricultural clienteles in the Pacific region.

Victor D. Phillips
Pacific Basin Coordinator
CROP MANAGEMENT

Sustainability of Taro-based Cropping Systems in Hawaii and the American-affiliated Pacific

Principal Investigators:
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Nature of Project
This project, in cooperation with the Soil Management Collaborative Research Support Program (TropSoils), has developed a computer model capable of simulating taro (Colocasia esculenta L.) along with a variety of other crops. The model, CropSys 2, will permit the long-term economic evaluations needed to estimate sustainability. Management strategies for improving sustainability, i.e., intercropping and green manures, can now be evaluated under the range of weather conditions expected at a site.

Much of the basic research underlying CropSys 2 comes from the International Benchmark Sites Network for Agrotechnology Transfer (IBSNAT) project and the Nitrogen Fixation in Tropical Agricultural Legumes (NifTAL) project. CropSys 2 represents a synthesis of science from three major international networks. This project has provided the "glue" to integrate the information and apply it to needs in Hawaii and the American-affiliated Pacific.

Major Achievements and Importance to Users
Millions of dollars have been spent developing crop models for sole crops, mostly in temperate regions. Although these single-species models represent a wealth of basic scientific information, the limits of their application have not always been clear. By linking sole crop models together to simulate intercrops, CropSys 2 provides a framework for testing many of the assumptions of the basic models.

Fieldwork performed by graduate students Leslie Poland (East-West Center grantee) and Falaniko Amosa (South Pacific Region Agricultural Development project) is producing some significant discoveries. Models for phenology (i.e., time of flowering and maturity) developed in sole crops do not work when applied to inter-crops. The shading of one crop by another provides a "daylength shift": rice, for example, planted under a taro canopy produced fewer leaves and flowered earlier than its counterpart planted in an open field. This response is consistent with an effective decrease in the daylength inside the taro canopy. Further research is now required to better quantify and model the effects.

Presentation of these results at meetings in Thailand and at Michigan State University created a great deal of interest among the sole crop modelers. Crops planted with other crops are subject to stresses that test plants—and plant models—to their limits. Sole-crop modelers benefit from intercrop research by better understanding the physiology of plants under exceptionally high stress. This research also is the basis for understanding and modeling taro grown under trees (i.e., on coconut plantations) and taro in competition with weeds.

Fig. 1. Intercropping (right) can severely reduce growth of taro. Computer simulations are helping us find more sustainable taro systems.
Optimum Fertilization of Horticultural Crops Based on Petiole Sap Diagnosis

Principal Investigators:
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R. R. Coltman, Dept. of Horticulture, UH
V. Santos, University of Guam

Nature of Project
An important way to monitor crop growth is to analyze plant tissues and determine the internal levels of important plant nutrients. In the past this analysis has required elaborate laboratory facilities; it is expensive, usually costing more than $10 per sample, and there is a delay while the sample is dried and analyzed, often at a distant laboratory. Recently, rapid analysis capabilities for major plant nutrients have become available to farmers. Analysis can be performed in the field by measuring nutrient levels in sap squeezed from the plant. Portable selective ion meters costing only a few hundred dollars have been developed, and test strips that change color in the presence of certain ions are available at a cost of less than a dollar per analysis. Previous work at the University of Hawaii established the utility of rapid techniques for plant sap analysis in the case of nitrate in tomato plants. In the present project a cooperator on Guam is studying nitrate levels in watermelon sap, and researchers in Hawaii are investigating nitrate and potassium levels in papaya plants.

Major Achievements
There are no major achievements at this time. Field preparations, including reduction of soil nitrogen levels, are complete, and planting will be in early 1992 for both the papaya and the watermelon experiments.

Importance to Users
Once the relationship is established between levels of sap nutrients during plant development and the eventual yield of fruits, papaya and watermelon farmers will have a convenient and inexpensive method to monitor the nutrient status of their crops. This is particularly vital for papaya in Hawaii, most of which is grown on porous, nutrient-poor lava soils, where fertilizers must be applied frequently to prevent nutrient deficiency and decreased yields. If the investigations are successful, not only will farmers be better able to ensure optimum nutrient levels in the crop, but also they will be able to avoid overapplication of fertilizers and thus minimize groundwater contamination.

Papaya Management Expert System

Nature of Project
Developing a papaya management expert system initially involved identifying common problems with papaya production encountered by experts in the disciplines of soil science, botany, plant nutrition, plant pathology, entomology, and weed science. Computer scientists then "captured" and integrated the knowledge and the problem-solving approaches of the agricultural scientists by incorporating the expertise into a software package capable of running on an IBM-PC-compatible computer.

Major Achievements
The "Professor Papaya" (PROPA) expert system has been a significant attempt to produce major innovations in the application of artificial intelligence to crop management. Past efforts with other crops were generally little more than

Fig. 1. Experts from the University of Hawaii, the Hawaii Department of Agriculture, and the Cooperative Extension Service meet with a papaya grower. Routine problems, however, can often be solved with the help of microcomputer-based expert systems, without the direct involvement of research personnel.
database-information retrieval systems. PROPA not only contains passive information (document library and research bibliography) but incorporates active processes of knowledge, electronically placing a multidisciplinary, interactive panel of experts at the service of the user.

PROPA’s key innovative capability is its problem-solving component. Users select descriptions of problems affecting papaya growth, yield, or fruit quality, and Professor Papaya’s panel of experts then requests additional information necessary to identify the causes of the problems and offer solutions.

**Importance to Users**

PROPA was designed without a limited clientele focus, avoiding a single-discipline approach and attempting to deal with many types of users from farmers and students to extension agents, specialists, and researchers. PROPA covers most homeowner-gardener problems as well as the problems most affecting the Hawaii papaya industry. PROPA can serve as a prototype for other personal computer–based expert systems.

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**Crop Microclimate: Key to Improved Cropping-system Management**

Principal Investigator:

Elizabeth A. Graser, Dept. of Agronomy & Soil Science, UH

**Nature of Project**

This project takes a unique approach to understanding and predicting a crop’s microclimate, a difficult and complex problem. Wide-row crops and intercrops are the study systems, so flow observations will show much clearer patterns than seen in more uniform crops. A grid of temperature sensors will allow visualization of flow in the crop under conditions with strong thermal turbulence types—thermal, mixed, or mechanical. Data from other sensors will help explain the flow processes by accounting for the energy available. Analysis will result in a model of canopy flow and the crop microclimate.

**Major Achievements**

We have built and are testing and calibrating a fast-response temperature-measuring grid and data acquisition system. We have devised a way of synchronizing operation of this equipment with our two three-dimensional sonic anemometers. We have planted appropriate cropping systems for field experiments.

**Importance to Users**

In the 1990s and beyond, improvements in cropping system management will mean efficient use and conservation of water, environmentally sound pest control, and, possibly, adaptation to climate change. To make these modifications to a cropping system in a predictable, scientific way, the crop’s microclimate—which controls transport of water vapor between the crop and the atmosphere, regulates the environment of pests, and is a crop’s interface to changing climate—will have to be understood adequately.

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**Production and Management of Vesicular-arbuscular Mycorrhizal Inocula for Large-scale Use in the Tropics**

Principal Investigator:

M. Habte, Dept. of Agronomy & Soil Science, UH

**Nature of Project**

Vesicular-arbuscular mycorrhizal (VAM) fungi improve plant growth largely by increasing the uptake of immobile nutrients, particularly P, through the extension of their hyphae from root surfaces to regions of soil that are beyond the reach of the unaided root. A major obstacle to the large-scale use of these fungi in agriculture is our inability to grow them in laboratory media because of their requirement for actively growing plants.

This project is concerned with the development of a standardized protocol for the production, preservation, and application of mass cultures of VAM fungi based on infected roots. The approaches involved include the identification of suitable nurse plants; a clear definition of the nutritional, cultural, and environmental factors for inoculum production; the evaluation of VAM fungal characteristics; and the influence of prestorage and storage conditions on the efficacy of infected root inocula.
Major Achievements

Several nurse plant species were screened during the initial phase of the project with respect to root productivity and rapid susceptibility to infection with VAM fungi. Corn was found to be the species with the largest quantity of root and the highest level of VAM colonization.

In subsequent studies corn (Fig. 1) was used to assess the influence of nitrogen, rock phosphate, and superphosphate on infected root inoculum production. These studies have shown that P levels ranging from 0.02 mg/l to 0.07 mg/l can be satisfactorily used for producing infected root inocula. If rock phosphate is available, it can effectively replace superphosphate when applied at the rate of 17 g/kg. Rock phosphate did not influence VAM colonization levels; its effect on infected root inocula is related largely to its effect on root mass.

The optimum level of nitrogen for infected root inocula was found to be 80 to 120 mg/l.

Results of studies we completed more recently have shown that root biomass and VAM colonization of roots can also be significantly enhanced through the management of starter inoculum density, nurse plant density, and duration of growth of nurse plants.

Results of inoculum dose–response studies have indicated that the amount of infected root inoculum associated with maximum host response lies between 0.5 and 1.0 g/kg of soil.

Importance to Users

Using the information we have already generated and that which we will be generating during the balance of the project, we will develop a protocol for the production, preservation, and application of infected root inocula. We anticipate that the availability of the protocol will enhance the widespread use of infected root inocula by mycorrhizal researchers and by those who are interested in the effective integration of VAM symbiosis in low-input agricultural systems.

Fig. 1. Response of corn to vesicular-arbuscular mycorrhizal infection. MYCO = inoculated; -MYCO = uninoculated.

Increasing Crop Productivity Through Soil and Plant Analysis, Using the Diagnosis and Recommendation Integrated System (DRIS)

Principal Investigators:
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R. S. Yost, Dept. of Agronomy & Soil Science, UH

Nature of Project

We are trying to improve crop productivity through more accurate methodologies of testing soils and diagnosing plants for nutritional needs. Specifically, our research has focused on coffee, guava, and macadamia crops, and on the diagnosis and recommendation integrated system (DRIS) approach, for the following reasons.

1. Coffee, guava, and macadamia are crops important to the tropics and to Hawaii in particular. These crops have served as alternatives to sugarcane and pineapple in Hawaii’s agriculture, and are being planted to lands abandoned by sugarcane. Such lands are mostly marginal for sugarcane; otherwise they would have remained in that crop. It is expected that alternative crops will encounter numerous nutritional problems and require a high degree of cultural management, including fertilization and soil amendment, to be successful.

2. The DRIS approach to predicting plant nutritional needs uses a holistic approach based on the principle of nutrient balances. This approach is thought to be more reliable than the conventional one of using critical or threshold levels.

3. No available DRIS data exist for coffee, guava, or macadamia.
**Major Achievements and Importance to Users**

Using DRIS values (norms) recently established by us, nutritional requirements of guava for maximum fruit production can be reliably estimated. As a result of our work, guava growers can apply correct fertilizers and increase profits through higher production; the environment also benefits, because nutrient pollution (e.g., nitrate or phosphate contamination) can be minimized.

**Aquafarm Structure and Size: A Dynamic Analysis Under Uncertainty**

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- Dept. of Agricultural & Resource Economics, UH (Visiting)
- Y. C. Shang, Dept. of Agricultural & Resource Economics, UH
- J. K. Wang, Dept. of Agricultural Engineering, UH

**Nature of Project**

Aquaculture is a new and emerging industry, particularly in the Pacific region. As with any new industry, not much is known about the desirable operational and investment levels. This project addresses the question of optimal farm size in aquaculture production. Farm size refers to the size of the total farm, which may consist of one or more farming units. A “farming unit” here refers to the basic unit of operation, such as a pond or a tank in aquaculture, or a field in agriculture. Farm size depends on many factors, including the scale economy of operation as well as the economic and sociological background of the region in which the farm will be operated. Size of the farming unit is primarily determined by the technical conditions of the operation. The size of the farming unit and its relationship to other farming units depend on the economics and management of the whole farm system, however. Many aquaculture operations are multicomponent in nature. For example, shrimp mariculture may include specialized facilities for maturation, hatchery, and nursery in addition to the grow-out operation. This complicates the process of determining the optimal farm size as these components are all interrelated, and determining optimal farm size would require the determination of the optimal size of each component. This problem involves managing long-run fixed assets and assuming risk from over- and under-capacity of each component due to random market and production fluctuations. For instance, a shrimp mariculture operation may want to have a hatchery that can produce more postlarvae than are needed by the nursery and grow-out phases. In this way, a consistent supply of postlarvae can be made available to the rest of the operation while avoiding the large fluctuations of postlarvae production in the hatchery. The excess postlarvae will have to be sold in the marketplace, however, where the producer generally faces random price fluctuations.

In summary, the problem of determining the optimal aquafarm size requires the determination of the optimal size of each of the production components and their interrelationships as related to the uncertain production and market environments.

**Major Achievements**

A conceptual framework in the form of a nonlinear programming model was developed to derive the optimal farm structure and size, considering the multicomponent production process of most aquaculture operations. In particular, the model takes into account the four distinct phases of aquaculture production: maturation, hatchery, nursery, and grow-out. Analytical solutions were derived for two special cases assuming markets for intermediate products (a) exist and (b) do not exist. The model has been implemented on GAMS/PC, a microcomputer-based mathematical programming system.

Costs of independent production of naupili, postlarvae, juvenile, and adult shrimp for various sizes of operation have been derived using production parameters from the Oceanic Institute. To derive the joint cost function, a synthetic experimental design has been used to simulate the production costs of various combinations of facility sizes for each of the four operations. After the joint cost function is estimated, it will be used together with the nonlinear programming model to determine the optimal size, structure, and operating strategies for shrimp mariculture in Hawaii and Guam.
Importance to Users

As mentioned earlier, aquaculture production has emerged as a potential new industry in the Pacific region. As in agriculture production, aquaculture production requires large initial investment outlays. In addition, the rigidity of the invested assets in aquaculture production is more serious than in agriculture. In facing an inelastic demand for agricultural and aquacultural products, the difficulty in adjustment would be more pronounced in the case of aquaculture. It is imperative in this early stage of the development of the aquaculture industry to identify the optimal size of operation to ensure a successful industry in the Pacific region. This would minimize the high cost of failure before investment takes place. A successful aquaculture industry can contribute to the diversification of the local economy, improvement in the balance of payments, and increase in employment opportunities in the region. Agricultural production economists have been concerned with farm size since the birth of agricultural economics. Optimal farm size is an ongoing concern of the profession. Development of the conceptual framework to solve for the optimal farm size in a multicomponent production system with production and market risks, as in aquaculture, will contribute to this ongoing quest.

Delineating the Hawaiian Climate for Crop Introduction, with Emphasis on Cocoa

Principal Investigator:
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Nature of the Project

Economic and environmental changes create the need to explore the feasibility of growing alternative crops in many agricultural areas of the world. Since soil and climatic factors are the most important determinants of crop productivity, the usual method for a new crop introduction is by field trials at a number of sites. Due to logistical considerations and convenience, the selected sites are generally limited to existing agricultural experiment stations, which very seldom represent all possible combinations of soil and climate conditions existing in a region. Conclusions based on these trials can be misleading. Therefore, an alternative methodology reducing total dependence on limited field experiments would be preferable. This is especially true for Hawaii, where climatic and soil conditions vary considerably over short distances. This research developed an analogous model coupled to a geographical information system for searching sites similar environmentally to sites where the crop under consideration has been grown successfully. This approach was used to evaluate the potential for growing cocoa in Hawaii.

Major Achievements

Basic requirements of cocoa for soil and climate were needed to develop this methodology. A comprehensive literature search was conducted to collect these data. The data were synthesized to establish the optimum conditions for cocoa production. Yield-affecting variables were also divided into unmanageable and manageable variables. The unmanageable ones were used to eliminate unsuitable cocoa-growing sites, and the manageable variables were used to predict input requirements. A comparative land productivity assessment methodology for a land-rating model was developed to predict cocoa yield and input needed to sustain the predicted yield with minimal field experimentation.

The methodology was applied to the island of Hawaii for identifying potential cocoa production sites. Fig. 2 shows areas suitable for cocoa production. There are about 49,000 hectares of land (based on unmanageable variables) where cocoa can be successfully produced. Fig. 3 shows the irrigation requirement for bringing potentially

![Fig. 1. Delineation of Hawaiian cocoa sites with respect to existing sites.](image-url)
suitable sites up to par with the reference site. Many island of Hawaii land parcels were predicted to have a potential yield of around 4000 to 4500 kg of dry beans per hectare after six years of planting.

Importance to Users
Agricultural planners and farm managers can use this methodology to identify potential sites for a new crop or to check the appropriateness of their existing site for growing a new crop. The methodology is not limited to any specific site or environment. It is capable of reducing the amount of trial and error research, as well as the risk of introducing a new crop to areas where the soil and weather data are available but no previous history of production of that crop exists. Its application to the mapping of suitable land for cocoa on the island of Hawaii should be useful to new growers as well as planners in the state. This methodology of analogous transfer to predict comparative land productivity in similar environments can be used throughout the world. The methodology reduces the cost of experimenting and time requirements for evaluating new crop introduction. Only readily available existing soil and weather data are needed. One important practical benefit of this study is the development of versatile interactive computer software for land-use planning for various crops.

Evaluation of the Potential of the Pejibaye (*Bactris gasipaes*, Palmae) for Palm Heart Production in Hawaii

Principal Investigator:
Richard Manshardt, Dept. of Horticulture, UH

Nature of the Project
Rising costs of land and labor are undermining the profitability of sugar and canned pineapple production in Hawaii. In order to remain economically viable, Hawaiian agriculture is diversifying into horticultural export crops with high unit value. The development of new crops for Hawaii, such as the tropical American peach palm, or pejibaye, is a part of the diversification trend. The pejibaye palm heart, consisting of the tender new leaves above the growing point, has potential as a gourmet fresh vegetable crop, both to supply the Hawaiian tourist’s desire for exotic foods and for export to U.S. West Coast markets. The United States currently imports 2000 tons of canned palm heart annually, valued at $4.5 million, of which 700 tons are pejibaye. Demand has grown continually during the past decade (+135 tons/year) and is expected to expand more rapidly as fresh palm heart, with wider culinary potential, becomes available. The growth habit of pejibaye, which produces a new shoot annually, is well suited to management as a perennial crop for palm heart production. This project is evaluating the potential of pejibaye palm heart to become a component of diversified agriculture in Hawaii.

Major Achievements
Eight open-pollinated progenies from both of two populations of pejibaye with spineless leaves and stems (from Yurimaguas, Peru, and Benjamin Constant, Brazil) have been introduced. Progenies of a third spineless population from San Carlos, Costa Rica, have been requested. Three palm heart progeny trials with plant densities ranging from 3333 to 6666 plants/ha are being established at three locations with environmental conditions representative of major Hawaiian agroecosystems. Two germplasm collections (on Oahu and Hawaii) will also be established to evaluate growth and yield for fruit and seed. Growth and yield will be evaluated to identify outstanding progenies. Physiological and genetic parameters and genotype–environment interactions will be estimated. The Honolulu Chefs...
d’Cuisine, a professional association of chefs, has been contacted and provided with small quantities of fresh palm heart for experimentation, so that when harvests from research plots become regular, the fresh product can be quickly and smoothly added to tourist and local menus to test consumer acceptance.

**Importance to Users**

This program provides a model for new crop introduction into the United States, as it gives priority to (1) obtaining a suitably wide genetic base, (2) evaluating germplasm adaptation under different environmental conditions, (3) preparing for a genetic improvement program in the event of farmer interest, and (4) developing culinary options to inspire local and tourist demand for the product.

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**Using Genetic Potentials of Crop Plants to Solve Problems of Acid Soils**

Principal Investigator:
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**Nature of Project**

Infertility of acid soils is one of the major factors that limit plant growth, affecting as much as 40 percent of the world’s cultivated soils. Plants grown on these impoverised soils suffer primarily from aluminum (Al) toxicity or manganese (Mn) toxicity. One solution to this problem is to lime to de-acidify the soils; however, this is not a feasible option for many resource-poor farmers of the tropics. An alternative solution is to select plant cultivars that tolerate these mineral stresses. Fortunately, there is wide genetic variability in the sensitivity of cultivars to soil acidity.

In this project, cultivars of two tropical crop species, taro and macadamia, are being characterized in their tolerance of Al and Mn toxicity. One of the goals of this research is to develop screening methods that plant breeders can use in their selection of cultivars that are tolerant of the mineral stresses of acid soils.

**Major Achievements**

A screening method has been developed to test taro cultivars for tolerance of Al toxicity. Taro cultivars are grown for approximately two months in hydroponic solutions containing 0 and 24 parts per million (ppm) of Al. Then, the changes in expansion growth parameters (fresh weights of leaves and roots, leaf areas, and root lengths) in response to increased Al are compared between cultivars.

Significant cultivar differences were found in Al tolerance, with ‘Lehua maoli’ exhibiting only a slight decrease in fresh weights of leaves and roots as Al increased from 0 to 24 ppm (Fig. 1), and ‘Bun-long’ showing a dramatic decrease in fresh
weights of leaves and roots between 0 and 24 ppm Al (Fig. 2).

Aluminum-tolerant 'Lehua maoli' is known to have a higher calcium oxalate content than Al-sensitive 'Bun-long'. Current research efforts, using ion chromatography and X-ray dispersive analysis, are aimed at determining whether the oxalate content of taro is related to Al tolerance.

**Importance to Users**

Plant breeders can use this screening method developed to select for and breed for taro cultivars that are tolerant of Al toxicity. A knowledge of the plant mechanisms involved in Al tolerance is critical, because without this information plant breeders could select inadvertently for cultivars that are more sensitive to acid soil infertility. For example, low acridity (irritation) in taro is considered a desirable trait; however, the same calcium oxalate crystals that could be partly responsible for acridity may also be involved in Al tolerance.

Fig. 1. Growth of Al-tolerant 'Lehua maoli' at 0 and 24 ppm Al.

Fig. 2. Growth of Al-sensitive 'Bun-long' at 0 and 24 ppm Al.
Synchronous Development of Tropical Fruit Trees

Principal Investigators:
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Leslie H. Fuchigami, Dept. of Horticulture, Oregon State U.

Nature of Project
In the tropics, a major constraint in the cultivation of tree fruits and nuts is the lack of synchrony in the production of vegetative flushes, flowers, and fruits. The lack of synchrony prevents the development of effective management systems for production of high and consistent yields and concentrated maturation of the crop. For example, nonuniform flowering of coffee and macadamia trees results in nonuniform fruit maturation. This leads to increased labor costs due to problems in implementing mechanical harvesting technology, and multiple harvests to gather the entire season’s crop. The objectives of this study are to develop production strategies for coffee, macadamia, and mango crops to stabilize production and regulate the timing of production.

Major Achievements
Identification of coffee varieties that are most suitable for mechanical harvesting can help to stabilize the labor input. Based on the amount of force (FRF) required to detach ripe fruits from trees, our studies show that the potential for greater selective mechanical harvesting of ripe berries is better with the Yellow Caturra, Catuai, and Guatemalan varieties. Greater FRF differences between ripe and green fruits early in the day show that morning harvesting increases selectivity. Guatemalan and Catuai are now considered the principal varieties suitable for mechanical harvesting, and several varieties have been eliminated as prospects for mechanization. Ethephon, a ripening chemical used on fruit crops, will stimulate the ripening of coffee berries. If it is applied when 25 percent of the berries are ripe, the remaining berries ripen within six days without a reduction in total tree yield, bean quality, or beverage quality. We have also been able to stimulate more uniform coffee flowering and fruit maturation when a growth regulator is applied to flower buds that are at the proper stage of development. The presence of suitable daylengths for flower induction during five months of the year is responsible for the prolonged flowering of coffee in Hawaii.

Macadamia trees exhibit vegetative flushing throughout the year; however, most of the flowers are associated with flushes that occur during late spring and early fall and within one to two years after the flushes develop. Research with mango has shown that a 2 or 4 percent solution of potassium nitrate fertilizer sprayed onto the foliage stimulates flowering within three weeks after application. The effect can vary with variety and growing environment.

Importance to Users
The results from this project have given coffee producers information to obtain more synchronous flowering and fruit ripening and have identified varieties most suitable for mechanized harvesting. This can result in more uniform ripening, a more concentrated harvest, and a stabilization in the amount of labor required for harvesting operations. To obtain maximum flowering and yield in macadamia, it is important for growers to encourage maximum vegetative growth during late spring and early fall through fertilization and supplemental irrigation. Results from mango experiments will enable growers to have more predictable yields during the normal harvest season and have the option of producing a crop during the off-season.

Fig. 1. Coffee beans of Guatemalan variety.
Development of Techniques for the Mass Production of Larval Rabbitfish (Siganidae) for Culture in Micronesia

Principal Investigator:
Stephen Nelson, University of Guam

Nature of Project
The objective of this project is to develop techniques for the mass production of juvenile siganids, also known as rabbitfish. These herbivorous fish are associated with coral reefs and are popular throughout the Pacific islands. Subsistence or commercial cultivation of rabbitfish has been considered within the U.S.-affiliated Pacific islands, including Guam, Pohnpei, Yap, American Samoa, the Northern Mariana Islands, and Belau.

A major constraint to the development of siganid mariculture in this region is the lack of juveniles for stocking ponds. In some areas where siganids are cultured commercially, such as the Philippines and Taiwan, fry are collected from the wild and stocked in ponds. On Guam, however, the wild stocks of juvenile siganids vary drastically from year to year and are not usually available in the large numbers that would be required for stocking ponds. The commercial production of siganids on Guam is dependent on either importing fry or developing techniques for the production of juveniles in the hatchery. Because of the potential hazards such as the spread of diseases and parasites, caused by continually importing fry from other areas, the latter strategy for supplying juveniles to growers is preferred. We hope that our work will allow the routine hatchery production of juvenile rabbitfish on Guam and, through this, will allow the development of commercial siganid aquaculture on Guam and other U.S.-affiliated Pacific islands.

Major Achievements
We have developed techniques for successfully spawning and collecting the eggs from several species of rabbitfish, and fertile eggs can be obtained year-round on a routine basis without the use of hormone injections. Although we have had successful spavings of three species of siganids, we have been most successful in rearing the larvae of one species in particular—Siganus randalli. This species is new to science and was only recently described. During the course of our investigation, the species was discovered to be present on Guam, and we have been able to rear the larvae from hatching to juveniles and to rear the juveniles to sexual maturity. We have thus been able to suggest this fish as a new candidate for aquaculture development on Guam and other Pacific islands.

We have identified the period of transition from the yolk-sac stage to first feeding as a particularly critical period in rearing larval siganids. This stage has therefore been the focus of our most recent work. We now have detailed information on how age and environmental temperature affect the rates of metabolism and development of siganid embryos and larvae. We have also acquired relevant information on the behavior of the larvae, and we know how prey density and previous experience affect the incidence of feeding and larval growth. This information will be used to improve our larval rearing procedures so that mortality is reduced and yield is increased.

Importance to Users
The primary users of the information generated by our study are most likely to be hatchery technicians or managers. The information will allow these personnel to be more efficient and to achieve a higher rate of productivity per unit effort. Eventually, commercial farmers will also benefit through the increased availability of siganid juveniles for stocking ponds or other grow-out enclosures. In addition, much of the information will be useful in developing hatchery techniques for production of other marine fishes that, like siganids, have small eggs and larvae.

Publications and technical reports of the results of this research will be available at a later date. In addition, the work done in conjunction with this project will serve as the basis of two M.S. theses in biology for students at the University of Guam and as the basis for a Ph.D. dissertation for a student from the University of Washington, College of Fisheries.
Taro Production, Handling, and Processing

Principal Investigators:
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Don Vargo, American Samoa Community College
Carol S. Whitaker, American Samoa Community College

Nature of Project
The main purpose of this project was to investigate the effect of production and handling practices on the production of taro flour for further processing into cookies and pizza crusts. Effects of variables such as soil types, harvesting dates, and storage conditions of the corms on the recovery of taro flour were examined. Storage stability of taro flour for taro cookies and pizza crust was also examined.

Major Achievements
Corm density was greatly influenced by soil types, suggesting that lighter soils produce lighter corms. Harvest age also influences corm density, but whether corm density increases or decreases with harvest age depends on soil type. Corm density was found to be independent of corm weight. Unidentified site-specific factors also influence these parameters. Interaction of soil type and corm density was significant.

A simple method of producing taro flour was developed. In general, 100 pounds of taro corms can yield 20 to 25 pounds of taro flour (moisture content about 6 percent). A formulation of snap-type cookies was developed using taro flour as one of the major ingredients, and the cookies made with this formulation were well accepted by a taste panel of about 80 people. The cookies packed in laminated bags under vacuum were stable for at least seven months. Taro flour packed in laminated bags and stored at room temperature (about 20°C) was stable for more than one year, as indicated by the cookies' quality measurements. Cookies made of taro flour from various sources showed significant differences in their quality. Chocolate-type cookies can use up to 100 percent taro flour in the formulation.

A formulation for pizza crust containing about 30 percent taro flour was also developed. Pizza crusts made from taro flour stored for three months did not show significant differences in crust size compared to freshly made crust.

Importance to Users
The results of this study indicated that taro corm density was influenced by various factors. More detailed work on production practices is needed to identify these variables, and it is hoped that they can be controlled. Technology for the production of taro flour from corms can be transferred to interested parties at this time. There is considerable interest in American and Western Samoa in producing taro flour in the near future. Because taro flours produced under various conditions caused significant differences in the taro cookies produced, producers will have to mix taro flours from various sources to come up with a "standard" flour. While taro flour can be applied successfully in cookie and pizza crust formulations, the acceptance of taro cookies was most encouraging; the test product was well accepted, and most of the tasters said they would buy the product in the market if available.

Removal of Suspended Particulates from Aquaculture Effluent

Principal Investigator:
J. K. Wang, Dept. of Agricultural Engineering, UH

Nature of Project
Settleable solids are a nagging problem in aquaculture. Settleable solids settle out of the water column and remain on the pond bottom, increasing both the cost and difficulty of water management for intensive or semi-intensive cultivation of bottom-dwelling aquatic animals such as shrimp and catfish (Boyd, 1984; Chamberlain, 1988; Fast, 1986; Wang, 1990). As much as 50 percent of the feed delivered to an aquaculture system is uneaten and, together with fecal waste, often finds its way to the bottom of the pond.

We wish to investigate the possibility of using the "secondary flow" phenomenon in fluid mechanics for the removal of settleable solids/sediments from the culturing system. The layer of organic sediment has a high biological oxygen demand (BOD), with oxygen often detectable only in the top few millimeters of the sediment. Pond
bottoms with a heavy accumulation of anoxic, reduced sediment material become undesirable habitats for bottom-dwelling animals. Our research entails qualifying the problem and investigating the use of rotating flow in circular containers to concentrate settleable solids at the container's bottom center (Burrows and Chenoweth, 1955; Larmoyeux et al., 1973). The concentrated solids can then be removed by syphoning. The combination of concentration and syphoning will result in the efficient removal of these settleable solids.

This investigation includes, as components, the development of a computer program to model the water flow pattern. Such a system will assist designers of aquaculture ponds and tanks, and laboratory experiments, to calibrate and verify the computer model.

**Major Achievements**

Both field and laboratory data gave clear indications that particles smaller than 0.1 millimeter in nominal diameter largely remain suspended in the water column, while larger particles settle very effectively. The critical size for settleability is identical to that reported for solids in municipal wastewater (Levine et al., 1991); thus, we have shown that vast developments in wastewater management are particle-size dependent and are likely to be applicable to aquaculture.

Our laboratory data showed that as many as two-thirds of the particles in the total mass concentration of solids at the bottom center of our tank were larger than the critical size. This indicates that settleable solids can be concentrated as expected, and that perhaps as much as two-thirds of the total average suspended solids mass (and the oxygen demand that it causes) might be removable if this tank design and flow pattern are properly exploited.

The other component of the project, a flow chart for the computer model, has been constructed, and coding is set to begin imminently. The experimental setup has been designed and the necessary components have been ordered. It is expected that the apparatus will be ready by mid-March.

**Importance to Users**

Aquaculture farmers in the world now rely exclusively on water exchange to maintain the water quality in their culturing systems. The importance of this research to aquaculturists lies not only in the potential water and energy savings, but also in the evidence it provides for taking an engineering approach to pond and tank design. In other words, there is much more to an aquaculture pond and tank than a place to keep water and animal together. Our research establishes that pond and tank shapes and flow patterns are significant parameters in water quality management.

In places where water supply and environmental, legal, economic, and other considerations require both intensive culture and intensive water treatment—Hawaii and other parts of the United States, for example—this research strongly suggests that relatively small circular ponds should be adopted and that a rotating flow be maintained in them. Also, this research provides the aquaculture industry with some hard numbers on the magnitude of the settleable solids problem, numbers that, for an acknowledged problem, have been notably scarce.
Nature of Project

Production of crucifer crops worldwide is limited by black rot, a bacterial disease caused by *Xanthomonas campestris* pv. *campestris*. The pathogen, which affects both leaves and stems, is spread by several means, including rain splash, irrigation, aerosol dispersal, infected plant debris, and seed. An extremely low level of seed infestation (one infested seed per 10,000) is sufficient to cause total crop loss in the production field by harvest, provided that environmental conditions during plant growth are conducive to disease. The major difficulty in controlling black rot is that the bacteria may persist, multiply, and be transmitted between symptomless plants for several months without any disease symptoms developing; nevertheless, severe disease outbreaks may occur in these plants only a few weeks before harvest, with resulting crop loss.

Analysis of the temporal and spatial distribution patterns of infected plants in the production field several weeks after transplanting indicated that significant spread of bacteria had occurred in the seedbed prior to transplanting, suggesting that control measures implemented at the seedling stage would have the greatest impact on disease reduction in the production field.

The purpose of this project is to develop methods of detecting very low numbers of pathogenic cells in and on symptomless plants, to study the nature of such latent (symptomless) infections, to monitor the spread of the pathogen on and between symptomless seedlings, and to develop practical control methods that would significantly reduce disease incidence in the production fields at harvest. Monoclonal antibodies (MAbs) were the tool of choice because of their high specificity for unique antigens present on bacterial cell surfaces.

Major Achievements

Monoclonal antibodies were produced to identify and to distinguish various strains of *X. campestris* pv. *campestris*. Most of the strains were detected with MAb X21, which reacts with a lipopolysaccharide antigen on the bacterial surfaces. Using MAb X21 in an immunofluorescence (IF) staining technique, individual bacterial cells can be detected when viewed with a fluorescence microscope. In practice, however, the IF assay lacked the sensitivity necessary to detect the pathogen in liquid obtained from seed and infected plant material. Furthermore, this technique does not distinguish between live and dead cells; thus, it cannot be used to predict the potential of such cells to cause disease. To resolve this problem we have amplified the number of cells detected per sample using enrichment culture followed by an immunofluorescent colony-staining technique (IFC). This method is several million times more sensitive than IF, and it can detect one to two viable bacterial cells in guttation droplets of symptomless seedlings (Fig. 1). Since the bacteria exude readily from young seedlings, this may be the major mode of disease spread in the seedbed.

To complement the IFC technique, we also have developed a genetically altered bioluminescent transconjugant of a virulent strain of the pathogen. This bacterium contains the "lux" gene that enables it to emit light in infected tissue and in culture, but is otherwise identical to the wild-type pathogen. By placing X-ray film over infected leaf tissues at various intervals, we are able to monitor the progress of infection nondestructively in symptomless plants. By using the IFC method in combination with bioluminescence, we now are able to measure very low bacterial populations in and on plants in the seedbed. Using bioluminescence, we have determined how mineral nutrition affects the degree to which the pathogen invades cabbage seedlings. Using selective media and IFC, we are determining how bacterial populations are spread via the guttation fluids of symptomless seedlings.

Importance to Users

A key to the epidemiology and control of black rot has already been provided by demonstrating that the pathogen does occur on symptomless plants in the seedbed and that internal infections of leaves extend far beyond the area of visible symptoms. Applying bacteriocides at critical intervals to prevent spread in the seedbed should minimize the need for chemical applications in production fields, reducing the cost of production
as well as the biohazard of chemicals to consumers. Furthermore, since nutrition of cabbage seedlings is directly related to disease severity during the first six weeks of plant growth, fertilizer rates might be modified accordingly with little cost to the grower while reducing disease incidence in mature plants at harvest.

**Fig. 1 (Right).** Sampling of guttation fluid from hydathodes of a cabbage seedling for subsequent assay by IFC.

**Prevention of Bacterial Wilt Through Early Detection and Strain Identification**

**Principal Investigators:**

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**Nature of Project**

Bacterial wilt is a widespread disease of tropical crops including tomato, ginger, banana, and heliconia. The disease kills infected plants (Fig. 1), and the bacteria survive for long periods in the soil, infecting the next crop through the roots. Once established in a new area, the disease is difficult to eradicate. Banana growers and other agriculturalists in the state of Hawaii are concerned by recent reports that bacterial wilt of banana ("moko") occurs in Hawaii and may have been introduced from Central America on symptomless heliconia cuttings. The banana pathogen that causes moko disease is caused only by particular strains (race 2) of the bacterial species *Pseudomonas solanacearum*. Heliconia plants, however, may harbor both race 1 (tomato race) and race 2 (banana race) strains. Race 1 strains are already present in Hawaii on tomato, pepper, eggplant, and other solanaceous hosts, as well as on ginger. In contrast, race 2 is not widespread in the Pacific; but repeated introductions on heliconia pose a serious threat to banana growers, because if race 2 becomes established in heliconia plantings, the pathogen could move into banana plantings as previously observed elsewhere. In Central America the disease was not serious initially, but with time, the mild strains mutated to highly infectious, insect-transmitted race 2, SFR-type strains that devastated the banana industry in certain regions of Costa Rica and Honduras.

The objectives of this project are to develop specific serological and genetic probes that identify the races of *P. solanacearum* and to use these probes to detect and rapidly identify race 2 strains extracted from plant tissues of symptomless plants. Ultimately, such methods will be used to develop procedures both to exclude the pathogen from entry and to eradicate it quickly if the pathogen is introduced on infected plant materials.

**Major Achievements**

During the first six months of this project we have produced several monoclonal antibodies (MAbs) that react with *P. solanacearum* but not with numerous other genera and species of bacteria. One MAb, designated Ps-1, has been used for rapid identification of *P. solanacearum* isolated from infected heliconia in Hawaii. Virulent strains recovered from heliconia rhizomes caused rapid wilt of banana as well as heliconia plants, and were subsequently identified as race 2. Likewise, virulent strains recovered from diseased banana plants in the Philippines reacted with MAb Ps-1 but not with avirulent strains. Since this MAb reacts with nearly all the *P. solanacearum* strains tested from an international collection, it has potential as a universal reagent in bacterial wilt disease detection.

Although numerous antibodies have been produced that react only with selected strains of this pathogen, none has yet been found that reacts exclusively with all race 2 strains. Thus, to delineate race 2 strains from other races of *P. solanacearum*, we have produced disease-free, tissue-cultured banana seedlings, which are
inoculated with colonies recovered from symptomless plants. Comparative pathogenicity tests also are performed on tomato and pepper as well as young tobacco and heliconia plants. These bioassays are reliable but require two weeks to read results. To shorten the assay time, we have attempted to identify unique DNA fragments of the race 2 strains. The DNA has been extracted from 14 strains of races 1, 2, and 3, and the RAPD technique has been applied to determine whether the race 2 strains have a unique DNA fingerprint. Currently, no DNA banding pattern has been uniformly associated with race 2. Thus, we are using another genetic technique involving production of a cosmid library that will be used to locate DNA fragments that clearly identify race 2.

**Importance to Users**

The banana industry represents a significant source of income to many small growers in Hawaii and the Pacific. Banana and plantain show the highest production figures among tropical fruits, according to FAO production figures, and 98.7 percent of the world crop is grown in the developing countries. Moreover, banana often is grown on hillsides and lands unsuited to other types of agriculture. We are applying new techniques of biotechnology to reduce the risk and uncertainty in the future of banana production in the Pacific. The introduction of ornamental planting stock from areas in Central America where moko disease is endemic into new production areas in Hawaii and the Pacific carries with it the risk of introducing latent pathogens, but the risks can be lowered by development of reliable techniques for detection and identification of pathogens harbored in symptomless plants. Production of specific MAbs and DNA probes that quickly identify the pathogen within mixtures of non-pathogenic microorganisms will foster implementation of appropriate eradication procedures and disease control practices.

**Genetic and Biological Control of the Leucaena Psyllid**

**Principal Investigators:**

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Jack W. Beardsley, Dept. of Entomology, UH

**Nature of Project**

Leucaena psyllids rapidly became a major economic pest of this important fodder tree throughout the Pacific Basin between 1984 and 1986. This research was conducted to identify sources of resistance to the insect pest, to establish a breeding program for its control, and to characterize the insect, its predators, and the nature of resistance.

**Major Achievements**

International collaboration was established that helped to identify the insect biology and sources of resistance to it, to increase and deploy resistant seeds, and to develop a long-range breeding program. Two major types of heritable psyllid tolerance were identified in the 15 species of *Leucaena*. Resistance scores were assigned to these species as parents and 60 of their viable hybrids, and to advanced progenies of two species. A primary mechanism of resistance was shown to be water-soluble leaf toxins.

The biology and life histories of leucaena psyllids and major predators were characterized on a wide range of resistant genotypes. During this period, predators became very effective in reducing seasonal damage by the pest. Parasitism by a wasp was also identified, but it proved ineffective in Hawaii due to its own hyperparasites.

Psyllid-tolerant species and species hybrids were entered in international leucaena psyllid trials (LPT) that showed high consistency in performance over sites. Seeds were released of a psyllid-tolerant composite, KX2, based on species hybrids of *Leucaena leucocephala* × *L. pallida*. The composite showed outstanding fodder perfor-
mance under psyllid pressure and was distributed by several agencies internationally.

Importance to Users
The psyllid is effectively no longer a pest problem where the resistant varieties are grown. In the meantime, the psyllid has continued its worldwide movement (and severe damage) westward from Asia to the Mascarene Islands, and it is expected in Africa in 1992. Seeds of the resistant leucaenas are now available at the University of Hawaii, and they are being grown throughout the tropics. Since this area is an estimated 5 million acres, the potential impact of this work is very great.

New international funds are becoming available to extend the UH studies and perhaps fund additional research here.

Fig. 1. An adult psyllid of *Heteropsylla cubana.*

Fig. 2. *Leucaena* trees under heavy psyllid infestation at Hemg. Chung, Taiwan in 1986. Left, susceptible “giant” K8 of *L. leucocephala*; right, tolerant K156 of *L. diversifolia.*

**Basis of the Chemosterilant Action of J2581 in Fruit Flies**

Principal Investigator:
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Eric Jang, USDA-ARS, Hilo, HI
Leonard Jurd, USDA-ARS, Berkeley, CA

Nature of Project
A program was begun in our laboratory in an attempt to determine whether new classes of biologically active compounds modeled after chemical constituents isolated from plant material could lead to safer insect control agents. In 1972, Dr. Jurd synthesized a series of chemicals modeled after compounds isolated and identified from a biologically active extract of Panamanian hardwood. In 1982, Drs. A. De Loof and H. Van Mellaert discovered that two derivatives of benzyl-1,3-benzodioxole synthesized by Dr. Jurd exhibited antijuvenile hormone activity in a simple bioassay developed to show juvenile hormone activity in insects. This finding was significant because the juvenile hormones are important in pre-adult insect development and in adult female reproduction. We have screened some 20 benzyl-1,3-benzodioxole derivatives (BBDs) and have found five to be active in interfering with reproduction in the Mediterranean fruit fly (Fig. 1). Research was begun to uncover the mode of action of the BBDs with the long-term objective of possibly developing these compounds or their modified derivatives into environmentally safe insect chemosterilants.

Major Achievements
Results obtained from this research strongly suggested that BBDs act by interfering with juvenile hormone biosynthesis and its release from the corpora allata. This would result in a failure of vitellogenesis, or yolk protein deposition, to occur since this process is dependent on the presence of juvenile hormone. Results also suggested that BBDs interfere with microtubule assembly, i.e., in the process leading to the formation of microtubules from smaller subunits known as tubulin. Microtubules are known to be important in the formation of the cytoskeleton of the cell, including spindle and muscle fiber formation. Also, interference with microtubule
assembly by BBD treatment may account for the improper development of the flagellar (tail) structure in insect spermatozoa. Binding of guanosine triphosphate (GTP) to tubulin receptor sites is a critical step in microtubule formation. BBDs were shown to compete with GTP for these receptor sites and thus may represent the molecular basis for their action.

**Importance to Users**

Although the BBDs have been shown to be negative in the Ames and Rec A assays for potential mutagens and are relatively nontoxic to mammals if orally administered, their effect on microtubule assembly is a cause for concern. The BBDs are easily amenable to chemical modification, and attempts are under way to design compounds that will be effective against insects with minimal effects on nontarget organisms. The goal is to develop environmentally safe insect chemosterilants, specifically against fruit flies, which can be incorporated in baits.

![Fig. 1. Light microscopy of terminal follicles from six-day-old female Mediterranean fruit flies. Typical follicle (right, above) from female treated with a BBD, J2581, showing lack of well-defined nurse and follicular cells, compared with (below) terminal follicle from control female.](image)

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**Biological Control of Liriomyza Leafminers with Entomopathogenic Nematodes**

**Principal Investigators:**

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**Nature of Project**

The *Liriomyza* leafminer (Fig. 1) is one of the most serious threats to world agriculture because of its shipment within plant material, wide host range, short generation time, high reproductive rate, and ability to rapidly develop pesticide resistance. The most damaging stage of this leafminer is the larva, which feeds on tissues between the upper and lower surfaces of leaves. *Liriomyza* leafminers affect crops in at least six ways: (1) reducing crop yield, (2) destroying young seedlings, (3) reducing the aesthetic value of ornamental plants, (4) accelerating leaf drop, (5) causing some plants species to be quarantined, and (6) serving as vectors of plant disease. In the Pacific, this pest has caused crop failures in a variety of vegetables (tomato, cucumber, celery, watermelon, and snap bean) and ornamentals (chrysanthemum, gypsophila, and gerbera).

The overall objective of this project is to exploit the biological potential of entomopathogenic nematodes against *Liriomyza* leafminers. Entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae are parasitic against hundreds of insect pest species but are harmless to mammals and plants. These insect parasitic nematodes can actively search for hosts and kill them in 48 hours. They appear most promising against insect stages inhabiting cryptic habitats (i.e., leafmines) and soil, which serve as their natural reservoir.

This project is directed towards (1) isolating naturally occurring insect parasitic nematodes in Hawaiian soils and determining their effectiveness against *Liriomyza* leafminers, (2) genetically selecting a nematode strain for
enhanced host preference to leafminers, and (3) determining laboratory and field effectiveness of these nematodes compared with other insect parasites and chemical insecticides.

**Major Achievements**

Soils from 351 sites representing ecologically diverse habitats from the six main Hawaiian Islands were assessed for insect parasitic nematodes using the waxworm baiting technique. Twenty-four sites (6.8 percent) were positive for insect parasitic nematodes. Twenty-two sites (6.3 percent) were positive for heterorhabditid nematodes on the islands of Kauai (six), Oahu (five), Maui (four), Molokai (one), and Hawaii (six), and two sites were positive for steinernematid nematodes from Maui. No insect parasitic nematodes were recovered from Lanai. Heterorhabditids were highly correlated with sandy beaches within 100 m of seashore. These positive sites contained sand grains from coral and shell. The steinernematid isolates came from inland areas in clay and loam soils. Preliminary studies indicate that the heterorhabditid isolates are a new species, while the steinernematid isolates are closely related to a previously described species. The natural insect hosts of these nematodes were not isolated, and studies are continuing to identify their hosts.

In laboratory tests, these Hawaiian isolates of heterorhabditid and steinernematid nematodes caused 84 to 98 percent mortality in *Liriomyza* leafminers. The Hawaiian isolates provided greater mortality rates among leafminers than did other tested temperate isolates of steinernematid and heterorhabditid nematodes. In greenhouse tests using foliar applications of nematodes, results were different, with the Hawaiian and temperate steinernematids performing better than the Hawaiian heterorhabditid, probably due to poor survival of heterorhabditids on the plant foliage. Mortality ranged from 33 to 67 percent and was not consistent, probably due to low humidity. To improve efficacy of foliar application, leafminer-infested plants were treated in an enclosed greenhouse equipped with a misting system producing cloud-fog droplets. Humidity was constantly maintained at about 80 percent. Results were much more consistent, with average mortality rates ranging from 69 to 77 percent. A genetically selected strain of a steinernematid nematode with highly improved host-finding and *Liriomyza*-penetrating properties was developed in the laboratory. This selected strain is presently being tested in the greenhouse.

**Importance to Users**

These results demonstrate that insect parasitic nematodes can provide a control alternative for leafminers in the Pacific and reduce the current reliance on expensive and toxic chemical insecticides. Moreover, the results are broadly applicable since this pest is widely distributed throughout the tropical and subtropical regions of the world. In addition, in view of the wide host range of these insect parasitic nematodes, successful development of application technology against leafminers will spawn expansion of their use against other insect pests in an integrated pest management program. These Hawaiian isolates serve as a resource for use in the tropics because they are likely to be more effective against tropical pest species. The use of naturally occurring Hawaiian isolates as biological control agents may also reduce the risk to nontarget organisms when compared with the use of exotic isolates, preventing possible disruption of the delicate island ecosystems.

![Cucumber leaf infested with Liriomyza leafminers.](image)
Genetic Improvement of Parasitoid Natural Enemies

Principal Investigators:
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Nature of Project

Biological control is successful against many pests in the Pacific Basin; however, it is extremely difficult to control all pests of a particular crop with natural enemies. Under ideal circumstances, natural enemies can be used to control certain pests while others are suppressed by pesticides. Biological and chemical controls are often incompatible, however, because pesticides are usually more toxic to natural enemies than to pests. Thus, insecticide applications can disrupt biological control and cause outbreaks of pests that were previously suppressed by natural enemies. This is a common problem in vegetable crops in Hawaii, which may have more than five pest species present at any one time.

A classic example of disruption of biological control by the application of insecticides is illustrated by the agromyzid leafminer complex (genus *Liriomyza*) and associated parasitoids on vegetables. Control of leafminers by natural enemies can reduce the number of insecticide applications required to keep these pests in check, thereby slowing development of insecticide resistance. Growers, in trying to control other susceptible pest species (e.g., aphids, thrips) attacking the crop, often continue to apply compounds to which the leafminers have developed resistance. Applications of these compounds can reduce the natural enemy populations and cause increases in the leafminers. If the natural enemies of leafminers were also resistant to pesticides, biological control would continue to be effective against leafminers while insecticides controlled the susceptible pests.

The major objectives of this project were to determine levels of insecticide resistance in the leafminer parasitoids *Diglyphus begini* and *Ganaspidium utilis* in Hawaii, and to determine the potential to increase pyrethroid resistance in *G. utilis* by repeated exposure to insecticides in the laboratory (artificial selection).

Major Achievements

Based on laboratory bioassays, *D. begini* from three locations in Hawaii were resistant to the commonly used insecticides methomyl, oxamyl, permethrin, and fenvalerate. Relative to a susceptible strain from California, the most highly resistant Hawaiian strain of *D. begini* was 22, 20, 13, and 17 times more resistant to methomyl, oxamyl, permethrin, and fenvalerate, respectively. Populations that had been treated frequently with insecticides were significantly more resistant to the insecticides compared with untreated populations. Our research showed that *D. begini* is one of the few parasitoids resistant to insecticides with LC$_{50}$ levels (those pesticide dosages causing 50 percent mortality) exceeding recommended field application rates. The maximum resistance ratios for *D. begini* are among the highest recorded for any parasitoids (including both naturally and artificially selected species). Our data strongly suggest that *D. begini* has evolved resistance to pyrethroids and carbamates in response to field application of these insecticides.

Five *G. utilis* populations from three islands (Oahu, Hawaii, and Maui) were screened for susceptibility to fenvalerate, permethrin, oxamyl, methomyl, and malathion. All test populations were partially, if not totally, susceptible to these compounds at the recommended field rates. With respect to fenvalerate, the LC$_{50}$ for the *G. utilis* laboratory colony was 128 mg ai/l (FL = 102–165 mg ai/l). A more resistant *G. utilis* population was found at Hilo, Hawaii (LC$_{50}$ = 406 mg ai/l with FL = 276–797 mg ai/l). As the recommended field dosage for fenvalerate is 240 mg ai/l, this is quite remarkable. Significant field mortality still occurs at the LC$_{50}$ exhibited by the Hilo population, population, our *G. utilis* Hilo colony was 16 times more resistant to fenvalerate.

Fig. 1. Tomato foliage infested with *Liriomyza* leafminer larvae, indicated by white serpentine mines on leaf surfaces.
however. Compared to the most resistant *D. begini* more susceptible to fenvalerate. Selection for fenvalerate resistance in *G. utilis* was begun in 1990 to increase fenvalerate resistance to a level 10 times that of the recommended field rate (240 mg ai/l). After eight generations of selection, the LC$_{50}$ increased about 2.0 and 3.0 for females and males, respectively. These levels were 5.4 times (females) and 3.2 times (males) the field rate (240 mg ai/l) for fenvalerate. The resistance was relatively stable in the *G. utilis* females, but not the males, when selection pressures were removed for several generations.

**Importance to Users**

These results provide evidence that it is possible for parasitoid natural enemies to evolve high levels of resistance to insecticides to which they are frequently exposed. Because of their unique ability to survive high rates of pyrethroids and carbamates, field-resistant strains of *D. begini* may be extremely useful for controlling *Liriomyza* leafminers in management programs that integrate biological and chemical controls. *Diglyphus begini* could be mass reared and released into crop systems where *Liriomyza* leafminers are commonly of minor importance but often increase in number after destruction of their natural enemies by pesticides applied for other pest species (e.g., thrips, aphids, spider mites) also inhabiting the crop. Selection of *G. utilis* for pesticide resistance will provide a second natural enemy of the leafminer, capable of surviving pesticide applications. This natural enemy is often found as a major parasitoid in crops that *D. begini* does not frequent or where it is not a dominant member of the natural enemy complex suppressing the leafminer.

**Sustainable Management of Tropical Pests with Genetically Improved Natural Enemies**

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**Nature of Project**

We have identified geographic strains of the *Liriomyza* leafminer parasitoid *Diglyphus begini* (Ashmead) in Hawaii that show extraordinary resistance to the pyrethroid insecticides and moderate resistance to other pesticides. This natural enemy causes major leafminer mortality in numerous crops. Three problems still exist in this system, however. The first is that known populations of highly resistant *D. begini* are isolated in various locations (e.g., Poamoho, Oahu, and Volcano, Hawaii), whereas the *Liriomyza* problem is widespread throughout vegetable and ornamental production areas of Hawaii.

Secondly, due to habitat preferences, *D. begini* does not effectively control *Liriomyza* leafminers in every crop system (i.e., onion, watermelon) where the leafminers occur, even in the absence of pesticides. Thus, complete biological control of *Liriomyza* spp. throughout all crop systems is not feasible by relying on pesticide-resistant *D. begini* strains alone.

Lastly, introduction of a pesticide-resistant parasitoid strain into a given area where susceptible members of the species exist does not guarantee that the resistant strain will become dominant in the area. This is due to the potential dilution of resistance genes of the resistant parasitoid strain when mating occurs with wild type (susceptible) individuals.

The optimum method of colonizing resistant strains in new locations is not obvious, and little is known about the types of biological or operational factors that determine successful implementation. Following the dispersal and establishment of resistant strains is difficult due to the number of pesticide bioassays that must be conducted to detect resistant individuals.

The proposed research will:(1) determine the potential for increasing insecticide resistance in a second leafminer parasitoid, *Ganaspidium utilis* (Hymenoptera: Eucoilidae), by laboratory selection, and (2) examine the feasibility of colonizing pesticide-resistant strains of *D. begini* and *G. utilis* throughout Hawaii and the Pacific Basin.

**Major Achievements**

Work was conducted on modifying a previously published population genetics model that simulates natural enemy dispersal and establishment following introductory releases (i.e., classical biological control). Two new components were added to the model: the inclusion of parasite–host interactions in multiple habitat
patches and the incorporation of partial reproductive incompatibilities. Both components should provide a more realistic viewpoint with respect to the success of introducing pesticide-resistant parasitoids into susceptible populations. The model is now being modified to include the biological parameters specific to D. begini and G. utilis.

Studies were begun to determine if the Random Amplified Polymorphic DNA (RAPD) method of the polymerase chain reaction (PCR) could provide a method for analyzing the DNA of arthropod natural enemies. The RAPD technique requires a method for extracting DNA in a consistent manner from individual insects. Once the DNA is extracted, appropriate parameters for the PCR reaction must be determined.

To perform the RAPD method, a DNA preparation technique (using the walnut aphid parasitoid Trioxys pallidus) was initially developed that allowed the preparation of large numbers of DNA samples quickly. This preparation technique provided consistent results with pesticide-susceptible D. begini collected in California. Next, primers must be screened to find those providing unique bands that can be used to differentiate susceptible D. begini individuals from pesticide-resistant individuals.

Work on this objective will continue. Importation permits were recently acquired granting us permission to ship pesticide-resistant D. begini individuals from Hawaii to California. Upon the production of a pesticide-resistant strain of G. utilis, efforts will be made to develop a RAPD technique for DNA marking of resistant and susceptible individuals.

**Importance to Users**

As applied research, the study will increase the potential for integrating biological and chemical controls, reduce growers' dependence on insecticides, slow development of insecticide resistance in the leafminers and other species, and reduce environmental contamination and human exposure to insecticides. Because D. begini and G. utilis are major parasitoids of Liriomyza sativae and Liriomyza trifolii, selected strains could be used worldwide to increase the effectiveness of biological control of this complex and thereby prove extremely valuable in pest management programs, particularly in the Pacific Basin. With respect to distributing selected strains, the study will provide a greater understanding of the fate of specific genetic strains when introduced into areas where large populations of wild type individuals exist. In addition, this study will produce methods to allow precise measurements of the spread of pesticide resistance genes in a population. Thus, it will help to determine how best to use genetically improved strains in the field. As basic research, the study will reduce gaps in knowledge of the biology of an organism of tropical importance. Large increases in insecticide resistance through selection of G. utilis would be a significant scientific achievement because it would demonstrate that parasitoids can achieve high levels of insecticide resistance through artificial selection, and it would subsequently be used in the field, as long theorized. If successful, the study may provide guidelines for future projects aimed at the genetic improvement of parasitoids and subsequent distribution of selected strains in the field.

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**Chemistry and Physiology of Sexuality in Pathogenic Species of Phytophthora**

**Principal Investigators:**

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**Nature of Project**

Each year fungal pathogens in the genus *Phytophthora* cause substantial losses in yields of macadamia, papaya, pineapple, avocado, and taro in Hawaii and other parts of the world. The sexual stage of this group of pathogens provides not only a means of propagation and survival in nature but also a potential source of genetic variation. The purpose of this project is to understand the biological activity and the nature of chemicals that regulate this basic phenomenon for eventual development of an effective integrated method for controlling diseases caused by these pathogens.

**Major Achievements**

Hormones essential for regulating sexual reproduction in *Phytophthora* are produced in minute quantities, and the method previously developed for isolation is very expensive and time consuming. A breakthrough in hormone isolation has been achieved, however. Previous isolation involved production and extraction of hormones from the Millipore filter. Now we are able to extract hormones directly from agar culture by...
grinding the culture with organic solvent in a homogenizer and removing the solid materials from the extract by centrifugation.

The crude $A_1$ hormone extract was dark orange, and the amount obtained from one culture plate was capable of inducing 20,000 to 100,000 oospores (the sexual propagules of Phytophthora). The crude $A_2$ hormone extract was yellow, and the amount obtained from one culture plate was capable of inducing 20,000 to 50,000 oospores.

A quantitative method has also been developed for determining the effect of nutritional factors required for sexual reproduction in Phytophthora. A small agarose block with a well in the center was prepared from the basal medium. Different amounts of the nutritional factor were added to the well before these blocks were used to grow the fungus for production of sexual propagules. With this method it was determined that each $\mu$g of lecithin was capable of inducing about 200 oospores of $P. \text{parasitica}$ and 500 oospores of $P. \text{cactorum}$ under the most favorable concentration used.

Light inhibition of sexual reproduction in Phytophthora is a well-known phenomenon. The mechanism of inhibition had not been elucidated prior to our study, however. Our results show that the inhibitory effect of light on sexual reproduction of $P. \text{parasitica}$ is mainly the result of light inhibition on production and biological activity of sex hormones. Light has no effect on the stability of hormones, formation and stability of hormone receptors, or oospore formation after hormone induction.

**Importance to Users**

The methods developed for extraction of sex hormones and for quantitative determination of the biological effect of nutritional factors in Phytophthora should be useful to other researchers working on physiology of other microorganisms. The experimental design developed by us for determining the mechanism involved in light inhibition of sexual reproduction in Phytophthora should also be useful to other researchers for elucidating the effect of environmental factors on the biological activity of other microorganisms.

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**Ethology of Fruit Fly Parasitoids: A Basis for Augmentative Biological Control**

**Principal Investigator:**
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**Nature of Project**

This project was designed to improve biological control of fruit flies by learning more about the parasites that attack them. By studying the flight, host-finding, and oviposition behaviors of these parasites, we hope to be able to enhance their ability to attack and control pests. This should enable us to grow a greater variety and abundance of fruit and vegetables in Hawaii, with less reliance on toxic chemical insecticides.

**Major Achievements**

So far, we have learned that the parasites tend to disperse by flying downwind, that they apparently remain longer and search more thoroughly in an orchard (canopy) situation than in a shrub (coffee) or open (grass field) habitat, that they prefer to fly within rows of vegetation rather than across open space, and that they fly at a height of about 1.5 meters. In multiple releases of 10,000 to 20,000 mass-reared parasites, most of them dispersed to a distance of only about 30 meters.

In searching for the fruit fly larvae that are their hosts, the parasites first orient towards cues from ripe fruit, the habitat of the larvae. Male parasites use visual cues (e.g., color) to orient towards fruit, while females have a greater tendency to use olfactory cues (fruit odor). Females show a distinct preference for certain fruit odors (e.g., orange, guava) rather than others (e.g., lychee, apple).

**Importance to Users**

So far, our results have been of use primarily to research entomologists. We now can use the most effective combination of color and odor to design traps that monitor parasites used in a large-scale augmentation project to test their effectiveness against the Oriental fruit fly in the field. In the future, we hope to be better able to manipulate parasite populations to enhance their effectiveness as fruit fly control agents.
Biological Control of *Lantana camara* in Micronesia

Principal Investigators:
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**M. Marutani**, University of Guam

**Nature of Project**

*Lantana camara* is one of the top 10 noxious weeds in the world. A native of the tropical and subtropical Americas, it was introduced to Micronesia as an ornamental plant at the turn of the century. Over the past 40 years various natural enemies of lantana have been introduced into Micronesia in an attempt to curb its spread.

Our surveys conducted in this region revealed that lantana is distributed on all the islands except Rota and Kosrae. There are four different color-morphs of lantana in Micronesia; of these, the pink and yellow flowering variety is the common one. Of the 13 species of natural enemies introduced in the past for control of lantana, only seven have been established. Not all were represented on all islands where lantana was present, and some species were more effective than others.

**Major Achievements**

A thorough survey has been made of lantana and the effectiveness of its introduced natural enemies in Micronesia. The five most important insect species, in terms of their distribution and effectiveness, were the tingid bug, *Teleonemia scrupulosa*; the hispine beetle, *Uroplata girardi*; the pterophorid moth, *Lantanophaga pusillidactyla*; the tortricid moth, *Epinotia lantana*; and the pod-fly, *Ophiomyia lantanae*. *Uroplata girardi* was introduced to Tinian, *T. scrupulosa* to Yap, and *O. lantanae* to Yap and Pohnpei.

Legislation was introduced in the Commonwealth of the Northern Marianas Legislature to ban any introduction of lantana on the island of Rota. Similar legislation has been recommended for Kosrae. A USDA permit has been obtained to introduce the lantana leafminer, *Calcomyza lantanae*, from Australia to Guam.

**Importance to Users**

The *Lantana camara* Control Act of 1990 passed by the Commonwealth of the Northern Marianas will prevent introduction of lantana to Rota. Introduction of additional natural enemies of lantana to Tinian, Yap, and Pohnpei should reduce the severity of this weed on these islands. The lantana leafminer, a new natural enemy of lantana yet to be introduced to Micronesia, will be imported from Australia in the near future.

Different natural enemies of lantana introduced to Micronesian islands and their distribution and effectiveness have been determined. This information will form a foundation for further programs for control of lantana in Micronesia.

![Fig. 1. Lantana flowers damaged by the pterophorid *Lantanophaga pusillidactyla*.](image)
Effect of Insect-induced Changes in Siam Weed on Three Trophic Levels

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Nature of Project
Siam weed, Chromolaena odorata, is an invasive neotropical weed established in most humid tropical regions of Asia, Africa, and Micronesia. An arctiid moth, Pareuchaetes pseudoinsulata, was introduced to Guam for biological control of this weed in 1985. Since then the weed has been suppressed on Guam, and now it occurs in scattered and sparse patches. The leaves of C. odorata turn yellow whenever larvae of P. pseudoinsulata feed on them. This insect-induced change in C. odorata is a defense against the caterpillars of P. pseudoinsulata. The yellow leaves turn green upon removal of the caterpillars. Although C. odorata is highly allelopathic, when it turns yellow it is possible that it loses this allelopathic property. The P. pseudoinsulata caterpillars normally feed at night on C. odorata and hide in the ground during the day to avoid diurnal natural enemies. Insect-induced change forces the caterpillars to remain on the foliage both day and night, resulting in exposure to diurnal natural enemies.

Major Achievements
Studies of insect-induced defense, allelopathy, and effect on natural enemies will be valuable for programs of pest management, biological control, and evolutionary biology. Chromolaena odorata has been suppressed on Guam, Rota, Tinian, Saipan, and Pohnpei. Shipments of P. pseudoinsulata have been sent to Kosrae, Yap, Belau, Indonesia, Thailand, the Philippines, Ghana, Ivory Coast, Germany, and South Africa from Guam. An International Network for Biological Control and Management of Chromolaena odorata has been set up with headquarters at the University of Guam. This is the only research network operating wherein north and south collaborate. Participation in and support for this program have come from donor international agencies such as the Australian Centre for International Agricultural Research, Australia; Food and Fertilizer Technology Center for the Asian and Pacific Region, Taiwan; Southeast Asian Regional Center for Tropical Biology, Indonesia; Institut de Research pour le Development en Cooperation, France; Food and Agricultural Organization, Rome; IRHO and CIRAD, France; and International Institute of Biological Control, United Kingdom. Chromolaena odorata Newsletter is being published from the University of Guam at irregular intervals.

The People's Republic of China is interested in introducing P. pseudoinsulata to the southern part of China for biological control of C. odorata.

Importance to Users
The results of this project have been successfully disseminated to Micronesia, Asia, and Africa. The Second International Workshop on Biological Control of Chromolaena odorata was held at Bogor, Indonesia, February 4–8, 1991. The proceedings of this workshop has been published as “Ecology and Management of Chromolaena odorata.” Five numbers of Chromolaena odorata Newsletter have been published. The Third International Workshop on Biological Control and Management of Chromolaena odorata has been scheduled for March 15–19, 1993, in Abidjan, Ivory Coast. The Food and Agricultural Organization has already authorized $24,000 for this purpose. Copies of the proceedings of the first and second international workshops and the newsletters are available from the senior author upon request.

Fig. 1. Adult moth Pareuchaetes pseudoinsulata.
Biology and Control of *Aulacophora similis* and *Diaphania indica*

Principal Investigators:
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Ilse H. Schreiner, University of Guam

Nature of Project
Cucurbits are widely grown crops in the Pacific region, and cucumbers, watermelons, and cantaloupes are among the most profitable crops. In this region, cucurbits are attacked by a variety of insect pests. Some of these, such as melon aphids, leafminers, and melon thrips, have wide distributions, and considerable work has been done on their biology and control. Others are restricted to the Asian Pacific region, and little information is available about them. Among these are two species that were studied in this project, the orange pumpkin beetle, *Aulacophora similis*, and the melonworm *Diaphania indica*. Both species can be serious pests of cucurbit crops, and integrated pest management systems for the region must incorporate management methods for these two pests.

Major Achievements
Orange pumpkin beetles were found to have many cucurbit hosts but were unable to use bittermelon, the most abundant wild cucurbit on Guam, as a host. Reports that crops such as maize and sweet potato might be hosts were not borne out in our tests. The life cycle of the beetles comprises about 11 days in the egg stage, 30–40 days as larvae, and 10 days as pupae. Adult beetles lived for months, and females laid about 200 eggs. Beetles preferred to oviposit in soil with at least some organic matter, and their survival was higher in these soils.

Beetle larvae were not easily controlled. Tests with soil insecticides applied preplant showed that none of them was capable of controlling beetle larvae through harvest. Plastic mulch influenced the number of larvae around cantaloupe plants. They were less abundant around mulched plants in the wet season but more abundant in the dry season. Soil water content apparently influenced beetles more than the mulch. Plastic mulch did protect individual melons, however. Melons lying on plastic were rarely damaged by beetle larvae. Adult beetles were readily controlled with carbaryl, and greater than 90 percent reductions could be achieved even when only every other or every fourth row of a field was treated. Reducing adult beetles reduced larval numbers, but an action threshold of one adult beetle per 10 plants still resulted in significant damage to cantaloupes by the beetle larvae.

Host plants of the melonworm were identified. Cucumber was their preferred host, but the melonworm was also successfully reared on bittermelon and will choose this host in the field. In India, melonworms cannot survive on bittermelon. A series of data was summarized and published during the course of this grant that pinpointed the injury thresholds for melonworm on cucumber. Little yield loss occurs as long as there is less than one caterpillar per leaf, but above that level yield loss increases linearly with increasing numbers of melonworms. Several studies on the effect of mulch were inconclusive, although in general melonworms were more numerous on cucumber plants mulched with plastic than on unmulched ones. Increases in yield due to other effects of the plastic mulch more than offset any yield losses due to increased numbers of caterpillars, however.

Importance to Users
A damage threshold has been determined for melonworms on cucumber. This will help farmers reduce the frequency of spraying and should save money. This threshold has been adapted to watermelon and cantaloupe by adjusting thresholds to account for the size differences of the leaves on the different crops. It was tested and verified as part of a revised Watermelon IPM system imported from Hawaii.

We have not developed successful systems for reducing larval beetle populations directly, but we did show that adult control reduced larval numbers. We also demonstrated that it is not necessary to spray the entire field to control adult pumpkin beetles. Because of their high mobility, spraying every other row results in just as good control as does full coverage. This should save farmers money and help conserve leafminer parasites and other important natural enemies of cucumber pests.
Biology and Control of Mango Pests on Guam

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Nature of Project
Before 1986, mango production on Guam was poor, and Guam was thought to have an unsuitable climate for it. There were, however, some important pests attacking mango on Guam that appeared to be significantly reducing production. One of these was the mango shoot caterpillar, *Penicillaria jocosatrix* Guenee, which is a minor pest of mango in Asia and the Pacific. The project was begun to determine the basic life cycle parameters of the mango shoot caterpillar, determine natural enemies of the caterpillar and assess their importance, and evaluate the importance of mango shoot moth feeding on the flowers.

A second pest of mango common on Guam is a species of cecydomyiid midge that produces shot holes in the foliage. For many years, the shot hole damage was attributed to anthracnose, *Colletotrichum gloeosporioides*. Anthracnose is a disease of mango that attacks the leaves even when the midge is absent, but on Guam the primary cause of foliar damage is feeding by the midge, and the disease is secondary. A further objective of this project was to determine the basic life cycle parameters of the cecydomyiid miner and assess its importance.

Major Achievements
The biology of the mango shoot caterpillar was studied in the field and laboratory. Development was rapid, as the eggs hatched in two to three days and the caterpillar completed feeding in five to six days. The pupal period lasted about 10 days. Survival was better on three- to nine-day-old leaves and flowers, and poor on older leaves. On Guam, 55 percent of the leaves initiated by mangoes, and most of the flowers, were eaten by the caterpillars. This level of destruction prevented good flowering and fruit production.

No significant natural enemies were found on Guam; four parasites were imported from abroad, and two of these became established. Populations of mango shoot caterpillars declined to one-fourth their previous levels and remained low over the three-year monitoring period. Leaf damage declined to 17 percent. Much of the damage found was caused by another caterpillar.

The gall midge attacking mango, *Procontarinia schreineri*, was a species new to science. The gall midge made tiny circular lesions on young leaves. The young midges fed on the leaves for about five days, and then dropped out and pupated in the soil. Severely attacked leaves were distorted or curled, and they fell prematurely from the tree. More importantly, the lesions provided entry sites and reservoirs for anthracnose, a disease that infected the twigs, flowers, and fruits. Trees differed in their susceptibility to the *Procontarinia*. Rainfall was the most important factor affecting midge infestation levels. During rainy weather, midge damage was more severe.

Importance to Users
After biological control of the mango shoot caterpillar, the percentage of branches initiating flowering steadily increased. Mango fruit production increased 20- to 80-fold, and abundant mangoes were present on backyard trees. Many trees that had never borne fruit flowered and fruited heavily. For the first time in many years, large amounts of locally produced fruit were available to consumers during the fruiting season.

Details on the life cycle of the mango shoot caterpillar showed that only leaves less than 10 days old were suitable as food. If treatment with insecticides is needed, it may be restricted to this period.

A new pest of mango, an undescribed species of *Procontarinia*, was described. Although it is not always abundant on mango, it allows anthracnose to infect the leaves. Spores from these leaf lesions can then infect flowers and fruit, decreasing yield and lowering product quality. The midge is only briefly on the tree as it attacks the young leaves, so treatment is difficult. Trees differ in susceptibility to the gall midge, suggesting that there is potential for finding resistant varieties. This factor must be considered if new varieties of mango are promoted on Guam.
Intraspecific Chemotypes and the Allelopathy of Tropical Weeds

Principal Investigators:
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M. Kawate, Dept. of Environmental Biochemistry, UH
R. K. Nishimoto, Dept. of Horticulture, UH

Nature of Project
Weeds now impose a crop loss of more than $10 billion each year in the United States. The cost of controlling weeds with herbicides reflects the seriousness of the problem; over 60 percent of the expenditure on pesticides in the United States was for herbicides. In addition, the hidden cost to the environment (e.g., groundwater contamination) and to health demands a novel solution in modern agriculture. It is therefore imperative to develop effective and environmentally sound methods of weed control. This goal cannot be achieved without an understanding of the mechanism and basic characteristics of weed–crop interaction.

The adverse effect of weeds on crops is caused mainly by interference, which consists of two types of interactions, competition and allelopathy. We have studied several chemotypes of purple nutsedge (*Cyperus rotundus* L.), known as the world’s worst weed. The four major types have a distinctive difference in their sesquiterpene composition, which accounts for the inhibitory activities of the essential oils against other plants through allelopathic interactions.

We theorize that a chemotype that contains less (or fewer) phytotoxic compound(s) would cause less damage to crops than a chemotype containing more phytotoxic compounds. It would be wasteful if a less aggressive chemotype of weed received the same level of weed control as an aggressive chemotype. Chemotypes, therefore, could provide vital information in the modern practice of weed management. To our knowledge, the intraspecific chemotypes of weeds have not been a subject of scientific pursuit, and we would like to pioneer in this new area of study.

Major Achievements
To confirm our hypothesis, we propose to conduct comparative studies on the allelopathic inhibition of crops in relation to the chemotypes of *C. rotundus*. Clones of tubers of the four chemotypes of purple nutsedge, *C. rotundus* L. were collected from Japan, Taiwan (Chaiyi, Tainan, and Taichong), the island of Hawaii (Black Sand Beach vicinity and Kamuela), and Oahu (Waimanalo).

White kyllinga (*C. kyllingia*, syn. *Kyllinga nemoralis* (J. R. Forster & G. Forster) Dandy ex Hutchinson & Dalziel) and green kyllinga (*C. brevifolia*, syn. *Kyllinga brevifolia* Rottb.) were collected from the University of Hawaii, Manoa campus.

The above clones of weed species are now grown and maintained in the greenhouse, with precautions against their escape. They will serve

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<th>Table 1. Effect of green and white kyllingas on growth of bermuda grass</th>
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| Green kyllinga | 3 | 7.0 | 13.2 | 19.4 | n.s. | *

*Significantly different by the t-test at more than the 95% confidence level.
Note: The t-tests were run to determine the difference between the green kyllinga and white kyllinga.
as a source of tubers, roots, and rhizomes for the comparative study of the essential oils and allelopathic activities.

We have decided that, although we do have the four chymotypes of *C. rotundus*, it would be a good strategy to compare the white and green kyllingas first, since the two have more significant quantitative and qualitative differences in their essential oils.

Common bermuda grass (*Cynodon dactylon* (L.) Pres.) foliar growth was reduced substantially when grown with white kyllinga, but not when grown with green kyllinga at same planting density in pots (Table 1). White and green kyllingas are morphologically similar; therefore, the competition for resources did not contribute to the difference. The logical explanation for these observations would be that the white kyllinga has more essential oils in the roots and rhizomes than does the green kyllinga. Furthermore, while the essential oil of the former contains mainly phytotoxic sesquiterpenes, the latter contains nontoxic waxes. Both weeds were established in the continuous root-exudate trapping system (CRETS) in the greenhouse. Root exudates were collected under undisturbed conditions, and their capillary chromatograms showed that the two weeds had distinctively different rhizospheric chemical environments. Chromatograms suggest that the white kyllinga has more hydrophobic root exudates than does the green kyllinga. These results will be repeated and confirmed.

The study of terpene composition in the tubers of *Cyperus* spp. has proved to be useful in chemotaxonomy. The sesquiterpene gas chromatogram (GC) profiles of *C. rotundus*, *C. tuberosus*, and *C. bulbosus* were determined and compared (Table 2). It is clear that reliable identification of these morphologically similar species can be made using these characteristic GC chromatograms.

### Table 2. Comparison of essential oils from components of the major sesquiterpenoids in tubers of *C. rotundus*, *C. tuberosus*, and *C. bulbosus*

<table>
<thead>
<tr>
<th>Peak no.*</th>
<th>Sesquiterpenoid</th>
<th>Percentage of total peak area</th>
<th>Method**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1</td>
<td>α-Copaene</td>
<td>t</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>Cyperene</td>
<td>8.0</td>
<td>31.3</td>
</tr>
<tr>
<td>3</td>
<td>β-Elemene</td>
<td>2.5</td>
<td>5.2</td>
</tr>
<tr>
<td>4</td>
<td>β-Caryophyllene</td>
<td>3.0</td>
<td>3.8</td>
</tr>
<tr>
<td>5</td>
<td>α-Humulene</td>
<td>2.2</td>
<td>4.1</td>
</tr>
<tr>
<td>6</td>
<td>β-Selinene</td>
<td>16.0</td>
<td>n</td>
</tr>
<tr>
<td>7</td>
<td>δ-Cadinene</td>
<td>3.1</td>
<td>3.0</td>
</tr>
<tr>
<td>8</td>
<td>Calamenene</td>
<td>t</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>Caryophyllene oxide</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>10</td>
<td>Humulene oxide</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>11</td>
<td>Cyperotundone</td>
<td>19.5</td>
<td>12.0</td>
</tr>
<tr>
<td>12</td>
<td>α-Cyperone</td>
<td>31.4</td>
<td>n</td>
</tr>
<tr>
<td>13</td>
<td>Patchoulenyl acetate</td>
<td>t</td>
<td>9.1</td>
</tr>
<tr>
<td>14</td>
<td>Sugeonyl acetate</td>
<td>0.5</td>
<td>8.5</td>
</tr>
<tr>
<td>15</td>
<td>Sugertriol triacetate</td>
<td>2.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>

I = *C. rotundus* M-type, II = *C. rotundus* K-type, III = *C. bulbosus*, IV = *C. tuberosus*.

*Sequence based on Rt.

**Methods used for identification: 1 = retention time on GC, 2 = GC-MS, 3 = cochromatography, 4 = IR, 5 = 13C-NMR.
Biological Control of Purple Nutsedge with Plant Pathogens

Principal Investigators:
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M. Aragaki, Dept. of Plant Pathology, UH
J. DeFrank, Dept. of Horticulture, UH

Nature of Project
Purple nutsedge (Cyperaceae), also known as nutgrass, is one of the world’s worst weeds and is mainly distributed throughout the tropical regions of the world. At high population densities, crop yields have been reduced by 50 percent or more. Herbicides have not effectively controlled this weed, and moreover they are expensive. Controlling this important economic weed by reducing populations to manageable levels with a pathogen would contribute significantly to agriculture of Hawaii and the southern United States.

Major Achievements
In a survey of purple nutsedge in Taiwan during December 1990, Puccinia canaliculata, a rust, was the only pathogen found. This rust is also known in Hawaii but does not appear to have significant effects on nutsedge. Dry weather preceding the survey period had a major impact on the absence of disease on nutsedge. It was noted that inundated rice paddies were free of purple nutsedge, suggesting that Oomycetes may be a factor. No significant disease was found in several surveys throughout Hawaii.

In February 1991, a heart rot disease of nutsedge, characterized by yellowing and subsequent death of the central leaves and abundant oospores of a Phycomycete in diseased tissue, was found in Waimanalo, Oahu. Incubation in water resulted in the development of proliferating sporangia, resembling certain Phytophthora spp. Repeated attempts using different methods have not resulted in successful isolation of the pathogen. The fungus is either a Phytophthora sp. or a downy mildew, which is an obligate parasite. Efforts to isolate the fungus in pure culture are continuing, and the disease is being maintained in the greenhouse, obviating the need for further field searches.

Importance to Users
The successful cultivation of this pathogen in artificial media will greatly facilitate studies. The resulting knowledge will allow multiplication in nutsedge tissue culture and be the basis for mass production of the fungus, in particular the oospores, which are resistant structures. The ready dissemination of this mycoherbicide and the control of purple nutsedge will bring long-term economic returns to the tropical agricultural community.

Nonpersistent Virus Epidemics in the Tropics: Vector Intensity, Virus Interactions, and a Basis for Control

Principal Investigators:
Diane E. Ullman, Dept. of Entomology, UH
John J. Cho, Dept. of Plant Pathology, UH

Nature of Project
Aphid-transmitted plant viruses pose a serious threat to production of food and fiber crops throughout the world. More than 60 percent of the known insect-borne plant viruses are transmitted by aphids. Estimates made in the late 1970s suggest that the combined costs of controlling aphid vectors, and crop losses due to the plant viruses they transmit, cost growers worldwide more than $200 million per year. In Hawaii, growers of cucurbit crops (zucchini, watermelon, cucumber) cite aphid-transmitted viruses as one of their most important pest problems. More than 60 percent of the cucurbits consumed in Hawaii are imported, largely due to the tremendous difficulty our growers experience with cucurbit viruses. The aim of this project has been to develop strategies for limiting the spread of aphid-transmitted viruses in cucurbits in Hawaii and elsewhere in the Pacific Basin.

Major Achievements
Since project inception in 1989, we have demonstrated that pesticides are ineffective in limiting virus spread, demonstrated that stylet oils and reflective mulches are helpful in limiting virus spread and are readily integrated into existing integrated pest management pro-
grams (IPM), demonstrated that mild virus strain cross-protection is highly successful in managing zucchini yellow mosaic virus (ZYMV) on at least one of the Hawaiian islands, and developed a cost-and labor-effective technique for determining aphid species importance as vectors.

Perhaps our greatest accomplishment has been in demonstrating the efficacy of mild strain cross-protection on the island of Maui. This management strategy involves mechanically inoculating seedlings with a strain of the virus that causes extremely mild symptoms. Protected seedlings are then protected from the wild virus strain that causes very severe symptoms. In field trials on Maui, cross-protected zucchini yielded up to 16 times more fruit than nonprotected plants. The research emerging from this USDA 406-funded project resulted in a technology transfer project funded by the state of Hawaii. Fourteen growers participated in the technology transfer project in which growers received cross-protected seedlings. In less than six months, these growers produced approximately 300,000 pounds of zucchini, or about 81 percent of the total state production in 1990. The success of this project has prompted requests from Guam, the Federated States of Micronesia, American Samoa, Western Samoa, the Kingdom of Tonga, and the New Zealand South Pacific Commission for assistance in conducting virus surveys and instituting mild strain cross-protection.

Importance to Users

The results of this project have provided growers with at least three environmentally sound, sustainable methods for managing epidemics of cucurbit viruses. Consumers will benefit by having access to locally grown, fresh produce. We have demonstrated to growers that pesticides are not useful in controlling aphid-transmitted viruses, which should reduce the use of pesticides that potentially have detrimental environmental and health effects. The control measures we provide, particularly mild strain cross-protection, may enable Hawaiian growers to respond to local market needs for fresh cucurbit produce and reduce the need for imports to the state. In addition, mild strain cross-protection may prove to be an important solution to problems beyond the Hawaiian Islands, potentially benefiting the entire Pacific Basin. For example, export of exotic pumpkins is extremely important to the economies of Western Samoa and the Kingdom of Tonga. This important agricultural industry is currently being limited by cucurbit viruses. Use of cross-protection may enable these islands to feed.

![Fig. 1. Yields of marketable zucchini from nonprotected plants and plants cross-protected with a mild strain of ZYMV. Green, yellow, and red portions of the stacked bar represent nondiseased fruit. Grade A (green), B (yellow), and offgrade (red) refer to the size of the fruit at picking. Under grower conditions most of this fruit would have been picked as Grade A. Under experimental conditions we were unable to harvest as frequently, which is why much of the harvest was Grade B or offgrade.](image)

![Fig. 2. Appearance of zucchini placed in Grades A, B, offgrade, or diseased.](image)
themselves as well as maintaining important export industries. Finally, we have acquired important information regarding the dynamics of aphid-transmitted virus epidemics and the relative importance of different aphid species in spreading cucurbit viruses. This information provides the foundation for developing grower-useable decision-making models that will help growers assess their risks of virus epidemics prior to planting.

**Physiological and Molecular Determinants of Thrips Infectivity: A Basis for Prediction and Control of Tomato Spotted Wilt Virus**

Principal Investigators:

Diane E. Ullman, Dept. of Entomology, UH
John J. Cho, Dept. of Plant Pathology, UH
Ronald F. L. Mau, Dept. of Entomology, UH

**Nature of Project**

The thrips-transmitted Tospoviruses, of which tomato spotted wilt virus (TSWV) is the type species, pose a serious threat to agricultural production worldwide. Spread of the Tospoviruses is especially difficult to control in tropical agricultural cropping systems, because mild climates favor continuous cropping and year-round presence of the viruses and populations of the minute insects (thrips) that transmit the viruses. The losses caused by Tospovirus infections cost growers worldwide billions of dollars in control costs and lost productivity. In Hawaii, since 1982, decreases in lettuce production up to 70 percent and in tomato up to 30 percent have been attributed to TSWV epidemics. Due to the very large plant host range of the Tospoviruses and their thrips vectors, lack of adequate control measures to manage TSWV spread is a major gap in virtually all integrated pest management (IPM) systems and low-input sustainable agriculture (LISA) systems for most vegetable and some ornamental crops in the tropics.

It is the aim of this project to use basic knowledge of the interactions occurring between TSWV and its thrips vectors to develop thrips infectivity indices. These infectivity indices will then be used to forecast TSWV epidemics for growers prior to planting of crops. This work, funded only by the USDA 406 program, promises to provide information that can be used directly to dramatize the pattern and rate of spread of TSWV in Hawaiian vegetable crops.

**Major Achievements**

Since project inception seven months ago, two major findings have emerged from our research. First, infectivity indices must be tailored to the crop type because transmission of TSWV by thrips is greatly influenced by plant host. Our findings indicate that the highest infection rates occur on less preferred plants and the lowest infection rates occur on plants the thrips prefer. This somewhat counter-intuitive result happens because, when an insect prefers a plant, it completely empties the cells of their contents and does not move much. These behaviors destroy plant cells so they do not serve as good media for virus replication and do not favor virus spread from cell to cell or plant to plant. In contrast, on a less preferred plant, insects make shallow probes into cells to “taste” the plant, salivate a lot (injecting the virus with the saliva), and move a lot, making many probes. These behaviors are very favorable for virus replication (viruses need living cells to replicate) and spread of virus between plants.

Second, detection of infective thrips may be enhanced through use of methods enabling detection of protein associated only with replication in plant or insect cells. Tests that detect TSWV in thrips cells lie at the foundation of developing thrips infectivity indices. Most of the tests we use involve use of antibodies specific to the proteins that make up a whole TSWV virion. Through collaboration with Drs. German and Sherwood (University of Wisconsin and Oklahoma State University, respectively) we have produced an antibody that detects the presence of virus non-structural proteins that are present only during replication. Preliminary assays suggest this antibody will refine our ability to test thrips for infectivity by enabling distinction between thrips that simply have filled their intestines with the virus and those that have acquired the virus and can transmit it.

**Importance to Users**

Our early accomplishments are important steps towards building a TSWV preplanting forecasting system. The greatest financial investment a grower makes in producing a vegetable crop is in land preparation, planting
material, and planting. Currently, growers can predict the severity of TSWV epidemics only after incurring these costs. A preplanting forecasting system based on thrips infectivity will allow growers to assess their risks prior to planting. Then they can plant at low-risk times and substantially decrease their losses. Another benefit of this system will be in reducing the amount of infected crop plant material serving as virus inoculum in the environment. Ultimately, reduction of virus inoculum should reduce TSWV virus pressure and lessen epidemic severity.

Development and Evaluation of Effective Control Techniques for the Papaya Ringspot Virus on Guam

Principal Investigators:
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L. S. Yudin, University of Guam
S. A. Ferreira, Dept. of Plant Pathology, UH
D. Gonsalves, Dept. of Plant Pathology, Cornell University

Nature of Project
Guam’s papaya production is seriously curtailed by the papaya ringspot virus (PRV). No resistant cultivars exist, and there are no known viricides. Gonsalves and other workers have shown that inoculating papaya seedlings with a mild strain of the virus, which does not reduce fruit production, causes the plants to become “immunized,” or cross-protected, against infection by the wild severe strain.

This technique is also showing good results in Hawaii, where Ferreira and others have some research still in progress. It is expected that this same technique and mild strains will also work against Guam’s severe strain of PRV. Not only are there differences in the virus strains from place to place, however, but papaya cultivars and environmental conditions also change, and these can have an effect on cross-protection. Therefore, it is necessary to test the technique under experimental conditions to evaluate its effectiveness on Guam, and that is the major objective of this project.

Major Achievements
An import permit was procured from USDA to introduce a mild strain of PRV from Cornell University to Guam. This mild strain was tested in the greenhouse against the wild severe strain of PRV from Guam. The tests were successful. This mild strain was able to provide cross-protection against our wild severe PRV strain when papaya seedlings were inoculated artificially under field conditions, and under natural disease pressure.

Interest in our research work among local growers is high; we have many who have offered land for experimental or demonstration plots. Naturally, we’re not ready for this until the technique has been tested successfully in our own experiment stations. But we’re already gathering valuable information on disease progress in growers’ fields. This information is being analyzed to help us better understand the nature of the disease, how it starts in a field, and how it spreads. Understanding this will be important to developing effective ways to help predict the rate of progress of an epidemic, and to finding additional ways of slowing it down.

Importance to Users
At the present time, papaya growers on Guam cannot reach the end of the first season without 100 percent infection by PRV. This means that they cannot sell their ripe fruit that was formed before infection, commonly less than half of their potential production. The rest is scarred and its taste is affected. Normally, a papaya plantation would last several years and produce constantly after eight to nine months. When the disease occurs, production costs are seriously affected because growers have to replant every year, and profits dwindle significantly if the majority of the fruit produced is scarred by PRV. With cross-protected plants, growers could produce close to their full potential each season, doubling their current yields. Demand for papaya is high, both at the local markets and the hotels and restaurants for tourists.
Tourism-induced Agricultural Development in the Pacific Islands

Principal Investigators:
Richard L. Bowen, Dept. of Agricultural & Resource Economics, UH
Linda J. Cox, Dept. of Agricultural & Resource Economics, UH
John R. Halloran, Dept. of Agricultural & Resource Economics, UH
Mort Fox, School of Tourism Industry Management, UH

Nature of Project
The project is an attempt to better understand how the development of tourism has affected agriculture in Hawaii and other Pacific island areas. A conceptual model of the various links between agriculture and tourism has been developed, based on previous and current research. Secondary data and surveys of agriculturally based visitor attractions, restaurants, and food manufacturers are being used to construct a tourism-agriculture impact model. Comparisons among island areas will assist in understanding how links between the two sectors develop over time and are influenced by size of the industries and other factors.

Major Achievements
The research has produced a balanced understanding of the links between agriculture and tourism, including both positive and negative influences. Declines in Hawaii’s agriculture have occurred in the plantation crops (sugar and pineapple), due to rising resource costs and a more competitive international trade environment. But owners of these resources, particularly landowners and laborers, have benefited from higher resource prices. Offsetting the decline in plantation agriculture is an increase in diversified agriculture, often because of strong links to tourism. A large number of firms based in agriculture that sell goods and services directly to visitors have been identified and characterized. Recently completed surveys of restaurants and food manufacturers will shed light on how tourism has affected the demand for agricultural produce in Hawaii.

Importance to Users
Results of this research will provide a balanced assessment of the effect of tourism on agriculture to policymakers and concerned residents in tourist destination areas. Our research shows how some agricultural industries in Hawaii have declined and others increased due to increased tourism. As a result, Hawaii’s agricultural sector has been transformed from one of large-scale production to a diverse mixture of industries producing goods and services for niche markets. The research will yield information on how positive links can be strengthened and negative links better managed in tourist destination areas.

Influence of Sediment Removal and Deposition on Soil and Water Quality in Hawaii

Principal Investigators:
S. A. El-Swaify, Dept. of Agronomy & Soil Science, UH
R. A. Sutherland, Dept. of Geography, UH

Nature of Project
The transport of erosional sediments and associated nutrients has been linked to many detrimental effects on ecosystems. Two major effects are the on-site decline in soil productivity and the off-site degradation of water quality. The severity of these effects depends on both the quantity and quality of sediments removed (erosion), suspended, or deposited (sedimentation). Sediment quality has both physical and chemical dimensions; these are exemplified by the size of “selectively” eroded particles or aggregates, and by their “load” of organic or inorganic chemicals, respectively. Limited information is available on the processes responsible for the selectivity of erosion, sediment transport, and deposition in tropical agricultural soils. This information is needed to design appropriate technologies for maintaining soil productivity, assuring long-term sustainability, and preventing nonpoint source pollution of surface water resources.
Major Achievements

The master study area for the project is at the Hawaii Institute of Tropical Agriculture and Human Resources Experiment Station at Poamoho, Central Oahu. The soil at the site is Wahiawa silty loam, an Oxisol (Tropeptic Eutrustox) that is typical of the well-weathered agricultural soils of the tropics.

A detailed history of land use and management practices has been compiled for the area. Detailed hillslope surveys have aided in the identification of crest, midslope, and footslope zones, with slope gradients ranging from 5 percent to 9 percent. Transect and grid soil sampling is under way on plots with slope lengths ranging from 12 to 44 meters. This baseline sampling will allow an initial characterization of spatial variability in soil chemical and physical properties before tillage and crop planting. After tillage, soil sampling will be repeated to determine the spatial uniformity of soils before the installation of sediment collection devices. Several bulk soil samples have also been collected for use in controlled studies of erosional sediment characteristics under simulated rainfall.

Fifty trough systems have been acquired for use as field collectors of runoff and sediment. Calibration and field pretesting of the systems has been successful; installation in the experimental plots with runoff storage containers will be conducted after land tillage. Instrumented plots will differ in slope gradient, slope length, soil nutrient status, surface residue cover, and vegetative cover.

Importance to Users

Results of this research will provide soil conservation planners and land managers with the following necessary information:

Processes causing the loss and affecting the quality of erosional sediments from tropical agricultural soils;

Field sources of sediments (e.g., with soils that are bare, poorly protected, rilled, etc.) and rates of sediment and nutrient removal associated with erosion from each;

Characteristics of sediments that affect their detachability, mobility, deposition, and load of organic matter and plant nutrients;

Management options for controlling sediment losses, preserving soil productivity, and maintaining surface water quality;

Inputs necessary for the application and validation in tropical settings of erosion and nonpoint source pollution models such as Chemical, Runoff, and Erosion from Agricultural Management Systems (CREAMS), Areal Nonpoint Source Watershed Environment Response Simulation (ANSWERS), Agricultural Nonpoint Source Pollution (AGNSP), and the Water Erosion Prediction Model (WEPM).

Role of Nitrogen Fixation in Sustainable Production of Agroforestry Systems

Principal Investigators:
James Fownes, Dept. of Agronomy & Soil Science, UH
Joann P. Roskoski, Dept. of Agronomy & Soil Science, UH
Jefren Demeterio, University of Guam

Nature of Project

This project sought to quantify the rate of biological nitrogen fixation by tropical leguminous trees in an "alleycropping" system and to assess how that nitrogen contributed to long-term soil fertility and crop growth without application of nitrogenous fertilizer. Evaluation of sustainability was an integral facet of the project, using computer simulation to make long-term projections and testing those projections with long-term field data.

The benefits of applications of tree prunings as green manures were evaluated for both short-term (affecting the current crop) and long-term (affecting soil organic N pools) effects.

Major Achievements

Corn production was sustained over three years by alleycropping with leguminous trees, with the application of tree prunings as green manure without added chemical nitrogen. A second treatment, involving removal of the prunings, and a control treatment (no trees) were not sustainable over the same period.

A non-nitrogen-fixing legume tree also sustained corn production through green manure application, suggesting that nitrogen cycling and availability were more important on this site than nitrogen inputs as such.

Significant correlations were found between chemical indices of green manure quality (ratio
of soluble polyphenols to nitrogen) and rate of nitrogen release (mineralization) from green manures.

Two to three years were required for the application of green manure to the soil to increase the long-term nitrogen capacity substantially.

The comparison of modeled versus measured nitrogen availability over three years indicated an additional source of organic nitrogen, hypothesized to be fine root shedding.

Importance to Users

Alleycropping with leguminous trees has been proven to be a sustainable option for relatively low-output (2 T/ha/crop) corn harvest, if the tree prunings are returned to the soil as green manure.

Farmers should not remove the tree prunings from their fields to feed to livestock or to mulch other plantings.

It may take several years for an increased organic nitrogen cycle to develop from green manuring practice.

Simple chemical indices (which can be done by fodder analysis labs) can be a useful tool for screening new potential green manure species.

Matching Drip-irrigation System Design and Operation to Soil Hydraulic Properties

Principal Investigators:
Richard E. Green, Dept. of Agronomy & Soil Science, UH
I-Pai Wu, Dept. of Agricultural Engineering, UH

Nature of Project

The rapidly expanding use of drip irrigation for sugarcane, pineapple, and vegetable crops in Hawaii will benefit from improved system design and water application methodology. Drip irrigation has become more important to agriculture with the implementation of stricter water conservation measures requiring greater water application efficiency. There has also been an increase in the use of chemigation, applying fertilizers and pest-control chemicals through drip irrigation. The goals of improved drip system design are to maximize water and agrochemical use to improve crop yields while minimizing groundwater contamination.

Little research exists concerning trickle system design in relation to crop requirements and soil hydraulic properties. Available soil-water flow models incorporate soil hydraulic properties in an attempt to simulate the wetted soil volume for different soils and emitter flow rates. Soil-water flow models should also include the effect of soil-water distribution on crop growth, and conversely, the crop effect on soil-water distribution. The adequacy of a single soil-water potential value midway between emitters should be evaluated against the spatial and temporal distribution of soil-water potential in the root zone.

Our first objective in this project was to apply water flow theory and measured soil hydraulic properties to determine optimum drip system design and operation (emitter spacing and flow rates) for tropical soils differing widely in structural and hydraulic properties. Soil hydraulic conductivity must be measured in the field or estimated from other soil properties. This study evaluated the Guelph Permeameter for characterizing soil hydraulic properties compared with the Simplified Drainage Flux Method.

Our second objective was to develop a simplified method for extension agents and farmers to implement the results of the first objective in matching drip system design to soil type and crop.

Major Achievements

For the first objective, a field experiment was carried out to measure wetting patterns under different point source discharge rates and different irrigation periods to compare the Bresler model approach of determining emitter spacing for various emitter discharge rates with field results. Emitter fluxes of 0.5, 1, 2, 4, and 8 l/hr for durations of 4, 8, 24, 48, and 72 hours were used. A bulk density of 1.13 was used to convert from gravimetric to volumetric water content, which was then compared with calculated water distributions. Water contents within the wetted volume were slightly greater for the higher flow rates than for lower flow rates for the same amount of water applied. Water content was highest under the point source and decreased with distance from the emitter. The shape of the wetted areas was similar for all tests.

The volume of water applied was more important than discharge rate in controlling water movement. To compare theoretical and field results, a reference water content was selected (35 percent), and the distance from the emitter to the reference water content contour was measured. The water potential for the reference water content...
was determined from the soil-water characteristic data for the same soil (−150 cm) and used in the model computation to estimate the emitter spacing. There was good agreement between the theoretical and measured results for low discharge rates of 0.5 and 1 l/hr. For higher discharge rates, the Bresler approach overestimated the emitter spacing.

Thomas (1991) addressed the utility of the midway soil-water potential parameter to represent crop water requirement in drip irrigation system design. For sweet corn, closer spacing of line sources (one line source per crop row) produced more uniformity than wider-spaced treatments.

Relationships between crop yield and soil-water potential at different depths and distances from a line source were also evaluated. Soil-water potential decreased with distance from the line source. Crop yield declined with increasing distance from the source. Both the representative value and midway soil-water potential at the 15 cm depth were correlated with crop growth and yield. In general, model results predicted narrower ranges of soil-water potential than field measured values. For the close line spacings, however, model predictions agreed with the field data. The model was suitable only where the variation in soil-water potential was relatively small.

The simplified unsteady drainage flux method, using a double ring infiltrometer, is a simple field method for mathematically describing the hydraulic conductivity as a function of volumetric water content or soil water pressure head. For the second objective in this project, the Guelph Permeameter (GP), which uses a simple method to estimate in situ field saturated hydraulic conductivity and the alpha parameter, was compared with the double ring infiltrometer method. Tensiometer and neutron moisture probe measurements were incorporated into the drainage flux method. Double ring infiltration and GP studies were conducted on two oxisols at two experimental sites on Oahu. These soils differed in their hydraulic properties and were representative of large tracts of agricultural land on Oahu. The results of the GP “best estimate” analysis and the double ring method were compared. Hydraulic conductivity values generated by the two methods were similar. The double ring provided repeatable measurements of soil alpha values. The GP produced highly variable alpha values, requiring numerous measurements to determine a statistical average. Tensiometers equipped with pressure transducers performed reliably and can be used to replace mercury manometer tensiometers. A neutron moisture probe adequately measured the soil moisture and density nondestructively in the surface soil. However, the neutron probe required calibration for each soil and was inappropriate for poorly drained soils or highly heterogeneous soils.

Importance to Users
Models serve as useful predictive tools and are a new approach in drip irrigation design. Designers can use this approach to calculate the wetted area and spacing between sources in combination with discharge rate for a particular soil and crop. The next logical step would be to combine drip hydraulic theory and spatial distribution of water within the soil. In this study we addressed the need for accurate measurement of soil hydraulic properties to create reliable data sets needed to calibrate and validate the numerous existing soil-water flow models.

We evaluated the Guelph Permeameter with the goal of simplifying soil hydraulic property measurements. Considerable error can result due to vertical soil heterogeneity, however, and it is not ideal for surface soil measurements. The simplified drainage flux method is more appropriate for determining near-surface soil hydraulic properties. However, this method is awkward compared with the Guelph Permeameter, due to the equipment, labor, time, and amount of water required, and its use is limited to relatively well drained soils. The surface neutron probe and tensiometers simplified the procedure, allowing numerous measurements to be made quickly at several sites.
Increasing Soil Productivity for the Humid Tropics
Through Organic Matter Management

Principal Investigator:
N. V. Hue, Dept. of Agronomy & Soil Science, UH

Nature of Project
Among the problems inherent to tropical soils, soil acidity, characterized by low pH, excessive aluminum, and deficient calcium, is the most serious. Tropical soils are also often unproductive because of a deficiency of phosphorus, a major nutrient essential for plant growth and reproduction. Soil acidity and mineral deficiencies can be corrected by lime and fertilizers. Unfortunately, lime and fertilizers are not options always available to resource-poor, subsistence farmers. Thus, in this project, we have evaluated organic residues as alternatives to processed fertilizers and amendments. More specifically, we have studied the interactions between components of organic matter and inorganic soil constituents to try to improve soil productivity and maintain environmental quality.

Major Achievements
We have found that in addition to providing plant nutrients, mostly nitrogen, green manures and animal wastes can be used as substitutes for lime to alleviate aluminum toxicity and calcium deficiency. This liming effect can last over one-and-a-half years (maximum time period that we evaluated the effect). Precautions must be taken, however, if the acid soil is high in manganese.

We also found that phosphorus became more available to crops through organic residue incorporation. This increased availability of phosphorus is probably caused by the reactions of organic matter-derived molecules with soil minerals.

Importance to Users
Our findings clearly indicate that farmers can significantly improve productivity of their soils by proper use of organic materials, which are usually available locally. The environment also markedly benefits through the reuse of organic wastes.

Prevention of Groundwater Contamination by Micro-irrigation Systems

Principal Investigators:
I-Pai Wu, Dept. of Agricultural Engineering, UH
Bernard Kratky, Dept. of Horticulture, UH

Nature of Project
Our objective was to evaluate the uniformity of water application in the soil by micro-irrigation systems and to determine quantitatively the amount of deep seepage caused by the nonuniformity of water application in the field. Optimal irrigation schedules can be determined to minimize the deep seepage water that is the source of groundwater contamination.

Major Achievements
A computer program was developed to evaluate the uniformity of irrigation of water in the field through micro-irrigation (drip) systems. The program contains hydraulic design, manufacturer's variations of drip emitters, plugging percentages, soil moisture wetting patterns, and emitter spacings. More than 3000 combinations of the above items were evaluated in the computer simulation.

The simulation results showed that plugging is the most significant item to affect the uniformity of irrigation water in soil, and emitter spacing is the second. Both the emitter flow variation, if hydraulically designed with 30 percent, and manufacturer's variation of emitter, as long as it is less than 20 percent, caused only minor changes in the uniformity of overlapped soil moisture patterns under micro-irrigation.

An optimal irrigation schedule equation was derived, based on the uniformity of water application, the price of crop yield, the cost of water, the loss of fertilizers and chemicals in the deep seepage water, and the cost of cleanup of contaminated groundwater per unit volume of deep seepage. A compute calculation was made by simulating different values of the above items affecting the optimal irrigation schedule. The simulation results showed that deficit irrigation should be scheduled when the cost of the damage by deep seepage is more than 10 percent of the price of the yield of crop. Deficit irrigation is defined as
an irrigation schedule in which less than the required amount is applied.

**Importance to Users**
The developed optimal irrigation schedule, including considerations of deep seepage losses and its associated damage to groundwater, can be used for irrigation management when the price of the damage caused by deep seepage can be identified.

**Bioimmobilized-microbial and Photo-chemical Treatment of Pesticide Waste and Wastewater**

Principal Investigators:
P. Y. Yang, Dept. of Agricultural Engineering, UH
Carl J. Miles, Dept. of Environmental Biochemistry, UH
Donald G. Crosby, Dept. of Environmental Toxicology, UC Davis

**Nature of Project**
Pesticide waste and wastewater are considered to be toxic and hazardous waste that may create a hazard and affect human health and the environment. In Hawaii, pesticides have been found as contaminants in the groundwater, and the unwanted pesticide wastes are required to be shipped to an approved mainland facility for treatment and disposal, at a significantly high cost.

This project has focused on the removal of pesticides from water and wastewater by biological, photo-chemical degradation processes. Treatability, process design and operation, and economics are the main focuses of this study.

**Major Achievements**
Selected pesticides, such as carbaryl, ethylene dibromide, and trichloropropane, were successfully treated by using the entrapped mixed microbial cell process. The process provides the ultimate oxidation or destruction of the pesticides into innocuous substances, such as carbon dioxide and water. Necessary design and operational criteria for removal of these selected pesticides using entrapped microbial cell process have been developed.

Three carbamate and four organophosphate pesticides were treated by using various chemical and photochemical conditions. Dark and light hypochlorite treatments were both found to enhance degradation for all pesticides used for this experiment except for chlorpyrifos in light. Also, dark and light peroxide treatments enhanced degradation in all pesticides used for this experiment except for aldoxycarb, carbaryl, and chlorpyrifos.

The degradation of pesticides through photo-chemical process does not mean that the toxicity ceases to exist. This requires further toxicity tests using the Microbics Microtox Toxicity Analyzer. This was based on the current results that were still not conclusive.

**Importance to Users**
The successful development of various processes, including biological and photo-chemical process for the treatment of pesticide water and waste in this project, can be very useful for further development of a prefabricated package treatment plant that may be fixed at a certain location or moved from one location to another. Producers or farmers will be able to treat and dispose of their pesticide waste and wastewater properly and will not pose any health or environmental problems. Also, shipping cost for the disposal of unwanted pesticide wastes to an approved mainland facility can be avoided, and pesticide-contaminated groundwater for water supply systems can be improved.

**Development of an On-site Small Wastewater Treatment Plant for Small and Rural Communities**

Principal Investigator
P. Y. Yang, Dept. of Agricultural Engineering, UH

**Nature of Project**
Many small communities rely on septic tanks for sewage treatment. Widespread failure of septic systems (including the state of Hawaii's) has led to odor problems and health hazards in some communities. With the cuts in federal aid
for wastewater treatment projects, the small communities and state environmental protection agencies are seeking alternative methods to enable the construction and upgrading of small wastewater treatment systems. This project focuses on the development of an alternative in replacing or upgrading the septic tank system for on-site small farm and an alternative in providing effective treatment of wastewater collected from the small-scale sewer systems in the farming and fishing villages.

Major Achievement
Both strictly and moderately land-limited small wastewater treatment systems were investigated. Since July 1991, a moderately land-limited biotreatment unit—a combined anaerobic biofixed film and aquatic plant (CBFFAP)—was designed for the treatment of flushing raw swine wastewater and anaerobically digested effluent. It was found that it could effectively remove both organics (biochemical and oxygen demand) and inorganics (nitrogen).

The system demonstrates that it is simple in construction and operation and requires only low-cost maintenance. The system eventually can be integrated into the proposed swine waste management system for the tropics as shown in Fig. 1. A detailed experiment for this moderate land approach and strict land approach is still in progress.

Importance to Users
With the development of appropriate on-site small wastewater systems, the small agricultural producers can relieve the immediate problems, such as the national emphasis on enhancement of environmental quality and lack of knowledge of how to deal adequately with the wastewater produced from the small agricultural production system.

For the long-term approach of agricultural production, it must emphasize not only the plant and animal production, but also management of the entire agricultural production system by including the maintenance of acceptable environmental quality for the entire society.

![Fig. 1. High-performance liquid chromatography of untreated and treated samples (A = carbaryl, B = 1-naphthol).](image)

Management of Anaerobically Digested Livestock Waste for Agricultural Production

Principal Investigators:
P.Y. Yang, Dept. of Agricultural Engineering, UH
R. Coltman, Dept. of Horticulture, UH
C. S. Tang, Dept. of Environmental Biochemistry, UH

Nature of Project
The primary purpose of this project was to investigate the management of anaerobically digested livestock waste (ADLW) for agricultural production and the development of a cost- and process-effective anaerobic treatment (methane fermentation) of livestock waste containing high organic solids. We also sought solutions to the problem of disposal of ADLW by recycling the livestock waste in an appropriate and sound ecological way and developing the adaptive mechanisms of plant growth in a soil environment containing high levels of ADLW in the tropical agricultural production system.

Major Achievements
A hybrid anaerobic treatment process for highly concentrated animal waste was developed,
based on a laboratory experimental unit. This provides an effective treatment system to reduce the reactor size and the amount of anaerobically digested effluent. Furthermore, since the system is a closed one, odor control is very effective. The energy (in the form of methane gas) generated from this system is also very useful for various types of farm use. The low cost and operational advantages make the hybrid anaerobic treatment system suitable for farms close to urban areas or cities, including those in the state of Hawaii, where land is expensive and restricted and disposal of effluent is a problem.

Anaerobically digested pig waste (ADPW) and anaerobically digested chicken waste (ADCW) were tested as liquid fertilizer on two ornamental crops, hibiscus and marigold. It was found that the height and weight of marigold and hibiscus increased linearly up to the highest slurry rate (20 percent by volume with tap water) of ADPW and 15 percent of ADCW rate for maximum number of open flowers and for flower diameter. The field yield response of sweet corn to ADCW was also investigated. It was found that the highest rate (70 m³/ha) of ADCW yielded as well as sweet corn fertilized with the recommended rate of chemical fertilizer. It was also found that the toxic effect of salt is more serious under the high concentration of ammonium nitrogen ion for the growth of marigold. The appropriate application rate of ADCW has been developed for proper crop production and disposal of ADCW problems.

**Importance to Users**

The livestock producers and poultry farmers will have an appropriate and cost-effective treatment and disposal or usage alternative for handling their animal wastes if methane fermentation (anaerobic treatment) process is considered or planned in order to protect environmental quality and reuse the byproducts (such as methane gas for energy and anaerobically digested sludge and effluent for fertilizer or soil conditioners).

![Fig. 1. Effect of concentration of anaerobically digested poultry waste on the height and weight production of marigolds.](image-url)
Effect of Solar Radiation on the Prenatal and Postnatal Growth of Beef Cattle

Principal Investigator:
Richard J. Early, Dept. of Animal Science, UH

Nature of Project
Environment and nutrition have a substantial effect on cattle production, particularly in the subtropics and tropics. Generally, the hotter tropical environments result in poorer cow breeding performance (fewer conceptions) and a higher incidence of embryonic death in pregnant cattle. Also, calf birth weights may be reduced in heat-stressed cattle, which in turn results in poorer performing calves. Poor nutrition probably compounds all of these effects, but research characterizing nutritional and environmental interactions on beef cattle production systems are meager.

In this project, the environmental (shade versus no shade) and nutritional (high versus low) effects on various factors influencing calf birth weight and growth were examined in cattle on dry lot and pasture feeding systems. Seasonal influences (hot, October; cool, February) on calf birth weights and growth in Hawaii also were examined. Measurements taken in addition to calf birth weight and growth included cow milking ability, colostrum and calf plasma immunoglobulin concentrations (immunoglobulins are the proteins that confer passive disease resistance to the calf from the mother), and cow body fluid pools. (Body fluid pools were used to monitor placental and fetal growth). This project thus gives a complete picture of the effects of environment and nutrition on the prenatal and postnatal growth of beef calves in a subtropical climate.

Major Achievements
The most interesting data derived from this project thus far center around the interaction of environment and nutrition on calf birth weights, cow colostrum and calf plasma immunoglobulin concentrations, cow milking ability, and the changes in cow body fluid spaces with stage of pregnancy. Calf birth weights tended to be lower in animals that were not shaded and were on low nutrition. That is, it appeared that when cows had enough shade, regardless of the nutritional status, birth weight was not affected. However, the dual stress of both low nutrition and no shade resulted in lowered birth weights. An additional factor that was thought to potentially affect calf performance was the cow's milk colostrum immunoglobulin concentrations. It was visually noted that mammary development was reduced in low-nutrition cows. However, when colostrum immunoglobulin concentrations were measured, some types of immunoglobulins were higher in the low-nutrition animals. This suggested that the underdeveloped udder of low-nutrition cows compensated for the lower colostrum content with greater concentrations of immunoglobulins. Passive immunity was therefore still conferred to the calf. Interestingly, once proper nutrition was restored to all cows during lactation, cow milking ability was regained in all animals, indicating that poor udder development before parturition did not permanently affect the animals' milking potential. The data on calf growth performance are still being analyzed.

Body fluid pool sizes also were measured over the gestational period as a means of monitoring placental and fetal development. Body fluid pools consisted of the total body water, water within the cells, water external to the cells, and water within the blood-vascular system. Calf birth weights were only moderately correlated with total body water and the water within the cells, and little difference could be detected between treatments. This system of measuring fetal and placental development did not prove to be as effective as desired.

Importance to Users
Reasons for characterizing environmental and nutritional interactions are to provide cattle producers with fundamental knowledge on how seasons of the year and nutrition availability affects their particular production systems. Many ranchers often use terrain for grazing that provides marginal nutrition and shade for their animals. These conditions, however, are often still the most economical way to use the resources available to them. Often, weather patterns result in drought, even in the tropics, which also reduces food availability.

It is hoped that this research will provide scientists and producers with more knowledge for making the best decisions related to the seasons and nutrition. Future research will explore ways...
to compensate for environmental and nutritional inadequacies, such as maximizing compensatory growth in low-birth-weight calves and examining the effects of poor nutrition and limited shade on the lactating cow’s milking ability and the subsequent effect on calf growth.

Efficacy of the Peptide Hormone Gonadotropin-releasing Hormone (GnRH) To Improve Reproductive Efficiency in Cattle in the Tropics.

Principal Investigators:
C. N. Lee, Dept. of Animal Sciences, UH
D. Vincent, Dept. of Animal Sciences, UH
C. Weems, Dept. of Animal Sciences, UH

Nature of Project
The project investigated the ability of GnRH administered at the time of breeding to improve conception rates. This peptide hormone is used routinely in the dairy industry across the United States. In the midwestern states, it has been shown to enhance conception rates in repeat breeder cows by some 25 percent. The high humidity and temperatures in the tropics result in heat stress conditions that have been documented to alter the hormonal profile of cows. Low fertility rates are common in hot months in the tropics. The objectives of the project were to investigate the hormonal profiles of cows in heat stress vs. nonheat-stress conditions, the efficacy of the GnRH during the cool vs. hot months, the ability of the hormone cortisol to inhibit the hormone luteinizing hormone (LH), and the ability of embryos to survive under heat-stress conditions.

Major Achievements
The experiment showed that cows without shade in the subtropics were under heat stress. These animals had longer intervals between estruses. Their hormonal profiles were altered. Unlike cows in studies conducted in environmental chambers, cows under natural heat-stress conditions had suppressed cortisol profile, lower LH, and elevated progesterone profiles. Subsequent studies indicated that cortisol did inhibit LH but elevated levels of progesterone did not. The field trials with GnRH administration at breeding confirmed the predicted outcome of lower conception rates in the summer months. In the cooler months, GnRH administration at first breeding improved conception rates by 15 percent. An improvement of only 5 percent was observed in the summer months. Subsequent studies also suggested that cows must be provided with shade more than 16 days after breeding if losses due to early embryonic mortality were to be reduced.

Importance to Users
These studies suggest there are differences in hormonal profiles in cows under natural heat stress versus those in studies done in environmental chambers. The major contributing factor to the differences was probably radiation load from the sun. The studies also indicated that providing shade to animals in the tropics could help to alleviate some of the stress factors confronting animal production in the tropics. The use of GnRH would only be cost-advantageous in the cool months. In addition, to reduce embryonic mortality, it would be helpful to provide cows with shade after breeding.

The studies provided useful information to dairy producers in the tropics on their management approaches to improve fertility rates in the hot months. Since poor fertility leads to lower milk production, alternative management systems that would improve conception rates should lead to higher milk production for cows in the tropics.
Purinergic Pathways in the Developing and Mature Corpus Luteum

Principal Investigators:
C. Weems, Dept. of Animal Sciences, UH
D. Vincent, Dept. of Animal Sciences, UH
C.N. Lee, Dept. of Animal Sciences, UH

Nature of Project
Deoxyribonucleic acid (DNA) is a double-stranded polymer (chain) made of nucleotides that consists of a nitrogenous base, a 5-carbon sugar, and a phosphate group. DNA is synthesized from four nucleoside triphosphates (adenosine triphosphate [ATP], guanosine triphosphate [GTP], cytosine triphosphate [CTP], and thymidine triphosphate [TTP]); two phosphates are lost in the coupling process to make DNA.

Genes are made of DNA and are parts of chromosomes that have stored information that controls the synthesis of specific proteins in cells. Much of DNA's regulation of protein synthesis is indirect and through ribonucleic acid (RNA). Less emphasis has been given to regulators of cellular function than DNA, RNA, and protein synthesis, although many cellular regulators are produced from the same precursor molecules (ATP, CTP, GTP, TTP, UTP) used to form DNA and RNA.

The best known of these cellular regulators in animal tissues is cyclic AMP, which comes from ATP and is a second messenger produced by hormone stimulation of cell membranes for regulation of cell function and growth. ATP is also used as the primary energy source in cells to drive biological activity.

Recently, another cellular regulator found to be produced from ATP at the cell membrane is adenosine, which consists of only a nitrogen base and a 5-carbon sugar but which has a short half-life. Adenosine affects many organs in rats and mice, and at least two types of receptors have been identified. Adenosine is the most potent dilator of arteries to increase blood flow to organs, and it is released by blood vessels during a stroke or heart attack.

The two best known clinical cases with adenosine are hairy cell leukemia, in which a five-day treatment with the stable synthetic analogue 2-chloroadenosine has brought remission in all cases for at least five years, and the classic immunodeficiency disease “The Boy in the Bubble.” The latter was due to a deficiency of the enzyme adenosine deaminase to break down adenosine. Thus, adenosine was not being broken down, and the immune system was so suppressed that exposure to any infectious microorganism would result in illness or death. Recently, the first clinical transfer of a gene in humans was the gene to produce adenosine deaminase to correct this immunodeficiency disease.

Roles for adenosine as a physiological and cellular regulator in livestock have not been explored. Growth of follicles for ovulation, formation of the corpus luteum to produce progesterone (which is necessary for a successful pregnancy), and implantation of embryos result in a 10-fold growth of these tissues in three to 10 days, which is associated with a rapid growth of blood vessels. Thirty percent of breedings result in early abortions due either to failure of the corpus luteum to develop and function normally or to a failure to implant. Thus, our work is involved with identifying effects of adenosine on corpus luteum function, using primarily sheep as an animal model and the synthetic pathways for the synthesis of the purines adenine and guanine on development of the corpus luteum.

Major Achievements
We have shown that adenosine in vitro enhances the response to hormones (luteinizing hormone, prostaglandin E1 and prostaglandin E2) that increase progesterone secretion by the corpus luteum of the cow, sheep, and pig. In addition, adenosine in vitro inhibits another hormone, prostaglandin F2α, which has a negative effect on secretion of progesterone by the corpus luteum. Also, we have shown that adenosine in vivo prevents prostaglandin F2α from destroying the corpus luteum. This has resulted in two refereed research journal articles; three more are in press.

Importance To Users
We have demonstrated that adenosine affects ovarian function in livestock. This should lead to clinical trials on its efficacy for fertility. If clinical trials are successful, FDA clearance would be required for their safety before use in production systems. Since adenine-containing nitrogenous bases are the only nitrogenous bases from food that are conserved, much work on dietary sources of adenosine on growth and DNA and RNA synthesis is needed.
MOLECULAR BIOLOGY AND PHYSIOLOGY

Molecular Mechanisms of Aluminum Toxicity

Principal Investigators:
Michael A. Dunn, Dept. of Food Science and Human Nutrition, UH
H. Michael Harrington, Dept. of Plant Molecular Physiology, UH

Nature of Project
In the tropics, environmental stresses created by such factors as soil aluminum (Al) and heat reduce agricultural production. Aluminum toxicity alone is considered one of the most important environmental stresses limiting food production worldwide. This is because the toxic effect of Al is a major factor limiting plant growth on acid soils.

Aluminum toxicity is especially severe in tropical regions, where acidic soils are common. Moreover, Al toxicity in plants is aggravated by high temperatures, intensifying the problem in tropical regions. The reason Al is toxic to plants is largely unknown. Aluminum is also toxic to animals used for food production in the tropics. Aluminum in the diet can decrease growth, feed efficiency, and egg production in poultry. Aluminum intake by grazing ruminants can be high and is correlated with reduced feed efficiency and altered mineral metabolism. Problems with agricultural productivity (both plant and animal) are worsened when different forms of environmental stress factors such as Al and heat combine in synergy to upset biological organisms. This will be the case as more marginal acidic lands are pressed into plant and animal production in the higher temperatures of the tropics.

To help overcome the negative effects of environmental stresses on food production, the molecular mechanisms by which stress factors such as Al and heat affect biological organisms need to be understood. This will help scientists develop strategies to prevent or alleviate toxicity through management, nutrition, or genetic manipulation.

Major Achievements
We have developed a growth medium containing aluminum that consistently reduces by 50 percent the growth of tobacco cells cultured in the medium. We will use this medium to mimic the exposure of plant cells to aluminum in agricultural systems. Cultured plant cells exposed to Al in this way will be studied to determine the toxic effects of Al on cell metabolism.

In the course of developing this medium, tobacco cells resistant to Al toxicity were discovered. The metabolism of these resistant cells will be studied to determine how they protect themselves against Al toxicity.

We are also studying the toxic effects of feeding Al as part of a normal diet to newly hatched chicks. When we determined the effect of dietary Al on cells of the small intestines of these chicks, we noted that it caused the disappearance of at least two proteins from these cells. We are trying to determine the identity and function of these missing proteins to help understand how their absence affects cell metabolism and the function of the intestine.

Importance to Users
Our achievements are of little practical importance to agricultural producers at present. However, we have established a laboratory cell culture medium to mimic Al toxicity to plant cells. This will enable scientists to study toxicity in the laboratory.

Our findings in the chick intestinal cells indicate that Al indeed has effects on cell proteins and cell metabolism. These are novel findings that will help scientists determine the molecular sites of Al toxicity.

As stated in the Nature of Project section above, the reasons Al is toxic to cells are unknown and will require studies like ours to fully understand.
Functional Analysis of a Heat Shock Protein in a Tropical Plant

Principal Investigator:
H. Michael Harrington, Dept. of Plant Molecular Physiology, UH

Nature of Project

The phenomenon of global warming through the "greenhouse effect" has received much attention in both the popular and scientific press. Of paramount importance to agriculture are the effects that elevated temperature has on the growth, development, and productivity of plants. Growth at elevated temperatures involves a form of stress that drastically limits productivity. Other forms of stress such as salt, heavy metals, drought, high light intensity, and ultraviolet irradiation negatively affect plant growth.

In tropical environments, combinations of different forms of stress are common, and these may interact synergistically with high temperatures to reduce plant productivity. These interactions suggest that there may be central features common to all forms of stress that would likely play key roles in plant responses to environmental stress. To ensure that future agricultural systems are able to meet the nutritional demands of a growing population, efforts must be made to understand the mechanisms by which plants respond to and tolerate stress.

All biological organisms, including field-grown crops, undergo what is termed the heat shock response (HSR) when subjected to a 5–6°C rise in growing temperature. The response is characterized by rapid shutdown of normal gene transcription and protein synthesis; induction of transcription from temperature-regulated heat shock genes and synthesis of as many as 50 heat shock proteins (HSPs); and development of thermotolerance to otherwise lethal high temperatures. Available evidence indicates that one or more of the HSPs are essential for the development and maintenance of thermotolerance. Despite intensive research on the HSR, essentially nothing is known about the role of HSPs in the molecular mechanisms of thermotolerance.

Major Achievements

The long-term goal of our research is to characterize the functions of specific HSPs and to learn their roles in the HSR. We have identified an HSP in sugarcane cells as the autophosphorylating protein kinase (APK).

As a class, protein kinases catalyze the transfer of the α-phosphate group from ATP to suitable protein substrates and in doing so act as metabolic switches. Some kinases amplify or transduce cellular signals, while others regulate metabolism by modulating the phosphorylation level of target proteins and enzymes. Protein kinases are, themselves, regulated by cellular signals such as hormone-receptor interactions, minute changes in calcium concentration or through binding of a calcium-dependent activator protein called calmodulin. Members of the protein kinase family are regulators of a variety of essential cellular functions, including cell division, motility, metabolite transport, gene activation, protein synthesis, muscle and energy metabolism, and cytoskeleton stability. Thus, it is likely that the HS-induced APK plays a significant role in the HSR.

The activity of the sugarcane APK is elevated five- to sevenfold by heat shock, and a similar enzyme is heat-shock-induced in tobacco cells. The induction and the decay of the elevated APK activity correlate the development and turnover of thermotolerance. The sugarcane protein is tentatively localized in a membrane fraction with a density similar to that of the plasma membrane, although appreciable amounts of APK activity are found in the soluble fraction. The kinase appears to be specific for membrane proteins. The protein has been purified greater than 500-fold. The sugarcane APK is of the serine kinase type; that is, the phosphorylated amino acid residue in the enzyme is serine. The highly purified enzyme preparation contains two major proteins and several minor components, all of which exhibit kinase activity.

The APK is unique in that it can also use GTP as a substrate, which may provide important clues as to its function. In addition to enzyme activation, a variety of other functions for the HS–induced APK may be suggested, including restoration of tubulin-phosphate linkages in the cytoskeleton, formation of phosphoinositides, stabilization of the plasma membrane; or mediation of the phosphatidylinositol and/or the calcium signal transduction pathways.

In related research, we have demonstrated that plant cells become thermotolerant much faster when heat-shocked in the presence of elevated levels of calcium or magnesium. These results suggest that nutritional status has an effect on the ability to withstand environmental stress. Heat
shock also causes perturbations of the sugarcane cell membrane, as evidenced by the rapid release of large quantities of inositol triphosphate into the cytoplasm. This compound is intimately linked to the perception and propagation of external stimuli. In the cytoplasm, it mobilizes Cas+ from intracellular stores, which could, in turn, activate a variety of metabolic events through the action of protein kinases and calmodulin. Such events could play important roles in the perception of, and responses to, environmental stress.

**Importance to Users**

Successful completion of this research will help to explain the molecular mechanisms by which environmental signals are perceived and transduced and will greatly enhance our understanding of signal propagation, metabolic regulation, and stress metabolism. The functional characterization of HSPs will provide a biochemical basis for the development of thermotolerance and help to clarify the mechanisms that act to stabilize cell components during stress. A clear understanding of the ways plants establish and maintain thermotolerance is essential for the development of management strategies that reduce stress in the field and for the development of improved plant varieties through biotechnology.

**Development of Gene Transfer Systems for Improvement of Dendrobium Orchid Hybrids**

**Principal Investigator:**

A. R. Kuehnle, Dept of Horticulture, UH

**Nature of Project**

Dendrobium orchid hybrids are among the leading cut and potted floricultural crops grown in the Pacific Basin. The global market remains very strong for this orchid. Hawaii alone produced about $7 million worth of dendrobium (wholesale value) in 1990. Thus the need for development of new and different varieties is strong. To address this need, new tools for orchid breeding and improvement must be explored. Genetic engineering, coupled with tissue culture techniques, offers a novel way to introduce specific genes into plants, as witnessed by the successful introduction of improved protein quality, novel flower color, and disease, insect, and pesticide resistance into various crops in relatively short periods. The development of a suitable gene transfer system for dendrobium orchids would provide the breeder with greater opportunity to produce commercially desirable dendrobium hybrids.

**Major Achievements**

Three major accomplishments have been made during this project.

A gene transfer system suitable for transformation of *Dendrobium* has been identified. Transgenic dendrobium plants—the first in the world—have been recovered after particle bombardment of protocorms.

A reliable procedure for the regeneration of dendrobium plants from tissue cultures has been developed, using etiolated shoot pieces derived...
from seeds or protocorm-like bodies germinated in the dark. A tissue culture system effective for gene transfer to dendrobium did not previously exist. This procedure is now being used for more efficient genetic engineering.

A protoplast isolation and culture procedure has been developed. Protoplast electroporation and culture will aid genetic engineering by permitting prior screening of various foreign genes for effectiveness in orchids.

As a result of this project, fruitful collaborations and correspondences have been initiated with other scientists in Hawaii and elsewhere (California, Florida, Thailand, New Zealand). These likely will facilitate future endeavors.

Importance to Users
Genetic engineering of dendrobium may prove to be a very powerful method for varietal breeding of dendrobium hybrids. Success with this project opens the way for the use of horticulturally desirable genes, such as genes for virus resistance being cloned in a recently approved 406 project. Results from this program may therefore have a significant effect on the dendrobium orchid industry throughout the tropics via eventual development of new plants for cultivation. In particular, effective genetic control of orchid disease will allow growers to improve the quality and quantity of orchid production without damaging our environment.

Virus-resistant Papaya Cultivars Through Genetically Engineered Cross-protection

Principal Investigators:
Richard Manshardt, Dept. of Horticulture, UH
Dennis Gonsalves, Dept. of Plant Pathology, Cornell University

Nature of Project
In most papaya-growing regions of the world, papaya ringspot virus (PRV) is a major limiting factor in production. In Hawaii, where papaya is the fourth most valuable crop ($15 million in 1990), PRV is responsible for the demise of the industry on Oahu, and production areas on the island of Hawaii are also threatened. Conventional breeding programs, relying on cross-pollination between selected papaya parents, have been limited by the modest levels of resistance available in papaya germplasm.

Since 1988, researchers at the University of Hawaii and Cornell University have been collaborating in a project to produce PRV-resistant papaya cultivars through genetic engineering. Genetic engineering, or transformation, involves the transfer of genetic information from one kind of organism to another in a fashion that permits stable expression of the foreign genes in the recipient organism. Other researchers have demonstrated that transfer of a viral gene, called the coat protein gene (CP), into virus-susceptible plants often suffices to produce resistance to the viral disease in the genetically transformed plants. The term "genetically engineered viral resistance" has been suggested to describe this strategy.

Major Achievements
In this collaborative project, the CP gene from PRV, which is the gene conferring resistance, was isolated and its DNA sequenced by Dennis Gonsalves of Cornell University. Jerry Slightom of Upjohn Co. put the CP gene in a transformation vector that allows the viral gene to be expressed in plant cells. The vector also contains "marker" genes that allow for the identification and selection of transformed cells. The vectors were shot into tissue-cultured papaya cells by an apparatus called a particle gun, provided by John Sanford of Cornell, one of its inventors. Transformed papaya plants were then recovered from tissue cultures through a regeneration system developed by Maureen Fitch, a UH graduate student.

Progress has been remarkably rapid, considering the complexity of the project. Within three years from the start of our collaboration, the various parts have been put together in the form of transformed papaya plants that express the marker genes, demonstrating that the genetic engineering technology works with papaya. More important, the presence of the CP gene has also been demonstrated in some of the transformed plants, and recently, the first PRV inoculation tests have indicated that some of the plants containing the CP gene are highly resistant to Hawaiian strains of PRV.

Importance to Users
Genetically engineered virus resistance may prove superior to current virus control methods in several important ways, including easier application, better and more durable resistance, and no detrimental side-effects. It will provide the Hawaiian papaya industry with effective protec-
tion against PRV. Finally, genetic engineering is a technology with broad applications in plant improvement. The methodology developed in this project may be helpful in overcoming other major breeding problems in papaya for which no useful variation exists within the species. Economically important improvements that may be achievable through genetic engineering include development of resistances to fruit fly infestation and fungal diseases, and extension of postharvest shelf life.

Rhizosphere Chemistry in Relation to Crop Productivity in Tropical Soils with Low P Concentrations

Principal Investigators:
C. L. Murdoch, Dept. of Horticulture, UH
C. S. Tang, Dept. of Environmental Biochemistry, UH
N. V. Hue, Dept. of Agronomy & Soil Science, UH

Nature of Project
The first objective was to identify and quantify organic compounds, particularly organic acids, produced by the root systems of mycorrhizal, normally mycorrhizal but uninoculated, and nonmycorrhizal but P-efficient plants, then to determine the effects of root exudates of these plants on P solubility in Hawaiian soils high in iron and aluminum oxides.

Root exudates from green mustard cabbage (Brassica juncea L. 'Waianae') were collected using a continuous hydrophobic exudate trapping apparatus.

Lettuce seed exposed to compounds eluded from the trapping resins from green mustard cabbage were inhibited (54.8 percent germination and 32.5 percent hypocotyl length). Green mustard cabbage grown in trapping apparatuses without trapping resins showed a 25 percent growth reduction compared with seedlings grown in similar apparatuses with the trapping resin, indicating that hydrophobic materials absorbed on the resins have a strong growth-inhibiting effect.

Gas chromatography and gas chromatography-mass spectrometry analysis of exudates trapped on XAD-4 resin showed that the main peak was an isothiocyanate (AITC). Isothiocyanates have been reported to have a wide range of biological activities on microorganisms, plants, and animals. We conclude that AITC was continuously released by germinating seed and the root system of established plants of green mustard cabbage. Cruciferous plants are unique in their ability to obtain phosphorus from low-P soils. They are also unique in their lack of a mycorrhizal relationship. The well established biological activity of AITC may have an inhibitory effect on fungi responsible for the mycorrhizal condition. At sufficient concentration, AITC affects the physiology of cohabitating plants and microorganisms.

We are in the process of determining the effect of isothiocyanates trapped from root exudates of green mustard cabbage on spore germination of Glomus aggregatum, an important vesicular arbuscular mycorrhizal fungus.

The second objective was to evaluate cultural systems for increasing P efficiency on soils high in iron and aluminum oxides, viz., inoculating crops with efficient strains of VAM fungi, intercropping VAM-dependent, P-inefficient crops with P-efficient, nonmycorrhizal crops, growing VAM-dependent, P-inefficient crops in rotations after nonmycorrhizal, P-efficient ones, and amending soils with green manure (residue of Ananas comosus).

Recent research has demonstrated that addition of green manures or animal wastes reduces, or even eliminates, Al toxicity and that the chemical processes responsible for the Al toxification appear to be primarily Al chelation by organic molecules, rather than Al precipitation.

It is also known that rock phosphate is most effective in acidic environments. Thus, it is logical to explore the beneficial effects of rock phosphate-green manure combinations. Nutritional potential of rock phosphate is of interest because it is often available in developing countries with highly weathered soils low in available P.

A greenhouse experiment was conducted using an acid, high P-fixing Ultisol (Humoxic Tropohumult, Paaloa Series) with the following treatments: coral lime (CaCO₃), at 0, 2, and 4 tons/ha; Florida phosphate rock at 0, 150, and 300 kg P/ha; cowpea green manure at 0, 10, and 20 tons/ha. The treatments were a 3 x 3 x 3 factorial set arranged in a completely random design, with three replications. Basal fertilizer treatments were applied to bring the soil solution up to minimum levels for growth of guava seedlings.
Guava seedlings ('Beaumont') were transplanted to individual pots containing 2 kg soil. We primarily tested the following hypotheses.

In the presence of organic matter, specifically readily decomposable green manures, crops are protected from Al toxicity in acid soils as a result of metal complexation.

Unlike liming, which raises soil pH and precipitates Al, complexation by organic matter does not raise pH, thereby keeping rock phosphate dissolution high according to this reaction:

\[ \text{Ca}_6(\text{PO}_4)_2\text{F}_2 \rightleftharpoons 10 \text{Ca}^{2+} + 6 \text{H}_2\text{PO}_4^- + 2\text{F}^- \]

Although the soil pH is low, plants grow well if Al is not toxic. Vigorous growth, in turn, allows the plant to take up more P. Thus, a positive feedback in growth results.

Phosphate rock dissolution in soil can also be increased as Ca activity in soil solution is decreased by chelation of Ca^{2+} by organic anions (e.g., tartrate, citrate) produced by green manures or soil organic matter.

Organic anions can compete with H$_2$PO$_4^-$ for adsorption sites on the soil mineral surface, thereby making P more available for plant uptake.

Organic manures also help incorporate inorganic P from phosphate rock into the organic pool of soil P, thereby creating an additional means of releasing P from phosphate rock for eventual plant uptake through mineralization.

Preliminary analysis showed that pH increased with increasing rates of lime and green manure and to a lesser extent with phosphate rock. Soil solution P showed an increase with increasing rock P and, surprisingly, an increase with increasing lime rates and a decrease with increasing green. Perhaps other, more sensitive parameters (e.g., plant response data) should be examined before conclusions can be made.

Postharvest Senescence and Physiology of Tropical Leafy Vegetables

Principal Investigator:
Robert Paull, Dept. of Plant Molecular Physiology, UH

Nature of Project
In many tropical areas, the more common, cheaper leafy vegetables available in markets are not the temperate transplants: cabbages, celery, lettuce, spinach, or various mustards. The leafy vegetables will be tropical in origin with higher yields and sometimes low maintenance, such as cassava leaf, taro leaves, ung choi, and leafy amaranth. These are always grown by small-scale agricultural units. However, little is known about the postharvest physiology, senescence, and optimum handling for maximum postharvest life and quality maintenance of these tropical vegetables.

This final stage in the agricultural food production system can significantly influence the price obtained by the producer and paid by the consumer. Lack of information on postharvest senescence, physiology, and handling greatly limits the spread of these commodities in highly developed markets. This lack can lead to high losses.

Hence, two problems are to be addressed by this project: what is the senescence process and physiological reason for loss of postharvest life and quality in tropical leafy vegetables, and what optimum postharvest practices are needed to reduce losses to tolerable levels, conserving the best qualities of the current handling system? The second objective follows from the findings of the first. The objectives are directed at both the basic and applied general aspects of tropical leaf senescence.

Major Achievements
The greatest extension of storage life at 12.5°C was achieved with an atmosphere of 1 percent oxygen with no additional carbon dioxide. Addition of 2.5 percent and 5 percent carbon dioxide led to more rapid loss of leaf appearance due to darkening and wilting of the leaves.

The major changes in protein complement have been characterized. Nine leaf proteins decreased. These results confirm our findings using translated mRNA showing increases in proteins having molecular weights of 65, 58, 56, and 39 kD. The major change in proteins was in a 44kD protein whose rate of loss during storage was reduced by storage in an atmosphere of 1 percent oxygen plus 5 percent carbon dioxide. Compared with tissue held in air, a 42kD protein increased under both 1 percent oxygen and 1 percent oxygen.
plus 5 percent carbon dioxide. After an initial decline, a 29kD protein increased under 1 percent oxygen plus 5 percent carbon dioxide; 27kD and 19kD proteins declined in all atmosphere treatments during storage.

**Importance to Users**

Because they provide basic data, the results are of importance to researchers interested in the postharvest physiology and senescence of leafy vegetables. Extension workers are assisted by the availability of information on optimum handling practices in the critical final phase of the agricultural system for tropical leafy vegetables. The clientele most affected by this information would be growers, wholesalers, and the ultimate consumer, who would obtain uniform, high quality tropical leafy vegetables. In addition, worldwide urbanization—and the need to store vegetables before consumer purchase—will increase the need for this information.

The above research, though primarily basic in nature, is providing information on the time-temperature relationship of storage, the length of storage possible, and the rate of decline in quality as determined both objectively (chlorophyll, fiber, loss of ascorbic acid, respiration, etc.) and subjectively (appearance). The water loss pattern and use of modified atmospheres generated through various packing procedures will also be available to help determine the optimum storage conditions.

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**Construction of a Restriction Fragment Length Polymorphism Map of Papaya**

Principal Investigators:

John I. Stiles, Dept. of Plant Molecular Physiology, UH  
Richard Manshardt, Dept. of Horticulture, UH

**Nature of Project**

We are using a new tool of biotechnology, Random Amplification of Polymorphic DNA (RAPD), to construct a detailed genetic map of papaya. RAPD technology is similar to DNA fingerprinting used in forensic science for genetic analysis. Although papaya is an important crop in tropical regions throughout the world, only modest improvements in its germplasm have been accomplished through plant breeding. Many significant problems remain, including viral and fungal diseases, fruit quality problems during certain times of the year, and other physiological factors that limit productivity. Because of the lack of extensive previous breeding work, we feel that the application of modern techniques of breeding and biotechnology can have a significant effect on the rapid development of improved cultivars.

**Major Achievements**

Using RAPD technology, we have analyzed the genomes of 10 different papaya cultivars, seven Solo varieties developed in Hawaii, two Asian cultivars (‘Saipan Red’ from the Mariana Islands and ‘Bentong’ from Malaysia) and a Central American cultivar, ‘Line 356’. As expected, the Hawaiian cultivars were all quite closely related. Although the Asian and Central American cultivars showed more genetic variability when compared with the Hawaiian cultivars, the total variability shown was quite small. This indicates that the commercial germplasm of papaya is quite narrow, and the introduction of wild type germplasm may be beneficial and necessary to obtain major improvements in cultivated papaya.

We are also developing a genetic map using RAPD technology. RAPD technology uses short pieces of synthetic DNA of random sequence to amplify arbitrary but yet specific segments of the papaya genome. The amplification products can be genetically mapped in a manner identical to traditional phenotypic markers, except that they are much more numerous and can be analyzed in a single cross. We have 43 markers mapped and anticipate a map of about 200 markers within the next year.

**Importance to Users**

We anticipate that our genetic map will allow us to identify genes that impart tolerance to papaya ringspot virus, a major disease in almost all regions of the world in which papaya is grown, and to incorporate these genes into Solo and other papaya varieties. We will also identify genes responsible for earlier flowering, fruit characteristics, and other plant characteristics that limit productivity. The application of these biotechnology-based genetic breeding techniques will speed the introduction of advanced cultivars and increase productivity for this important tropical fruit.
Molecular Approach for Improving Protein Quality in Tropical Legume and Root Crops

Principal Investigator:
Samuel S. M. Sun, Dept. of Plant Molecular Physiology, UH

Nature of Project
Plants are the primary source of all protein consumed by man and livestock. Tropical legume and root crops provide significant amounts of dietary protein to the people in the tropics. However, the protein quality of these crops, like most of other legumes and vegetables, is not high. This is due to a deficiency of their proteins in the essential sulfur amino acids, methionine and cysteine. Conventional plant breeding efforts to correct this deficiency have met with little success thus far. This project aims at improving the protein quality of these crops through genetic engineering. The approach is to transfer and express in these crops a gene encoding a seed protein containing a high level of the sulfur amino acids. Optimum expression of this protein should enhance the level of the essential amino acids in these tropical crops.

Major Achievements
The 2S proteins in cannonball (Couroupita guianensis) and paradise nut (Lecythis spp.) have been purified and characterized as to their physical and chemical properties. These proteins, like the Brazil nut 2S protein, are rich in the sulfur amino acids (16 percent met and 8 percent cys). Five cDNAs encoding these sulfur-rich proteins (SRP) were cloned and sequenced. Modifications in the SRP coding sequences to further increase the met content (by 8-63 percent) have been generated via mutagenesis and sequence insertion. Chimeric genes containing these modified and the wild type SRP coding sequences and the CaMV 35S promoter were constructed and have been transferred into potato cells using an agrobacterium gene transfer system. Potato plants are being regenerated from these cells. Preliminary results from histochemical analysis of the regenerated plantlets for marker gene expression indicate that transformation has been successful (Fig. 1). Potato tubers will be produced from these transgenic plants in the future and analyzed for improved protein quality. Alfalfa, as an example of legume plant, will also be transformed using these chimeric genes.

Importance to Users
The importance of this work is severalfold. First, proper expression of the SRP gene would increase the methionine content of the plant storage organs. This would enhance the quality of plant protein and contribute to the nutritional well-being of people in the tropics. Second, expression of the SRP derivatives would be of particular interest to future protein engineers in terms of understanding what types of changes a protein can undergo without interfering with its structure and function. Last, expression of these chimeras would demonstrate the feasibility and utility of genetic engineering in crop improvement.
Molecular Approaches To Protect Plants from Tomato Spotted Wilt Virus Infection

Principal Investigators:
Samuel S. M. Sun, Dept. of Plant Molecular Physiology, UH
John Cho, Dept. of Plant Pathology, UH

Nature of Project
It has been demonstrated in several plant virus-host systems that transgenic plants that expressed viral capsid protein or its antisense mRNA gain resistance to the same or related viruses. These approaches were applied in this research to tomato spotted wilt virus (TSWV), a major threat to vegetable and ornamental crop production in Hawaii. A distinct difference between TSWV and other viruses is that TSWV is a membrane-enclosed virus. Thus, it is of basic interest also to find out whether this capsid protein-mediated protection can be applied to TSWV. Tobacco plant was chosen to test the feasibility of this protection and the efficiency of the chimeric gene constructs and transformation procedures. Later attempts will be made on tomato, the target plant.

Major Achievements
Tomato spotted wilt virus capsid protein (N) gene was cloned from Hawaiian L isolate. The complete nucleotide sequence of this gene was obtained, and the identity of this gene was confirmed by serological method and by sequence

Fig. 1. Untransformed tobacco plants nine days after inoculation with tomato spotted wilt virus; large numbers of local necrotic lesions have merged, and the leaf has started to wilt.

Fig. 2. Transformed tobacco plants nine days after inoculation with tomato spotted wilt virus; fewer local necrotic lesions have developed, and at a much slower rate.
comparison with two different isolates. Over 95 percent sequence identity was found among these isolates, suggesting a close relationship.

A chimeric gene containing the N gene driven by the CaMV 35S promoter was constructed and introduced into tobacco cells by an agrobacterium gene transfer system. Tobacco plants were then regenerated from these cells. Twenty-nine transgenic plants were identified and shown to express the N gene. Some of the first generation transgenic tobacco plants were tested for viral resistance. Results indicate that the transgenic plants show slower and milder symptom development as compared with the wild type tobacco (Figs. 1, 2).

Importance to Users

Tomato spotted wilt virus affects production of several important vegetables and ornamental crops in Hawaii. It has caused 50–90 percent yield loss of tomato and lettuce. Because of its wide host range, development of feasible control of TSWV has been difficult. Extensive survey of germplasm of tomato and lettuce for resistance to TSWV was not satisfactory, especially in the case of lettuce. Capsid protein-mediated protection represents a novel biotechnology approach in producing TSWV-resistant plants. Preliminary results from transgenic tobacco test plants indicate that this approach is feasible and promising.

Molecular Cloning of Organ-specific Promoters and Construction of Expression Vectors for Tropical Root and Tuber Crops

Principal Investigator:
Samuel S. M. Sun, Dept. of Plant Molecular Physiology, UH

Nature of Project

Root and tuber crops are grown widely throughout the tropical and subtropical regions and are staple food for 400–500 million people. They supply significant portions of carbohydrate, protein, vitamins, and minerals to the diet of this segment of the world population. Conventional plant breeding techniques have been used to improve these crops for better nutritional quality and yield. Although significant improvements have been obtained in the past, problems of nutritional deficiency and low yield persist. Recent advances in biotechnology offer opportunities for further improvement of these crops. To apply this technology, it is essential to have available organ-specific promoters and a gene transfer system for the root and tuber crops. This project aims at developing such genetic materials and molecular tools.

Major Achievements

This research project is at its very early stage. We have carried out preliminary experiments to identify target genes from roots and tubers for construction of expression vectors. Specifically, we have analyzed the total protein profiles and the distribution of these proteins in five major root and tuber crops. This information will enable us to select suitable target genes. A 21-kD protein in the cassava root appears a major protein component and thus a good candidate for cloning and isolation.

Importance to Users

Developing countries produced 376 million metric tons of root and tuber crops in 1988, representing 66 percent of the world's production. This major production demonstrates the importance of these crops to the well-being and the economy of these regions. However, the production and average yield of these crops in the regions are generally low as a result of many intrinsic genetic characteristics and external biotic and abiotic stresses. This project aims at developing necessary genetic materials and molecular tools that can be used to improve these crops via biotechnological approaches. New varieties of root and tuber crops with improved quality and productivity should benefit the nutritional well-being and health of the people in these regions.
Genetic Basis of Resistance to Biorational and Conventional Insecticides

Principal Investigators:
Bruce Tabashnik, Department of Entomology, UH
Marshall Johnson, Department of Entomology, UH

Nature of Project
One of the most promising alternatives to conventional pesticides is the biopesticide *Bacillus thuringiensis* (*B.t.*). *B.t.* is a common soil microbe that produces proteins that are highly toxic to certain insect pests yet are harmless to people, beneficial insects, and other nontarget organisms. Recent advances in biotechnology, including transfer of toxin-encoding genes directly into several major crop plants, will dramatically increase the use of *B.t.* for crop protection. Some projections indicate that annual sales of *B.t.* products could top $1 billion by the year 2000. The most serious threat to the continued success of *B.t.* is the potential for resistance development in pest populations. Resistance to pesticides is a problem in more than 500 species of pests, yet until recently, no cases had been documented of resistance to *B.t.* in field populations.

The goal of our project is to prolong the usefulness of biorational and conventional insecticides through increased understanding of the genetic basis of resistance. Our research targets the diamondback moth, a global pest of cabbage and related vegetables (Fig. 1). In some areas of North America and the Pacific Basin, this damaging pest has developed resistance to all major classes of conventional insecticides. By understanding the genetic basis of resistance, we can devise strategies to delay, avoid, or even reverse resistance development in diamondback moth and other pests.

Major Achievements
Our results provided the first rigorous evidence for development of resistance to *B.t.* in the field. The concentration of *B.t.* required to kill 50 percent (LC50) of the most resistant field population was about 30-fold greater than the LC50 of susceptible strains. Laboratory selection experiments showed that resistance increased to 800-fold after nine generations of continuous exposure to *B.t.* Resistance declined slowly when treatments with *B.t.* were stopped.

Importance to Users
Our discovery of resistance in diamondback moth provides an early warning that pests can develop resistance to *B.t.* in the field. These findings have received widespread media attention, including coverage by the Wall Street Journal, the BBC, and Science. Our results, in conjunction with data from other laboratories, are changing strategies for implementing *B.t.* Scientists in academia, government, and industry now realize that heavy reliance on intensive and extensive use of *B.t.* can cause pest resistance and thus shorten the effective life of an extraordinarily useful and environmentally safe product. The information we are obtaining will facilitate development of strategies to slow resistance. This will help to optimize use of *B.t.*, maintain competitiveness of U. S. agriculture, and help preserve the environment.

Fig. 1. Diamondback moth caterpillars (larvae) devour cabbage. Resistant caterpillars are no longer controlled by *B.t.*
Light Stress (Photoinhibition) and the Violaxanthin Cycle

Principal Investigator:
Harry Y. Yamamoto, Dept. of Plant Molecular Physiology, UH

Nature of Project
It paradoxical that light, which is essential for photosynthesis, can at the same time be harmful to plants. Light can be harmful whenever there is more light energy than can be used photosynthetically. Surprisingly, this condition exists throughout the course of a normal sunny day. The harmful effects of light are seen as reduced photosynthetic efficiency or, in the extreme, irreversible damage to plants.

Recently, it has been found that higher plants have a way to dissipate excess light energy harmlessly as heat. This mechanism is still not completely understood but involves a carotenoid pigment, zeaxanthin, which is formed in a biochemical pathway known as the violaxanthin or xanthophyll cycle, and a "high-energy" state or acidification of the lumen (the inner space of chloroplasts). The objectives of this project are to clarify the biochemical mechanisms by which zeaxanthin dissipates excess energy in response to excess light.

Major Achievements
We perfected a system by which zeaxanthin formation and nonradiative dissipation of energy can be examined in chloroplasts isolated from lettuce and peas. This isolated \textit{(in vitro)} system is ideally suited for biochemical and mechanistic studies, as the steps involved can be dissected and studied in detail. We also developed a superior high-performance chromatographic system for the analysis of the plant pigments. We showed through the use of various biochemical reagents that zeaxanthin-dependent nonradiative dissipation of light energy requires not only zeaxanthin and acidification of the lumen but also other, still undefined, slow changes, possibly conformational changes in protein or membrane structure.

We also showed that nonradiative dissipation of energy was linearly correlated with the amount of zeaxanthin.

On the basis of the above accomplishments, we have been awarded two nationally competitive grants to continue these studies, one from the USDA Competitive Grants Program, entitled "Mechanisms of Xanthophyll Responses that Protect Plants Against Excess Light" and another from the Department of Energy, Division of Energy Biosciences, entitled "Violaxanthin De-epoxidase: Biogenesis and Structure." In addition, the Ph.D. student working on this project was awarded an F. Matsuda Scholarship from the Research Corporation of the University of Hawaii.

Importance to Users
Clarification of the biochemistry of zeaxanthin-dependent nonradiative dissipation of energy is important to both the scientific community and the public. The advances made clearly show that energy dissipation is biochemically regulated and can be studied by classical biochemical methods. Most studies to date have been physiological, using intact leaves. These methods, while useful, have limited capacity for revealing biochemical mechanisms. Understanding the biochemical mechanisms for nonreactive dissipation will help determine the limits of this system to protect plants. In view of the environmental changes being caused by human activities, it is important to know to what extent plants can tolerate such changes. Knowledge of the biochemistry may also make it possible to increase the protective capacity and efficiency of light usage. From the evolutionary viewpoint, this research will ultimately answer the question, why do all higher plants have this system but not all photosynthetic organisms?
HUMAN NUTRITION

Determination of Minimum-cost Diets

Principal Investigator:
Basile P. Goungetas, Dept. of Agricultural & Resource Economics, UH

Nature of Project
Designing and implementing successful nutrition enhancement programs requires a thorough understanding of the way individuals buy and consume foods. Unfortunately, many individuals make poor food choices, partly because of inadequate knowledge about proper nutrition and partly because of insufficient income. As a result, attempts have been made to find the least costly combination of basic food items (flour, rice, etc.) needed to meet the minimum nutritional requirements of an individual of specified age and sex.

These so-called minimum-cost diet programs have three deficiencies: they lack variety, i.e., only a small number of food items (usually 6-10) appear in the selection; they lack palatability, i.e., the selection contains food items that are usually unappetizing; and it is very difficult, if not impossible, for individuals to make meals from a very small number of food items.

In this project, we circumvent these deficiencies by looking for the least costly combination of recipes (complete meals) rather than food items.

Major Achievements
This is a relatively new project, and there is no major achievement to report so far. However, a computer algorithm needed for the optimization has been developed and tested using 217 recipes from Hawaii, and we are expanding the computerized recipe database and the coding of food items, using the Universal Product Code.

Importance to Users
The model under study and the accompanying computerized database of recipes and food items can serve as an alternative for the design of low-cost diet plans in Hawaii, the Pacific Basin, and elsewhere. Implementation elsewhere, however, will require that the computerized database of recipes and food prices be revised to reflect regional food tastes and prices.

Physical and Chemical Properties of Taro Gums and Degummed Taro Products

Principal Investigator:
Alvin S. Huang, Dept. of Food Science and Human Nutrition, UH

Nature of Project
Today's health-conscious consumers demand more dietary fiber in foods they eat. However, they do not want “rabbit foods,” and the high-fiber food items must be tasty as well. In responding to this consumer demand, food scientists have discovered that water-soluble dietary fibers, or “gums,” possess all the health benefits of regular fibers but taste much better. This discovery triggered the so-called oats war not long ago, because oats are rich in gums. Taro, a tropical crop that grows well in Hawaii, is also rich in gums. But the natures of taro gums are practically unknown. Consumers are not aware of the benefits of eating taro as a source of dietary fiber. The purpose of this project is to isolate and identify the physical and chemical properties of taro gums. With this knowledge, we can better use this tropical crop to benefit local producers as well as general consumers.

Major Achievements
The isolation of gums from taro corms turned out to be more difficult than I had expected. Taro corms are mainly starch, which is similar to gums in many aspects and therefore hard to separate from gums. We developed an innovative process that includes a precisely controlled slicing step and a freezing-thawing step to soak gums out of starch. The resulting gum fraction is starch-free. The process can be easily scaled up to production size with minimum capital investment in the beginning. We are filing a patent application with the hope that this technology will stay to benefit tropical regions, where taro grows well.

By obtaining purified taro gums, we have successfully determined that these gums have two major components. Their molecular weight distributions and sugar compositions were deter-
mined. The viscosities of taro gums are temperature-dependent but not affected by acidity. A strong synergistic effect between taro gums and starch in terms of viscosity has been observed. This explains the strong gels formed in taro foods when refrigerated. Degummed taro flour forms a soft gel that is more palatable. It was interesting to note that the acridity of degummed taro flour is much lower than regular taro flour’s. The exact cause of taro acridity is still unclear. However, the removal of acridity can definitely make the uses of taro flour in many food products much easier.

Importance to Users
With the development of a process that can isolate taro gums as well as make better quality taro flour, we would like to help the local taro industry to develop more taro food products. Taro gums may be used as a stabilizer in ice cream and other frozen deserts. Degummed taro flour can replace up to 100 percent of wheat flour in bakery goods such as cookies and bread. By properly controlling the starch/gum ratio, we can make taro products with longer shelf life and better taste. In all these applications, we still maintain the health benefits of dietary fiber when gums are added to, or left with, the flour. With the publication of our results in fact sheets and newsletters, taro chip producers already are adding dietary fiber content on their product bags. I believe an array of new taro food products will come out to satisfy consumers’ appetites in the near future.

Development of Methodology for Culture-specific Food Groupings and Assessment

Principal Investigators:
N. E. Johnson, Dept. of Food Science and Human Nutrition, UH
S. Shimabukuro, Dept. of Food Science and Human Nutrition, UH
J. Derrickson, Dept. of Food Science and Human Nutrition, UH

Nature of Project
The purpose of the study was to determine nutrient intakes of some EFNEP (Expanded Food and Nutrition Education Program) participants, examine their food consumption patterns, and construct a food guide based on those patterns. Dietary intake was determined by 24-hour food recalls, and nutrient content was calculated by computer. The sample of homemakers was composed of 41 percent Hawaiian, 23 percent Caucasian, 21 percent Filipino, 9 percent Polynesian, and 6 percent Oriental.

Major Achievements
Results from the study indicated that average intakes of magnesium, calcium, zinc, iron, copper, manganese, and vitamins A and B6 were less than 70 percent of recommended amounts for women 23–50 years of age. As a result of examining the data, using regression analysis and indexing nutrients, the limiting nutrients of the diet were identified. Since there are intercorrelations among nutrients, those nutrients that needed to be emphasized were identified; they became the basis for the food guide we developed.

A culturally sensitive food guide was constructed. The guide included foods most frequently used by the population groups and emphasized limiting nutrients and dietary quality. Serving sizes of specific food groups were developed. The guide provided a nutrient adequacy score of 90 out of a possible 100 when typical menus were developed. A scoring system also was developed to evaluate nutrient quality. The taro plant was selected as a symbol of the dietary guide.

Fig. 1. Food guide for Hawaii.
Some testing of the guide was conducted among EFNEP paraprofessionals.

**Importance to Users**

The importance of this research is that a statistically based methodology for developing food guides for different cultural groups has been developed. The process can be used by others to develop a guide that focuses on limiting or deficient nutrients in a specific population. Nutrition education can thus be targeted to be more effective than broad recommendations that do not consider specific shortages and that often tend to amplify excesses and omit deficiencies.

**Noncholinergic Neurotoxicity of Organophosphate and Methylcarbamate Insecticides**

**Principal Investigator:**

J. Seifert, Dept. of Environmental Biochemistry, UH

**Nature of Project**

Insecticides are an important factor in reducing human and animal diseases caused by insect vectors and increasing agricultural production by preventing losses due to insect pests. Organophosphorous acid triesters (OP) and methylcarbamates (MC) are major types of currently used insecticides. While most of them are environmentally safer than the traditionally used chlorinated hydrocarbons, their toxic potential is more eminent for nontarget species. The hazard is especially high for the countries of the Pacific Basin area, partly because of the lack of expertise among the local population in dealing with modern agricultural practices and partly because of the unique nature of the tropical island environment. This project considers noncholinergic toxicity due to acute and subchronic exposure to OP and MC insecticides. The research focuses on mechanisms and factors by which OP and MC insecticides affect L-tryptophan metabolism.

**Major Achievements**

Our research revealed a novel mechanism of OP and MC action in mice used as an animal model system. Kynurenine formamidase, an enzyme important for L-tryptophan metabolism, was most severely inhibited by several OP and MC insecticides in livers. Enzyme inhibition resulted in reduced production of L-kynurenine, a key intermediate in biosynthesis of several physiologically active L-tryptophan metabolites. The inhibition was accompanied by accumulation of N-formyl-L-kynurenine. In contrast to the reaction in livers, the plasma L-kynurenine increased by OP treatment, due to a novel synthesis or a release from an extrahepatic tissue. Urinary kynurenic and xanthurenic acid excretion was enhanced as a consequence of altered N-formyl and L-kynurenine formation. The pyrimidyl phosphorothioates diazinon and ethyl pirimiphos were the most potent in increasing plasma L-kynurenine and excretion of urinary xanthurenic acid and in inhibiting liver kynurenine formamidase. The crotonamide phosphates dicrotophos and monocrotophos were of intermediate potency, while the MC carbaryl was least potent. The observed phenomena showed a sexual-dimorphism. The cholinesterase reactivator 2-PAM, the transaminase cofactor pyridoxin, and the protein biosynthesis inhibitor cycloheximide did not alleviate L-tryptophan metabolism altered by OP and MC insecticides.

**Importance to Users**

This project provides several pieces of new information important for assessment of OP and MC insecticide noncholinergic toxicity. Alteration of L-tryptophan metabolism in mice by some OP and MC compounds can result in severe toxic manifestations. First, increase in xanthurenic acid must be considered with respect to possible development of bladder malignancies. Studies in rats identified xanthurenic acid as an initiator of bladder cancer. Second, xanthurenic acid is diabetogenic, due to its ability to reduce insulin availability. Its increase due to OP or MC poisoning would be detrimental particularly for the Samoan and Hawaiian populations, who are recognized as having a high incidence of diabetes. Third, changes in plasma L-kynurenine affect the rate of its transport into the brain. Consequently, biosyntheses of several neuroactive compounds that are dependent on L-kynurenine may be affected. Some OP and MC noncholinergic toxic symptoms originated in the central nerve system can be traced to this novel mechanism. This project must be further expanded across animal species to be able to extrapolate its conclusions to humans.
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