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The following format is designed for Primary Research Articles, and should be modified appropriately for manuscripts in other categories. The manuscript should be arranged as follows, with each section beginning on a separate page:

Title page: Title should be concise and informative; List of authors; Institute or laboratory of origin: Where authors have different addresses, use numbered superscripts to refer to each address provided; Corresponding author: include their telephone and email details.

Keywords: key words or phrases to enable retrieval and indexing by searching techniques; not to exceed 8 words.

Abstract: This should provide a concise statement of the motivation for the work done, the scope of the work and the principal findings. The abstract should be less than 300 words for Primary Research Articles and Reviews, and 150 words for other categories.

Introduction: This should argue the case for your study, outlining only essential background, but should not include either the findings or the conclusions. It should not be a review of the subject area, but should finish with a clear statement of the question being addressed.

Methods: This should allow replication of all experiments described and demonstrate the validity of those experiments for the research being conducted.

Results: This should not include material appropriate to the Discussion section.

Discussion: This should highlight the significance of the results and place them in the context of other work. It should not introduce new material, be over-speculative, reiterate the results, or exceed 20% of the total length. The Results and Discussion sections may be combined for Technical Advances papers.

References: The reference list should be in alphabetical order and include the full title with the name of the journal given in full. When there are eight or more authors only the first three should be listed, following the et al.

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Pacific Agriculture and Natural Resources

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Editor's Words of Welcome

Welcome to the first volume of the new journal entitled ***Pacific Agriculture and Natural Resources*** (PANR)!

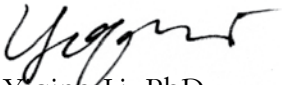
The PANR evolved from the ***Journal for Hawaiian and Pacific Agriculture*** (JHPA) established in 1988 for the promotion of tropical agriculture. Previous editors including Drs. Marcel Tsang, Bruce Mathews, William Sakai, Sheldon Furutani, and Lorna Arita-Tsutsumi are highly professional and served admirably. The JHPA played an important role in disseminating quality information to the growers, ranchers and farmers around the Pacific Rim. All papers from JHPA are available free online: (http://www.uhh.hawaii.edu/academics/cafnrm/research).

I am truly honored to have been selected by my colleagues at the University of Hawaii at Hilo as senior editor of PANR. I wish to further improve upon the previous editors' efforts and make PANR the highly esteemed applied agricultural and natural resources management journal for the tropical Pacific Islands.

The PANR publishes peer-reviewed original research, reviews, and professional commentaries on all aspects of agricultural science and natural resource management in the tropical Pacific. The journal covers a broad scope including production agriculture, agroecology, agricultural and environmental engineering, disease and pest management, food science, fisheries and aquaculture, forestry and agroforestry, resource economics, conservation biology, and natural resource management. The PANR concentrates on primarily original research articles, but operates a flexible policy with the editorial board's discretion regarding other submissions, such as reviews, professional commentaries, book reviews, letters, technical notes, as well as educational methods for life science and natural resource management.

The PANR welcomes submission of manuscripts in all aspects described above and subsequent volumes will be published annually in October.

Respectfully,



Yiqing Li, PhD
Editor, PANR
College of Agriculture, Forestry &
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Contents

Pacific Agriculture and Natural Resources

In vitro Germplasm Storage of Gardenia Brighamii

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Abstract: This study demonstrated that *Gardenia brighamii,* a plant listed as endangered, can be micropropagated and maintained *in vitro* using an economical in vitro medium. Greenwood stems with 4 – 6 axillary buds were surface disinfected with Laboratory Disinfectant (10:1:1) and used as initial explanting material. Murashige and Skoog (M&S) medium supplemented with 30 g/L sucrose, 8.9 µM 6-Benzylamino purine (BA) and 2 mg·L^{-1} Plant Preservative Medium (PPM) was used as the initial explanting medium. This medium formulation promoted development of axillary buds into microshoots which were used for further studies on *in vitro* germplasm storage. Vessel sealing is commonly practiced in Hawaii as a means of reducing casual contamination during culture storage. Our study showed that the use of parafilm for vessel sealing significantly increased vitrification. It also decreased plantlet growth and rhizogenesis. Germplasm storage requires a long term commitment in providing the necessary conditions for sustaining the plant material. As such, effective strategies for reducing costs are paramount to the success of the program. Maui LCF (LCF), a fertilizer formulation for stimulating *ex vitro* plant growth was evaluated as a potential low cost *in vitro* germplasm storage medium. This formulation was selected because of its low cost when compared to the M& S salts and promising observations made when it was used in a preliminary study on *Nephroplepis exaltata* in this laboratory. This study demonstrated that LCF at a concentration of 2560 mg·L^{-1} was the most effective medium treatment in reducing general chlorosis.

Keywords: *Gardenia brighamii*, micropropagation, *in vitro* propagation, plant tissue culture, germplasm storage.

Introduction

Gardenia brighamii, or nanu, is listed as endangered on the Endangered Species Act and as critically endangered on the IUCN relist (U.S. Fish and Wildlife Service, 1985). Currently, 15 to 19 individual plants are known to exist on the islands of Hawai'i, Lana'i, Maui, Moloka'i and O'ahu (Koob, 1999; Wagner, 1999). Urban encroachment continues to present a serious threat to the nanu. Other threats include grazing and trampling by animals and competition with invasive plant species.

Conservation strategies include both protection of plant populations as well as regeneration for replanting purposes.

Plants may be regenerated by seminal propagation, stem cuttings, air layers and grafting (Stratton, et.al., 1998). These methods are variable with regard to success and each mother plant can produce only a limited number of propagulas. Additionally, regenerated field grown (*ex vitro*) plants are prone to environmental stress problems related to moisture, temperature, light, pests and diseases. These situations pose great challenges for storing germplasm material. Aseptic micropropagation has the potential to regenerate mass numbers of cloned plants from a single stock plant. There are, however, "stumbling blocks" that tend to discourage the use of this technology. One of the most common problems in the State of Hawai'i is contamination. This problem is especially prevalent with field grown plants that are exposed to high levels of contaminants, free-moisture and high temperatures.

The concerns and disadvantages of growing and maintaining plants ex vitro are greatly reduced in vitro. In vitro conditions provide a very high level of control with regard to moisture, temperature, light, nutrition, plant regulators, pests and diseases. However, labor and materials costs can be high. The in vitro culture is typically subcultured (transferred to new media) every 1-2 months; dependent on culture growth rate. Faster growing cultures must be subcultured more frequently because they deplete growth factors and release undesirable compounds into the culture medium. Frequent transfers results in higher labor and materials costs. Conditions that promote rapid plantlet growth also predispose the cultures to higher somaclonal variation. This condition is undesirable when attempting to maintain the genetic integrity of the plant during germplasm storage.

The objectives of this project were to: 1) develop an initial explanting protocol (placement of the plant organ into the sterile culture medium); 2) evaluate LCF as an economical medium for germplasm storage and 3)evaluate the influence of vessel sealing on the photoautotrophic growth of explants.

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Materials and Methods

Development of initial explanting protocol

Stock field grown plants grown on the UHH campus were used as a source of explants.Vigorous growing greenwood stems were selected for initial explanting. The typical stem cutting contained 4-6 axillary buds. All leaves were removed and the terminal cuttings were soaked in Laboratory Disinfectant (LD) at the concentration of 10 parts water: 1 part base: 1 part activator for 30 minutes (Tanabe and Matsumoto, 1992). Approximately 5 mm at the base of the cutting was removed and the remainder of the stem was cut into individual explants with each explant having 2 axillary buds. These explants were soaked in LD for 2 minutes and immediately placed into separate tubes of sterile water. The explants were transferred into either full or half salts Murashige and Skoog (M&S) basal media (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose, 8.9 μM 6-Benzylamino purine (BA) and 2 $mg \cdot L^{-1}$ Plant Preservative Medium (PPM).

LCF germplasm storage media treatments

LCF is a compost tea super concentrate. It is derived from pineapple, papaya, sugarcane products, and mushroom spawn. It also contains 0.3% Nitrogen, 0.075% P_2O_5 and 2.25% K_2O. Preliminary studies with the *Nephrolepis exaltata* indicated that LCF has the potential to maintain plant life over an extended period of time. As such, it was evaluated in this project as a potential economical germplasm storage medium.

The following LCF media treatments were used: 0 $mg \cdot L^{-1}$; 320 $mg \cdot L^{-1}$; 640 $mg \cdot L^{-1}$; 1280 $mg \cdot L^{-1}$; 2560 $mg \cdot L^{-1}$; 5120 $mg \cdot L^{-1}$; 10240 $mg \cdot L^{-1}$. PPM was included in all media treatments at 2 $mg \cdot L^{-1}$.

All media were adjusted to a pH of 4.0 – 4.3 and sucrose was eliminated to discourage microbial growth and encourage photoautotrophy. The media were solidified with 0.3% gellan gum. Ten milliliters of culture medium was dispensed into each 25 mm x 150 mm culture vessel and sealed with a kaput. Parafilm was wrapped at the base of the kaputs for parafilm sealed treatments (Tanabe and Horiuchi, 2006; Tanabe et.al., 1993 and Tanabe et.al., 1992).

The culture vessels were autoclaved at 121° C at 15 psi for 15 minutes. Cultures were maintained at 22°C under a 16 hr photoperiod provided by Philips cool-white fluorescent tubes, at a light intensity of 50 $\mu mol\ m^{-2}s^{-1}$. Each treatmeant consisted of 8 replicate cultures with 1 explant per culture vessel.

Observations

Shoot growth was determined by measuring the distance from the crown of the plantlet to the most distal growing point. General chlorosis was evaluated with a visual rating system. The assignment of numbers ranged from 1 for a plantlet with normal green leaves to 5 for a plantlet with highly chlorotic leaves. Rhizogenesis was determined by counting the number of roots produced per plantlet. A plantlet was considered vitrified if it showed foliage "water logging" symptoms. Advanced cases generally expressed necrosis.

Results and Discussion

Contamination and oversterilization were less than 5% when a combination of LD soak and PPM were used. Plant material collected during dry weather conditions were less prone to contamination, possibly because fungal and bacterial contaminants are highly reliant on free moisture for their survival. Explant growth and development was very similar for ½ and full salts M&S over a 2 month period. There was no difference in plant height and foliage color.

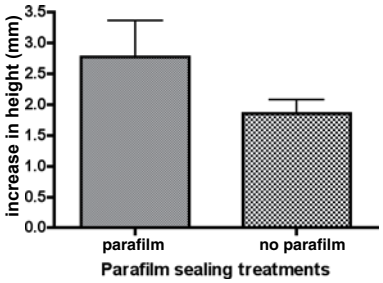

Fig.1. Nanu plantlet growth at 8 weeks as influenced by parafilm sealing. Data are means ± SE.

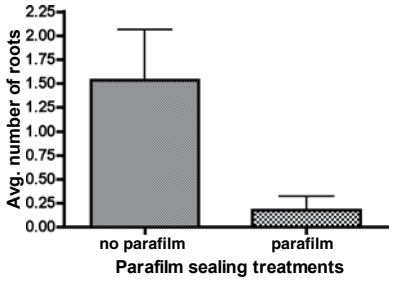

Fig.2. Nanu rhizogenesis at 8 weeks as influenced by parafilm sealing. Data are means ± SE.

The inclusion of 8.90 μM BA did not significantly increase plantlet regeneration over a 2 month period of time but did promote axillary bud breaks. These shoots were excised from the stock plant and served as microcuttings for the in vitro LCF germplasm storage treatments.

Plantlet growth and rhizogenesis were significantly less when culture vessels were parafilmed (Figs.1&2). All cultures were placed into a photoautotrophic *in vitro* environment. As such, the plantlets were required to photosynthesize to produce sugars for growth and development. The vessels that were parafilmed probably did not allow for adequate gaseous exchange required to support normal photosynthesis.

Plantlet growth as determined by explant height, was influenced by the level of LCF (Fig. 3). Plantlet growth was significantly greater for the 10,240 mg · L^{-1} LCF treatment as compared to all other LCF treatments. This response may be undesirable when storing the plants for germplasm storage. The increased plantlet growth predisposes the culture to frequent subcultures due to the increased utilization of growth factors and the possible release of undesirable compounds into the culture medium.

Sealing the culture vessels with parafilm greatly increased vitrification (Fig.4). Vitrification is generally increased with an increase in free moisture and this condition was probably created by sealing the culture vessel with the water impervious parafilm. In general, explants with young leaves were more prone to vitrification than explants with older leaves.

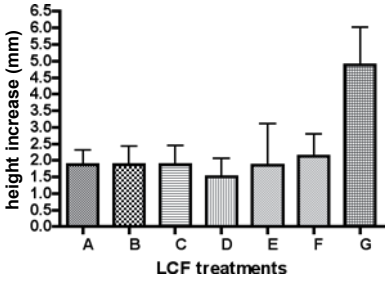

Fig.3. Nanu plantlet growth as influenced by LCF after 8 wks. A = 0 mg·L^{-1}; B = 320 mg·L^{-1}; C = 640 mg·L^{-1}; D = 1280 mg·L^{-1}; E = 2560 mg·L^{-1}; F = 5120 mg·L^{-1}; G = 10240 mg·L^{-1}. Data are means ± SE.

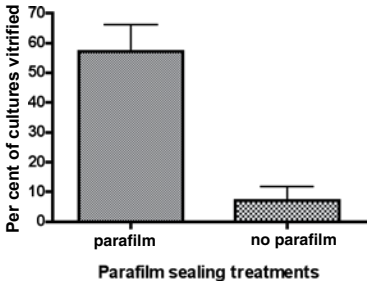

Fig. 4. Per cent nanu cultures vitrified as influenced by parafilm sealing. Data are means ± SE.

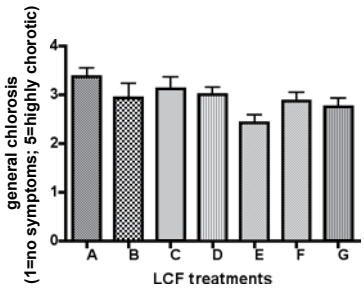

Fig. 5. Nanu plantlet general chlorosis as influenced by LCF after 8 weeks. A= 0 mg·L^{-1}; B = 320 mg·L^{-1}; C = 640 mg·L^{-1}; D = 1280 mg·L^{-1}; E = 2560 mg·L^{-1}; F = 5120 mg·L^{-1}; G = 10240 mg·L^{-1}. Data are means ± SE.

The level of general chlorosis was lowest with 2560 mg·L^{-1} LCF (fig. 5). This condition was desirable for germplasm storage as it suggested adequate levels of inorganic nutrients were available even after 2 months without subculturing into new medium.

Conclusion

This study demonstrated that nanu can be micropropagated by using a combination of LD surface disinfectant and PPM, a culture medium biocide. A culture medium comprised of full salts or ½ salts M&S with 8.90 µM BA promoted axillary bud development into microshoots that can be used as initial explant material.

An *in vitro* germplasm storage medium with 2560 mg·L^{-1} LCF produced plantlets with the least amount of general chlorosis over a 2 month period. Sealing the culture vessel with parafilm should be avoided as this increased vitrification.

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Pacific Agriculture and Natural Resources

Isolation and Characterization of *Erwinia* spp. Causing Bacterial Soft Rot on Orchids in Hawaii

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Abstract: Bacteria continue to cause serious disease problems on orchids in Hawaii. During a survey between 2002 and 2004 on the windward side of Hawaii Island, samples of leaves with soft rot of orchid genera including *Dendrobium*, *Miltonia*, *Cattleya* and *Oncidium* were collected. Fourteen strains of *Erwinia* were isolated and purified from these samples. Bacteria were identified using Analytical Profile Index (API) strips and standard physiological and biochemical tests, and confirmed by species-specific PCR. Three gram-negative, facultatively anaerobic, rod-shaped bacterium that produced pale cream, irregular colonies with undulate margins on nutrient broth yeast extract agar (NBY) were identified as *Erwinia chrysanthemi* (*Dickeya* sp.). These strains produced dark brownish blue colonies on nutrient agar supplemented with 1% glycerol and 2mM $MnCl_2.4H_2O$ (NGM). Pathogenicity of the *E. chrysanthemi* strains was confirmed on orchids with reisolation of the strains from symptomatic tissue. The *E. chrysanthemi* strains were resistant to copper and sensitive to streptomycin (Cu^r Sm^s). Field data suggests that *E. chrysanthemi* may not be as prevalent as once thought and laboratory data suggests the frequent use of copper in nurseries may have inadvertently resulted in the development of copper resistance in *E. chrysanthemi* from orchids.

Keywords: *Orchidaceae*, *Dickeya*

Introduction

Orchids are comprised of over 800 genera and thousands of hybrids. In countries such as the Netherlands, Germany, China, Taiwan, the USA and Japan, orchids are considered international business (Chan *et al.* 2005). Orchid crops in Hawaii, which include potted plants and cut flower production, had a commercial value of $22.2 million in 2006 (Anonymous 2007). Limiting productivity, however, is the persistence of phytopathogenic bacteria that results in losses at economically damaging levels during disease outbreaks (Chan *et al.* 2005). Worldwide, the most common and important bacterial disease problems of orchids include soft rot caused by *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi*, brown rot caused by *Erwinia cypripedii*, and corm and basal leaf rot caused by *Acidovorax avenae* subsp. *cattleya* (Chan *et al.* 2005; Simone & Burnett 2002). In Hawaii, *B. gladioli* and *E. chrysanthemi* have been reported as bacterial pathogens of orchids (Keith *et al.* 2005; Uchida 1995). *E. chrysanthemi* (*Dickeya* spp.) (Samson *et al.* 2005), originally isolated from chrysanthemum by Burkholder *et al.* (1953), is a pathogenic enterobacteria causing soft rot, stunting, and wilting on a wide range of hosts including ornamental plants (Dye 1969; Lee & Yu 2006; Munnecke 1960; Thomson *et al.* 1981) and is widely distributed in many temperate and tropical areas around the world (Bradbury 1968; Dickey 1979; Lee & Yu 2006; Lumb *et al.* 1986; Perombelon & Kelman 1980). Popular species of orchids, including *Phalaenopsis*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Oncidium*, and *Vandas*, are very susceptible to bacterial soft rot. In Taiwan, *Phalaenopsis* soft rot caused by *E. chrysanthemi* is an endemic disease that occurs in most orchid cultivation areas (Lee & Yu 2006). Similar symptoms are commonly observed on *Dendrobium* and *Phalaenopsis* sp. in Korea (Lee *et al.* 1999). *E. chrysanthemi* has been reported as a pathogen of *Dendrobium* orchids in Hawaii (Uchida 1995), but the rate of occurrence, the genetic diversity, and the extent of the bacterium's ability to cause severe rot on various orchid genera remains unknown. The objectives of this research were to determine the extent of *E. chrysanthemi* as a causal agent of orchid disease in Hawaii, to determine the effectiveness of current molecular techniques in identifying and distinguishing *E. chrysanthemi* from other bacteria, and to determine the levels of copper and streptomycin resistance present in the population. We believe this is the first study conducted to evaluate the importance of *E. chrysanthemi* as a pathogen on orchids in Hawaii and the effectiveness of chemicals used by growers for bacterial disease control.

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Materials and Methods

Field survey and sampling. From May 2002 to February 2004, leaves were collected from various orchid genera with soft rot symptoms from 6 farms located on the windward side of the island of Hawaii. Fourteen samples were collected during farm visits and from disease samples submitted by growers. Digital photographs of leaf symptoms were recorded using a Nikon Coolpix 995 model digital camera (Melville, NY). Typical symptoms were noted and described.

Isolation and identification of the pathogen. Diseased samples were processed within 24 h of collection. Small pieces (~ 10 mm^2) of leaf tissue were cut from the edge of the advancing portion of a lesion, surface sterilized in 0.5% sodium hypochlorite for 2 min, rinsed in sterile distilled water, and macerated in a sterile mortar or Petri dish with approximately 2 ml sterile water. A 20-µl sample was streaked onto plates containing nutrient broth yeast extract agar (NBY)(Vidaver 1967) supplemented with a filter-sterilized (0.2 µm pore size, Nalgene, Rochester, NY) solution of cycloheximide (final concentration, 50 µg/ml). Plates were incubated at 28°C. Individual bacterial colonies from plates were purified and stored at -80°C in 15% aqueous glycerol, and working cultures were kept on NBY agar. For all isolates, Gram-reactions were determined by the lysis of bacteria in 3% KOH (Gregersen 1978); cytochrome C oxidase was tested by using filter paper impregnated with NNN'N'-tetramethyl-p-phenylenediamine dihydrochloride (Kovacs 1956); oxidative/fermentation metabolism was evaluated as previously described (Huch & Leifson 1953); pyocyanin production was tested on King's Medium B (KMB)(King *et al.* 1954); and growth on the following selective media: Yeast dextrose calcium carbonate (YDC)(Wilson *et al.* 1967), Nutrient agar supplemented with 1% glycerol and 2 mM $MnCl_2.4H_2O$ (NGM) (Lee & Yu 2006) and Miller Schroth (MS)(Miller & Schroth 1972). *Erwinia* spp. were initially identified using Analytical Profile Index (API) 20E test strips (bioMerieux, Inc., USA office, Durham, NC) incubated at 37°C for 18 to 24 h. The API 20E test strips combine conventional and assimilation tests for the identification of *Enterobacteriaceae* and other non-fastidious Gram-negative rods. Putative *E. chrysanthemi* strains were characterized and compared to the results found in the Analytical Profile Index (bioMerieux, Inc.). Information from Bergey's Manual as described for the genus and as listed in Tables 5.26-5.28, pgs. 469-476 (Lelliot & Dickey 1984) was also used for comparative purposes. For positive identification, the rapid agglutination Express test from Adgen (Scotland) specific for *E. chrysanthemi* was used according to manufacturer recommendations.

Pathogenicity tests. Bacteria isolated were grown on NBY agar for 48 h. Healthy orchid plants of the genera *Phalaenopsis*, *Oncidium*, *Dendrobium*, *Miltonia* (approximately 2.5 yr old in 10 cm pots) and *Cattleya* (approximately 3 yr old in 10 cm pots), were used for the inoculation tests. To fulfill Koch's postulates, individual orchid leaves were inoculated (multiple inoculations on two leaves) by wounding with a sterile toothpick containing a single colony of *E. chrysanthemi* strains L8, L9 and L11 (Table 1). Sterile water was used for control inoculation. The inoculated plants were covered with plastic bags or placed in a dew chamber in the laboratory at 24°C with 70% RH for 48-72 h. Disease development was evaluated beginning at 2 to 3 days after inoculation by measuring the length of the water-soaked lesion that formed at the inoculation site. Lesion development was monitored for 7 days before bacteria were reisolated. Three plants were inoculated in each experiment with two replicates.

Tab.1. Sources of the strains of *Erwinia* spp. Recovered from diseased orchid plants in Hawaii

Strain	Host	Tissue source[a]	Location collected
L1	*Miltonia* hybrid	Spike, P	Hilo
L2	*Miltonia* hybrid	Leaf, P	Hilo
L3	*Miltonia* hybrid	Leaf, P	Hilo
L4	*Cattleya* hybrid	Leaf, P	Hakalau
L5	*Cattleya* hybrid	Leaf, P	Hakalau
L6	*Cattleya* hybrid	Leaf, P	Hakalau
L7	*Cattleya* hybrid	Leaf, P	Hakalau
L8	*Cattleya* hybrid	Leaf, P	Hakalau
L9	*Cattleya* hybrid	Leaf, P	Hakalau
L10	*Dendrobium* sp.	Leaf, P	Paradise Park
L11	*Oncidium* sp.	Pseudobulb, B	Pahoa
L12	*Dendrobium* sp.	Leaf, P	Pahoa
L13	*Oncidium* sp.	Leaf, P	Pahoa
L14	*Oncidium* sp.	Leaf, P	Pahoa

[a]Tissue source: P = potted plant, 5-inch round; B = potted plant, 12-inch round plastic bag.

Tab.2. Physiological tests for the identification of soft rot *Erwinias* isolated from orchids

	Bacterial strain													
Characteristic	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14
Gram stain	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidation/fermentation of glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acetoin production	+	+	-	+	+	-	+	+	+	-	+	+	+	+
Indole production	-	-	+	-	-	-	-	+	+	+	+	+	+	-
Growth at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease production	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beta-galactosidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine dihydrolase	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tryptophane deaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) positive reaction, (-) negative reaction

Tab.3. Acid production from carbohydrates used for the identification of soft rot *Erwinias* isolated from orchids

	Bacterial strain													
Substrate	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14
Sodium citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	+	+	-	-	-	-	-	-	-	-	+	+	-
Rhamnose	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+	+	-	-	-	-	-	+	+	+	+	-	+	+
Amygdalin	+	+	+	-	-	-	-	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(+) positive reaction, (-) negative reaction

Species-specific PCR. Molecular methods were used to confirm identification of the *E. chrysanthemi* strains using the primer pairs ADE1 (5'-GATCAGAAAGCCCGCAGCCAGAT-3') and ADE2 (5'-CTGTGGCCGATCAGGATGGTTTTGTCGTGC-3'), directed toward a species-specific region of the *pel*ADE genes (Nassar *et al.* 1996) or ERWFOR (5'-ACGCATGAAATCGGCCATGC-3') and CHRREV (5'-AGTGCTGCCGTACAGCACGT-3'), directed toward a species-specific region of the 16S rRNA gene (Smid *et al.* 1995). PCR for the ADE1-ADE2 primer pair had an initial denaturation of 94°C for 3 min with a subsequent 25-cycle amplification, and the PCR mixture contained 100 pmol of each primer, genomic DNA template, 200 µM of each dNTP, and 1.25 U of *Taq* DNA polymerase, in a total volume of 50-µl. PCR parameters included 30 cycles of denaturation at 94°C for 1 min followed by annealing and extension at 72°C for 2 min with a final extension at 72°C for 10 min. PCR for the ERWFOR-CHRREV primer pair had an initial denaturation of 94°C for 3 min, and the PCR mixture contained 100 pmol of each primer, genomic DNA template, 200 µM of each dNTP, and 1.25 U of *Taq* DNA polymerase, in a total volume of 50-µl. PCR parameters included annealing at 65°C for 1 min, denaturation at 94°C for 1 min, and extension at 72°C for 1 min with a final extension at 72°C for 10 min. Template DNA was prepared by picking individual bacterial colonies from agar plates using a sterile toothpick and re-suspending in 50-µl sterile distilled water. The suspension was boiled for 5 min and the cellular debris was pelleted by centrifugation. A 10-µl aliquot of the supernatant was used in 50-µl PCR reactions. All reactions used 2 mM $MgCl_2$, 200 µM dNTPs (New England Biolabs, Beverly, MA) and 1 U of *Taq* polymerase (Promega, Madison, WI). PCR reactions were performed in a MJ Scientific PTC-100 machine. A 10-µl aliquot of the reaction was analyzed by electrophoresis on a 1% agarose gel (Invitrogen Co., San Diego, CA) at 70 V for 1.5 h in Tris acetate buffer (Sambrook *et al.* 1989). Gels were stained with ethidium bromide. A 1-kb ladder (Invitrogen Co., San Diego, CA) was used as a size marker. The amplified bands were 420 and 450 bp in length, respectively.

Phenotypic characterization of copper and streptomycin resistance. Resistance to copper and streptomycin was tested for

the three strains of *E. chrysanthemi* (Table 2). Cultures were grown for 48 h on mannitol-glutamate medium (MG)(12) containing 0.025% yeast extract (MGY) at 28°C, and were subsequently streaked onto MGY plates containing cupric sulfate ($CuSO_4$)(MGYCu) (Sigma, St. Louis, MO) or streptomycin (MGYSm)(Sigma, St. Louis, MO). Minimum inhibitory concentrations (MICs) of cupric sulfate and streptomycin were determined for the *E. chrysanthemi* strains by inoculating them to 20 ml of MGY agar amended with 200 to 1000 μg/ml of $CuSO_4$ or 25 to 400 μg/ml of streptomycin. Strains that exhibited growth on MGY containing $CuSO_4$ or streptomycin equivalent to that on MGY alone after 72 h at 28°C were considered insensitive. The experiments were conducted twice with 3 replicates per experiment.

Results

Isolation and identification. Colonies were examined culturally and biochemically. On NBY, strains L8, L9 and L11 produced pale cream, irregular colonies with undulate margins. The other strains ranged in color from pale yellow to yellow. On YDC, strains L8, L9 and L11 produced pale-yellow, non-mucoid colonies and on NGM agar, colonies were blue. On MS media all strains turned orange indicating an enteric nature, and on KMB the strains did not produce a diffusible, green pigment. All 14 isolates were negative for oxidase and arginine dihydrolase (except for L1 and L2) and positive for □-galactosidase and citrate utilization (Tables 2 and 3). They fermented glucose and grew at 37°C. The isolates produced acid from utilizing glucose, mannitol, rhamnose (except L10), sucrose and arabinose. On the basis of their morphological, physiological, and biochemical characteristics, 3 isolates were identified as *E. chrysanthemi*. Results from descriptions in Bergey's Manual of Systematic Bacteriology (Lelliot & Dickey 1984) and the *E. chrysanthemi*-specific rapid agglutination Express test provided further evidence in support of the identification of the isolates. The other 11 strains could only be identified to the genus level.

Pathogenicity tests and cross-inoculation studies. Plants artificially inoculated with *E. chrysanthemi* developed symptoms similar to the commonly observed field symptoms, indicating that they are pathogenic to multiple orchid genera. The 3 *E. chrysanthemi* strains tested caused lesions around the inoculation sites with necrosis around the wound. The development of a necrotic zone or lesion of at least 2.0 mm beyond the inoculation site was considered positive for pathogenicity on the various orchid genera. On *Cattleya* hybrids, *Oncidium* hybrids, and *Phalaenopsis* sp. the water-soaked lesions appeared 1 to 2 days after inoculation (Fig. 2). Within 3 days after inoculation, lesions were very large (>9 mm diam.), dark brown to black, and water-soaked, and the soft rot expanded rapidly to resemble natural infection on almost the entire leaf (Fig. 3). On *Miltonia* hybrids at 3 days, the brown lesions continued to expand beyond 12 mm. diam. (Fig. 3). Within 6 days, all inoculated leaves on *Cattleya* hybrids, *Oncidium* hybrids, and *Phalaenopsis* sp. were completely rotten (Fig. 4a). *Dendrobium* sp. appeared to be most resistant to *E. chrysanthemi* infection. Lesions remained small (mean size 3 mm diam.) even after 7 days (Fig. 4b). Re-isolations from the artificially infected plants yielded *E. chrysanthemi*. Bacterial identification was confirmed with API 20E strips and Express tests, thus fulfilling Koch's postulates. Control plants inoculated with a sterile toothpick remained healthy (data not shown). Lesions were consistent in size and shape in repeated inoculation experiments for all *E. chrysanthemi* strains and orchid genera tested. Circular or ellipsoidal lesions appeared within 1 to 2 days and continued to expand.

Species-specific PCR. The identity of the isolated bacterial strains was also confirmed by PCR using *E. chrysanthemi*-specific primers (Nassar *et al.* 1996; Smid *et al.* 1995). An approximately 420-bp amplified fragment of the *pel*ADE genes or an approximately 450-bp amplified fragment of the 16S rRNA genes was obtained for the 3 *E. chrysanthemi* strains tested (Fig. 5). No amplified fragment was observed with the 11 other bacteria tested belonging to the *Erwinia/Pantoea* genera or the negative control (*B. gladioli*) (Fig 6). Results were similar for both primer sets; data is only shown for primer set ADE1/ADE2. Thus, the species-specific-PCR test with ADE1-ADE2 or ERWFOR-CHRREV could be used to accurately identify the *E. chrysanthemi* isolates.

Copper and streptomycin sensitivity determination. A summary of the bactericide-resistant *E. chrysanthemi* strains is presented in Table 4. Resistance to streptomycin was not present among the strains of *E. chrysanthemi* isolated from orchids in this study; however, copper resistance was. The MIC of cupric sulfate varied among Cu^r strains from 500 to 750 μg/ml, indicating a high level of resistance to the copper salt. The MIC of streptomycin was 25 μg/ml for all strains tested. The MICs remained constant for all *E. chrysanthemi* strains when bactericide-sensitivity experiments were repeated

Tab.4. Copper- and streptomycin-resistant strains of *Erwinia chrysanthemi* recovered from orchids in Hawaii

Concentration	Number of strains with growth at indicated concentration	
(μg/ml)	Cupric sulfate	Streptomycin
0	3	3
25	3	0
100	3	0
200	3	0
500	2	0
750	0	nd[z]
1000	0	nd

[z]nd = not determined

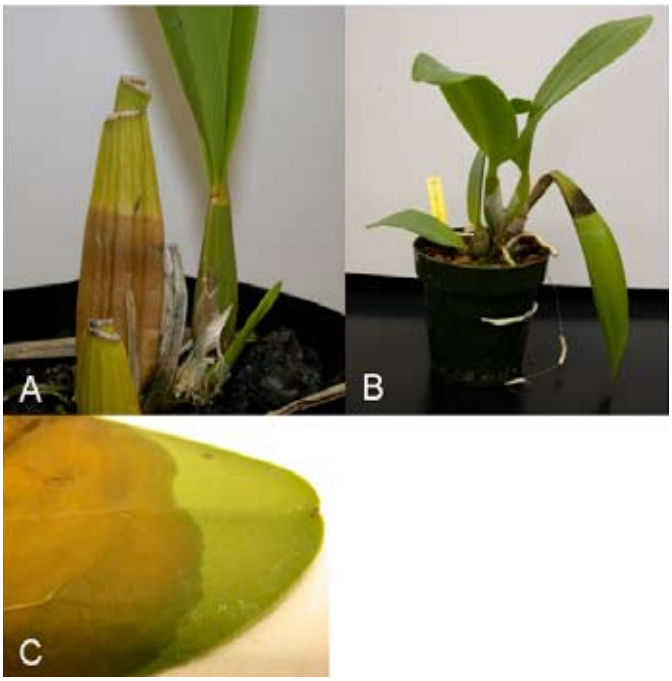

Fig. 1. Natural symptoms of bacterial leaf rot caused by *Erwinia chrysanthemi*. A, *Oncidium* sp. pseudobulb. B, *Cattleya* sp. pseudobulb/leaf junction. C, *Cattleya* sp. leaf.

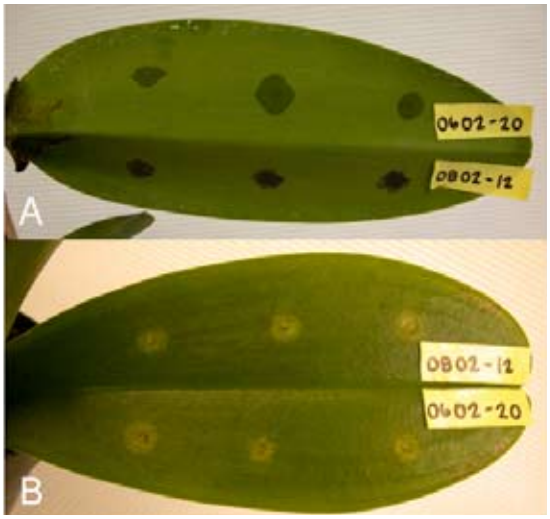

Fig. 2. Symptoms of inoculation studies of *E. chrysanthemi* on alternative hosts. A, Artificially inoculated *Cattleya* sp. leaf, front side, 1 day post inoculation. B, Artificially inoculated *Phalaenopsis* sp. leaf, front side, 1 day post inoculation.

Fig. 3. Symptoms of inoculation studies of *E. chrysanthemi* on alternative hosts. A, Artificially inoculated *Oncidium* sp. leaf, front side, 3 days post inoculation. B, Artificially inoculated *Miltonia* sp. leaf, front side, 4 days post inoculation. C, Artificially inoculated *Phalaenopsis* sp. leaf, front side, 3 days post inoculation. D, Artificially inoculated *Cattleya* sp. leaf, back side, 2 days post inoculation.

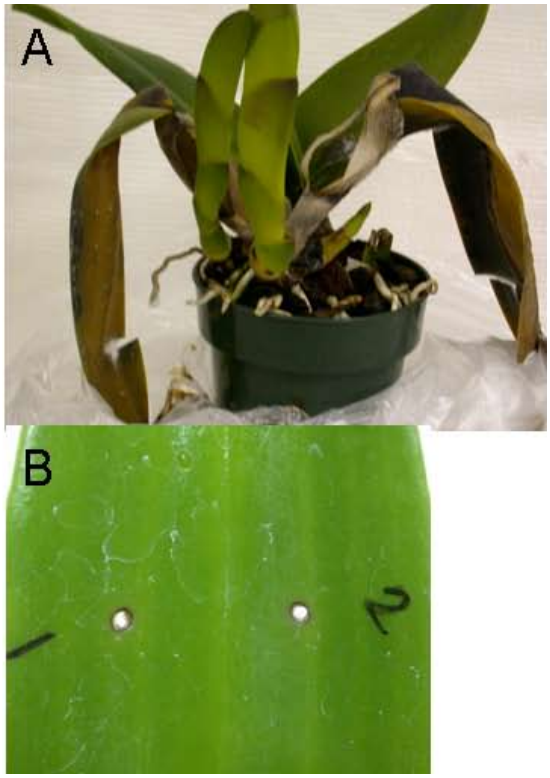

Fig. 4. Symptoms of inoculation studies of *E. chrysanthemi* on alternative hosts. A, Artificially inoculated *Cattleya* sp. 4 days post inoculation. B, Artificially inoculated *Dendrobium* sp. leaf, front side, 7 days post inoculation.

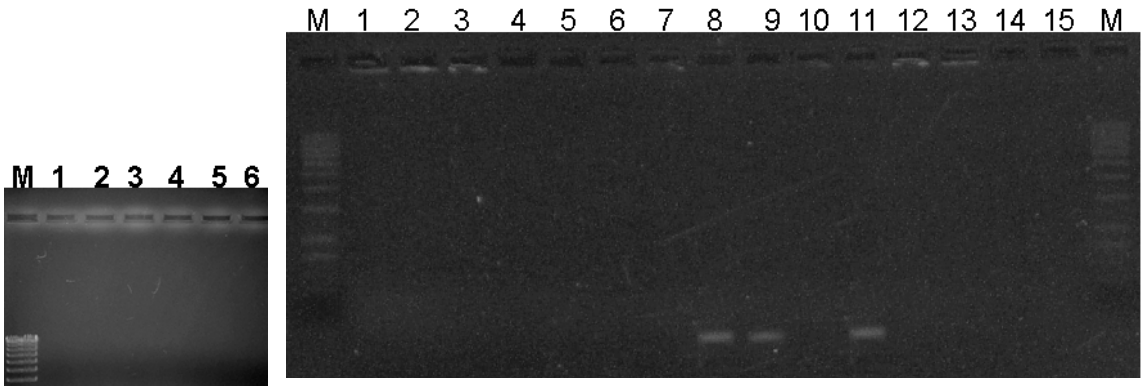

Fig. 6. PCR detection of *E. chrysanthemi* with specific primer pairs. Primer set ADE1/ADE2 was used for standard PCR. Aliquots of 10 µl from each amplified PCR mixture were separated by electophoresis on 1% agarose gel. Lane 1, strain L1; lane 2, L2; lane 3, L3; lane 4, L4; lane 5, L5; Lane 6, L6; Lane 7, L7; Lane 8, L8; Lane 9, L9; Lane 10, L10; Lane 11, L11; Lane 12, L12; Lane 13, L13; Lane 14, L14; Lane 15, *B. gladioli*; M: Invitrogen 1 kb DNA ladder.

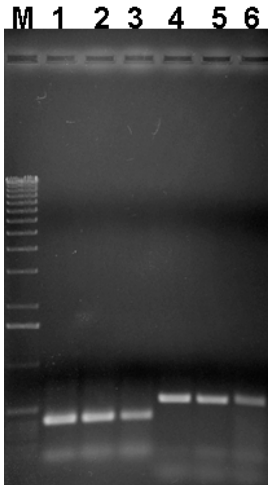

Fig. 5. Ethidium-bromide-stained 0.8% agarose gel displaying the 420 bp amplification product of the pectate lyase encoding genes (lanes 1-3) and the 450 bp amplification product of the 16S rRNA gene from *Erwinia chrysanthemi* (lanes 4-6) using species-specific PCR primers. Lane 1, strain L8; lane 2, L9; lane 3, L11; lane 4, L8; lane 5, L9; Lane 6, L11; M: Invitrogen 1 kb DNA ladder.

Discussion

In the United States, more than 130 plant diseases are reported to affect orchids. In Hawaii, orchid samples with symptoms resembling soft rots are consistently observed by farmers and brought in to the local diagnostic clinic. During the survey, small, water-soaked spots appeared on leaves of orchid plants. If not removed, these spots expanded rapidly and caused the entire leaf to collapse and rot. Symptoms appeared anywhere on the plant, with water-soaked lesions appearing on the leaves, spike or pseudobulb, similar to those observed by Lee *et al.* (1999). This study was initiated to obtain a better understanding of the typical symptoms, pathogen biology, and host-pathogen interactions of bacterial orchid diseases that will result in the development of more efficient control measures and management strategies to alleviate crop loss. Until now, *Erwinia chrysanthemi* was thought to be a major bacterial problem on orchids in Hawaii. Over a period of 18 months, *E. chrysanthemi* was detected in only a few of the diseased samples tested using traditional and molecular techniques. Strains of *E. chrysanthemi* isolated from orchids were similar to each other and to the type-strain used in the API 20E test from bioMerieux in most biochemical reactions evaluated and were similar to descriptions of the bacterium in Bergey's Manual of Systematic Bacteriology (Lelliott & Dickey 1984). The strains of *E. chrysanthemi* (L8, L9 and L11) produced similar-looking colonies on NBY, KMB, YDC, NGM and MS and were visually different from the 11 additional strains. In addition to morphology, the isolates were characterized at the molecular level by the detection of genes in this bacterium by PCR. Nassar *et al.* (1996) successfully developed a procedure for the identification of *E. chrysanthemi* using species-specific PCR primers derived from *pel*ADE, which are pectate lyase encoding genes of *E. chrysanthemi*. This primer pair was able to differentiate the 3 strains of *E. chrysanthemi* from the other bacteria identified as *Erwinia* by the API20E test. The primer pair ERWFOR and CHRREV, directed toward a species-specific region of the *E. chrysanthemi* 16S rRNA gene (Smid *et al.* 1995) also produced similar results.

E. chrysanthemi strains isolated from diseased greenhouse-grown orchids were inoculated into healthy plants, found to reproduce the symptoms of soft rot, and were re-isolated and re-characterized as being identical to the parental strains. This confirmed, using Koch's postulates, that *E. chrysanthemi* is a pathogen of orchids. The host range of the pathogen, which was previously unknown in Hawaii, was also determined and the 3 strains produced symptoms of soft rot on five different orchid genera (*Phalaenopsis*, *Miltonia*, *Cattleya*, *Dendrobium*, and *Oncidium*). These results clearly indicate the potentially damaging nature of this organism. *E. chrysanthemi* isolate L11 produced darker brown lesions on *Cattleya* than the other two strains. This could indicate a difference in virulence of this

particular strain or the result of a more rapid tissue death. Further investigation is necessary to determine the cause of this discoloration. The *Dendrobium* sp. used in this study showed some resistance to *E. chrysanthemi* L8 and L9. As for this particular *Dendrobium* sp. (Den. Odom's Imperial Topaz Penny's Luck), symptoms resembling a hypersensitive response were seen and re-isolation of *E. chrysanthemi* from these samples was difficult.

Additional factors may contribute to the prevalence and onset of bacterial diseases in Hawaii, including insect and snail vectors, warm and humid environments, low light levels, contaminated water, media and supplies, and contact between plants (Uchida 1995). The primary mechanisms of bacteria movement are via splashing water, contaminated tools, soil and insects (Hoppe & Kelman 1969; Uchida 1995). Once in contact with a susceptible plant, the bacteria enter through small wounds caused by plant handling or by insects. Irrigation and storm water runoff (using recycled water) are also potential sources of introduction. In a study by Norman *et al.* (2003), soft rot *Erwinia* populations in water resulted in the rapid spread and extensive crop loss to tropical foliage crops such as *Dieffenbachia* spp., *Syngonium* spp., and *Aglaonema* spp.

Chemicals are generally unsatisfactory for controlling bacterial diseases; therefore, the industry relies on early detection followed by sanitation for disease management (Chase 1987). There are no compounds that can rid infected plants of *Erwinia*; however, fungicides containing copper can help slow the spread of the pathogen (Norman *et al.* 2003). Copper is sometimes used for disease control on orchids, but this practice can contribute to the resistance found in phytopathogenic bacteria. The results of this study indicate that copper resistance exists among strains of *E. chrysanthemi* causing rot on orchids in Hawaii. The MIC was designated as the concentration of copper sulfate which prevented growth of the culture after a 72-h incubation at 28°C. Levels of resistance were in the normal range commonly recommended for use in disease control. The background levels of copper resistance found in *Erwinia* species is similar to what we observed for the *E. chrysanthemi* strains in this study indicating that copper would not be recommended as an efficient method of control. The efficient control of phytopathogens affecting economically important orchid crop species represents one of the major challenges in the floriculture industry (Chan *et al.* 2005).

This report is the first description of the characteristics of *E. chrysanthemi* isolated from orchids in Hawaii. The results of this study indicate that *E. chrysanthemi*, while not a prevalent pathogen or the only species causing soft rot on orchids in Hawaii, still has the potential to cause serious damage if not monitored and controlled. Because of limited available bactericides and the presence of genetic elements which confer copper resistance, prevention of plant disease caused by *E. chrysanthemi* using consistent practices such as sanitation and rouging, is a much better option than chemical control once the problem has presented itself.

The data presented described the phenotypic and morphological characteristics of the soft-rot *Erwinia* species found in Hawaii. Copper resistance of *E. chrysanthemi* in Hawaii is reported for the first time. A major reason for the failures in controlling the bacterial disease in many plant production areas may be the inadvertent selection of copper-resistant strains from continuous application of copper-based bactericides by growers (Bender *et al.* 1990). A better understanding of the etiology of soft rot disease on orchids will further the knowledge of the mechanisms of pathogenicity of *Erwinia* and may lead to more appropriate disease control strategies.

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Pacific Agriculture and Natural Resources

Characterization of *Pestalotiopsis* spp. Associated with Rambutan Fruit Disease in Hawaii

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Abstract: Rambutan (*Nephelium lappaceum*) is one of a group of tropical fruits in Hawaii that has the potential to expand in the niche market of exotic fruits. However, the primary bottleneck in pre-harvest and post-harvest quality is the occurrence of fungal diseases of the fruit. A survey of rambutan disease was conducted at the USDA/ARS Tropical Plant Genetic Resource Management Unit in Hilo, Hawaii and surrounding local farms. Fruit (rind and spinterns) were affected by brown to black lesions that progressed to blackening and drying of the fruit that frequently became totally mummified. One fungal genera isolated from symptomatic fruit was *Pestalotiopsis*. Sixteen isolates were collected from 117 infected fruit samples (% disease incidence = 13.7%). Morphology, colony characteristics, and pathogenicity of the isolates were examined. Molecular methods were used to identify the *Pestalotiopsis* taxa on rambutan in Hawaii. This is the first in-depth report of traditional and molecular methods being used for identification and characterization of fungal pathogens of rambutan.

Keyword: *Nephelium lappaceum*

Introduction

Rambutan (*Nephelium lappaceum* Linn.) is native to Malaysia and Indonesia and is commonly cultivated throughout Southeast Asia (Morton 1987). Seeds were imported into the United States from Java in 1906 (Morton 1987). Rambutan flourishes from sea-level to 600 m in tropical, humid regions having well-distributed rainfall (Morton 1987). Depending on the location, rambutan can produce up to two crops a year (Laksmi *et al.* 1987).

Hawaii tropical specialty fruit production and sales (fresh and processed) for 2005 totaled 1.5 million pounds, up 41 percent from the previous year, according to the Hawaii Agricultural Statistics (HAS) (Anonymous 2006). More acreage and maturing plantings contributed toward the boost in output; compared with 2004, an increase in the value of sales was reported for rambutan (Anonymous 2006). The 2005 farm value of tropical specialty fruits was estimated at $2.7 million, 28 percent higher than the 2004 value; rambutan was the top contributor to the 2005 value (Anonymous 2006). According to Hawaii Agricultural Statistics Service (HASS) rambutan production utilized for fresh sales and processing totaled 400,000 pounds in 2005, with statewide farm values totaling $1,005,000 (Anonymous 2006). In 2005 the number of rambutan trees totaled 11,200 of which only 71% were bearing (NAAS 2005).

Long distance transportation of rambutan has been impeded by its very short shelf life due to fruit diseases which may begin in the field or postharvest on fruit damaged during harvesting, packaging or transportation (Farungsang *et al.* 1994). Fruit rot can be caused by various fungal pathogens (Chayasombat & Sangchote 1983; Visarathanonth 1988). Farungsang *et al.* (1991) have isolated *Pestalotiopsis* sp. as a causal agent of postharvest rots in Thailand. *Pestalotiopsis* sp. has also been isolated from fruit stored at 2.5-12.5°C in Australia (Johnson *et al.* 1989).

In Hawaii, rambutan is found throughout the state and disease symptoms on leaves and fruits are consistently observed. Thus, rambutan is an excellent model for studying disease to better understand the interaction of susceptible host and pathogen, and the effects of environment on disease development. The objectives of this study were to: (i) identify the causal agent(s) of commonly observed symptoms of fruit disease on rambutan; (ii) compare *Pestalotiopsis* isolates by studying pathogenicity, morphological, cultural and molecular characteristics; (iii) examine the effect of environmental factors (e.g. temperature) on growth of the pathogen; and (iv) establish pathogenicity and potential control methods of *Pestalotiopsis* spp. on rambutan.

Materials and Methods

Field observations and symptoms. Plants were examined in the USDA-ARS germplasm repository located at the

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University of Hawaii, Waiakea Agricultural Experiment Station, Hilo, Hawaii and in surrounding areas. The primary site is 9.7 km south of Hilo, with an elevation ranging from 175 to 227 m. Maximum and minimum mean temperatures are 28°C and 16°C, respectively. Annual rainfall averages 4,445 mm and is most abundant during October to February. The soil consists of an extremely stony Papai muck with organic soils formed over mostly fragmental a'a lava. Digital photographs of leaf and fruit symptoms were recorded using a Nikon Coolpix 995 model digital camera.

Collection of isolates. Naturally infected fruit of 9 rambutan cultivars were collected from fields in Hilo and the surrounding areas. All fungi were grown on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) at 24°C under continuous illumination, and single spore cultures were derived from each isolate and stored at room temperature (20°C) in sterile distilled water (SDW). Cultures were maintained on PDA.

Initial identification and cultural characteristics. The 16 *Pestalotiopsis* isolates derived from single spores were grown on PDA. Cultures were incubated at 24°C in continuous light and cultural morphology was examined after seven to thirty days. Colony color was defined according to Raynor (1970). Spore size was determined by measuring the length and width of 32 to 40 arbitrarily selected conidia from a conidial suspension of each isolate that was prepared in SDW. The isolates were initially identified by comparing morphological and cultural characteristics (i.e. size of the conidia, color and length of median cells, length and number of apical appendages, and length of basal appendage) to those described in Guba's monograph of Monochaetia and Pestalotia (1961).

Temperature effects on mycelial growth. Five replicate, 10 cm diameter Petri dishes containing PDA (25 ml) were inoculated centrally with an agar disc (5 mm diameter) of a subset of isolates of *Pestalotiopsis* (2 strains), cut from the edge of an actively growing colony on PDA. The isolates were selected based on morphological characters. The effect of temperature on colony diameter was determined after 4, and 7 days at 10, 15, 22, 26, 30, and 35°C. All temperature experiments were conducted twice.

Pathogenicity tests. Pathogenicity tests consisted of inoculations of rambutan fruit removed from the tree. Before inoculation, fruit were surface disinfested by immersion in 10% bleach solution (0.5% sodium hypochlorite) for 2 min, rinsed in SDW, and then air dried in a laminar flow hood. Fruit were placed in plastic chambers containing moistened paper towels. Fruit (*Nephelium lappaceum* cv. R134) were wounded with a sterile cork borer, and inoculated with mycelial discs (3 mm diameter) of *Pestalotiopsis* isolates 2, 3, 4 and 5 grown for 5 days at 28°C. Controls were inoculated with PDA discs only. Fruit were observed for 7 days. To fulfill Koch's postulates, diseased tissue was placed on water agar and PDA and observed for colonies typical of the pathogen. Experiments were repeated at least twice with similar results.

Molecular characterization. Pestalotiopsis isolates were identified using molecular techniques. Total genomic DNA was extracted following the protocol found in Keith *et al.* (2006). The PCR reaction to amplify the ITS1/5.8 S rRNA/ITS4 region was largely based on Caetano-Anolles *et al.* (2001) and White *et al.* (1990). The PCR primers had the following base sequences: ITS1: TCCGTAGGTGAACCTGCGG, and ITS4: TCCTCCGCTTATTGATATGC. Primers were synthesized by Integrated DNA Technology (IA). PCR amplification was performed in a 50 □l reaction mixture containing 4 µl template DNA, 3 µM of each primer, 1X PCR buffer (supplied with *Taq* polymerase), 25 mM $MgCl_2$, 2 mM dNTPs, and 0.5 U *Taq* polymerase. After an initial hot start (95°C for 5 min), 35 PCR cycles were performed on a MJ Scientific PTC-100 thermocycler using the following conditions: a denaturation step of 95°C for 30 sec followed by annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension of 72°C for 6 min. Analysis was carried out by adding 3 µl loading buffer to 10 µl PCR products which were loaded onto a 2% agarose gel (Invitrogen Co., San Diego, CA) with electrophoresis at 5 Vcm^{-1} and visualized under UV after staining the gel with ethidium bromide. A 1-kb ladder (Invitrogen Co., San Diego, CA) was used as a size marker. PCR products were cloned with the TA cloning kit (Invitrogen Co., San Diego, CA) according to the manufacturer's recommendations. Plasmid DNA for sequencing was prepared with the Qiagen plasmid miniprep kit according to the recommendations of the manufacturer (Qiagen, Inc., Chatsworth, CA). DNA sequencing was performed at MWG Biotech Inc. (High Point, NC). Sequence data was aligned and similarity searches of the GenBank database were determined using the National Center for Biotechnology Information Blast Network Server (Altschul *et al.* 1990). A multiple sequence alignment was constructed using CLUSTALX (Thompson *et al.* 1997) and BOXSHADE 3.21 (Hoffman & Baron 1998). Phylogenetic analysis was constructed by the Neighbor-Joining method (Saitou & Nei 1987) derived from the ITS1/5.8S rDNA/ITS2 sequences.

Determination of sensitivity of Pestalotiopsis isolates to fungicides. The capacities of different fungicides (F) to inhibit fungal growth were determined using amended media. For all studies, commercial formulations of the products were used: azoxystrobin, Abound 2.08 EC (Syngenta Crop Protection, Greensboro, NC); clarified hydrophobic extract of Neem Oil, Trilogy (Certis USA, Columbia, MO); and QST 713 strain of *Bacillus subtilis*, Serenade (Agraquest, Inc., Davis, CA). These fungicides were diluted in sterile water and added to molten PDA to obtain final concentrations.

Concentrations used were those recommended by the manufacturer. Sensitivities of *Pestalotiopsis* isolates to Abound, Trilogy and Serenade were tested using radial growth (RG) assays. Each isolate was cultured on PDA at 24°C for 7 days. Five-millimeter-diameter mycelial plugs were cut from the margins of colonies and transferred onto 3 PDA dishes amended with each concentration of fungicide. Plates were maintained at 24°C with constant lighting. Colony diameter (RG) was measured across two axes, averaged, and the diameter of the mycelial plug subtracted from the average after 4 and 7 days. Percent growth inhibition in response to each fungicide at varying concentrations was calculated with the control plate as a reference such that percent inhibition is [1 - (RG of the experimental/RG of the control)] * 100. Fungicide resistance was classified as follows: A = highly resistant (RG on PDA+F plates was more than 2/3 of that on PDA plates); B = moderately resistant (RG on PDA+F plates was more than 1/3 of that on PDA plates); C = sensitive (RG on PDA+F plates was less than 1/3 of that on PDA plates). Each experiment was conducted twice.

Results

Field observations and symptoms. A *Pestalotiopsis* disease survey on rambutan was conducted at the Waiakea Agricultural Experiment Station and at a local farm. Disease symptoms were visible on leaves during non-fruiting seasons and on the exocarp of mature and immature fruits (pinhead size) which progressed as fruits got larger. Typical symptoms included tiny, brown to black spots on the spinterns or on the surface of the fruit (Fig. 1A, B). The spots darkened in color and became crusty looking in appearance. Over time, the tiny spots expanded to discrete, circular, dark brown to black, spots which dry out and eventually crack (Fig. 1C). Symptoms on leaves began as small dark brown spots that expanded to become grey/light brown circles surrounded by a dark brown border (Fig. 1D).

Isolation and identification. Sixteen isolates of *Pestalotiopsis* were collected from a range of known rambutan accessions from the Hilo field and surrounding areas (Table 1). Isolates were obtained from immature and mature fruit with disease symptoms. The isolates were named and given a corresponding number. The fungi were maintained on PDA and stored in SDW for additional studies. All isolates were identified as *Pestalotiopsis* sp. on the basis of conidial morphology. All had 5-celled conidia, of which apical and basal cells were hyaline, and the three median cells ranged from light brown to varying shades of olive green. Measurements of conidia revealed few differences in size between isolates. Seven *Pestalotiopsis* strains were compared (Table 3). Conidia varied from 20.3 $\pm$ 0.1 (standard error) to 24.5 $\pm$ 0.1 □m mean length and 5.8 $\pm$ 0.1 to 7.0 $\pm$ 0.1 □m mean width. Basal appendages were hyaline, straight or slightly curved and varied from 3.8 $\pm$ 0.1 to 8.5 $\pm$ 0.1 □m mean length. Numbers of apical appendages ranged from two to four, with three being the most common. The appendages showed the most size variation with mean lengths that ranged from 13.5 $\pm$ 0.2 to 26.5 $\pm$ 0.3 □m. The isolated fungi were tentatively identified as *P. virgatula* (Kleb.) Stey. based on morphological and cultural characteristics using Guba's monograph (1961).

Fig. 1. Most common symptoms of fruit disease of rambutan seen in the field. A, Immature fruit with small, brown, lesions on spinterns. B, Discrete dark brown lesions on fruit surface. C, Dark brown to black, coalescing lesions giving rise to dried and cracked appearance. D, Symptoms on mature fruit and leaves.

Tab.1. Isolates of *Pestalotiopsis* used in this study, their hosts, and sample origin

Isolate	Name	Cultivar	Plant part
1	12-2	Jitlee	Mature rambutan fruit
2	15-1	R167	Mature rambutan fruit
3	15-2	R167	Mature rambutan fruit
4	15-2 R12T12	R167	Mature rambutan fruit
5	15-5 R12T12	R167	Mature rambutan fruit
6	20-1	Daun Hijau	Mature spintern
7	BJ-01-A	Binjai	Mature spintern
8	BJ-01-C	Binjai	Immature rambutan fruit
9	BJ-01-D	Binjai	Immature spintern
10	BJ-02-A	Binjai	Immature spintern
11	BJ-02-E	Binjai	Immature spintern
12	JL-01-A	Jitlee	Immature rambutan fruit
13	BJ-0108-3C	Binjai	Mature rambutan fruit
14	JL-0108-1C	Jitlee	Mature rambutan fruit
15	JL-0108-3B	Jitlee	Immature mummified fruit
16	JL-0108-3C	Jitlee	Mature spintern

Cultural characteristics. On PDA plates, most of the isolates first developed grayish to white zonate colonies that later developed color and small, acervular conidiomata. The colors got darker as fungal age increased. A representative subsample of the isolates of *Pestalotiopsis* (10 strains) could be separated into 3 main groups based on their color on PDA. The first group, buff (front) and pale luteous (reverse), contained rambutan isolate 2. The second group, pale buff (front) and pale saffron (reverse), contained rambutan isolates 9 and 14. While isolates 10 and 16 also fell into this category, these fungal isolates contained greenish, glaucous patches scattered on their surface. The third group contained isolates 3, 4, 5, 8 and 15 which were pale buff (front) and pale luteous (reverse). Acervuli were black for all isolates examined (Table 2). However, production varied from few (isolates 9 and 14), to moderate (concentrated in the center of the PDA plate) (isolates 2, 3, 4, 5 and 10), to abundant (throughout the entire PDA plate) (isolates 8, 15 and 16) (Table 2).

Tab.2. Colony morphology in culture[a] of isolates of *Pestalotiopsis* and BLASTN results of the ITS and rDNA sequences

	Name	Front view	Reverse view	Acervuli	BLAST search ID
2	15-1	Buff	Pale luteous	Black; moderate	*Pestalotiopsis* sp. TA-59
3	15-2	Pale buff	Pale luteous	Black, moderate	*Pestalotiopsis virgatula*
4	15-2 R12T12	Pale buff	Pale luteous	Black, moderate	*Pestalotiopsis* sp. LK23
5	15-5 R12T12	Pale buff	Pale luteous	Black, moderate	*Pestalotiopsis virgatula*
8	BJ-01-C	Pale buff	Pale luteous	Black; abundant	nd[b]
9	BJ-01-D	Pale buff	Pale saffron	Black; few	nd
10	BJ-02-A	Pale buff w/greenish glaucous	Pale saffron	Black, moderate	nd
14	JL-0108-1C	Pale buff	Pale saffron	Black; few	nd
15	JL-0108-3B	Pale buff	Pale luteous	Black; abundant	nd
16	JL-0108-3C	Pale buff w/greenish glaucous	Pale saffron	Black; abundant	nd

[a] Fungi were grown on PDA with continuous light for 7-30 days.
[b] nd = not determined

Tab.3. Characteristics of conidia of *Pestalotiopsis* isolates

Isolate	Conidia length (□ m)	Conidia width (□ m)	Apical Appendage length (□ m)	Basal appendage length (□ m)	No. of apical appendages (range)
3	20.3 ± 0.1[a]	6.8 ± 0.1	16.8 ± 0.2	3.8 ± 0.1	2 - 3
5	23.5 ± 0.1	5.8 ± 0.1	26.5 ± 0.3	7.8 ± 0.1	2 - 4
8	24.3 ± 0.2	6.0 ± 0.1	14.5 ± 0.3	2.5 ± 0.1	2 - 3
9	22.0 ± 0.1	7.0 ± 0.1	13.5 ± 0.2	6.5 ± 0.1	2 - 3
10	24.5 ± 0.1	6.5 ± 0.1	15.0 ± 0.2	6.0 ± 0.1	2 - 3
15	24.0 ± 0.1	6.0 ± 0.1	14.5 ± 0.2	5.3 ± 0.1	2 - 3
16	22.3 ± 0.1	6.8 ± 0.1	21.0 ± 0.3	8.5 ± 0.1	2 - 3

[a] Each value is the mean ± SE from measurements of 32-40 conidia

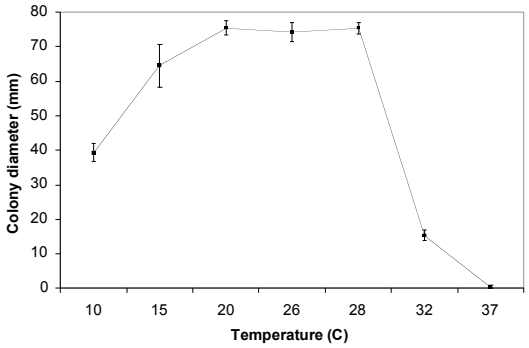

Fig. 2. Influence of temperature on mycelial growth of *Pestalotiopsis* isolated from diseased Hawaiian rambutan fruit. Growth after seven days on PDA. Each bar represents mean ± SEM (n = 2).

Temperature effects. Temperature had a significant affect on the colony diameter of the 2 isolates of *Pestalotiopsis* examined (Fig. 2). When grown on PDA, both isolates grew at a temperature range of 10° to 35°C, with optimum growth at 22°C to 28°C (Fig. 2). Within 7 days the majority of the diameter of mycelial growth of the strains measured more than 70 mm. When temperature experiments were repeated, colony diameters were virtually identical for all *Pestalotiopsis* strains tested.

Pathogenicity tests. To confirm pathogenicity and to determine if wounding was necessary for infection, mycelial plugs containing conidia were used as inoculum. Mature rambutan fruit (cv. R134) were inoculated in the laboratory with four *Pestalotiopsis* isolates (2, 3, 4 and 5) and maintained in a moist chamber for 7 to 10 days. Dark brown to black lesions resembling symptoms that occurred in the field were observed surrounding the inoculation sites beginning around day 5 (Fig. 3A, B). A wound appeared necessary for rapid penetration and severe infection by *Pestalotiopsis*. Wounded and inoculated fruit exhibited extensive necrosis. Fungal mycelia could be observed growing on the lesions. Symptoms were not observed on non-wounded fruit (Fig. 3A, B). Symptoms were not observed on control fruit inoculated with agar media (data not shown). The fungi were reisolated from the lesions of the diseased fruit and were identical to the original isolates.

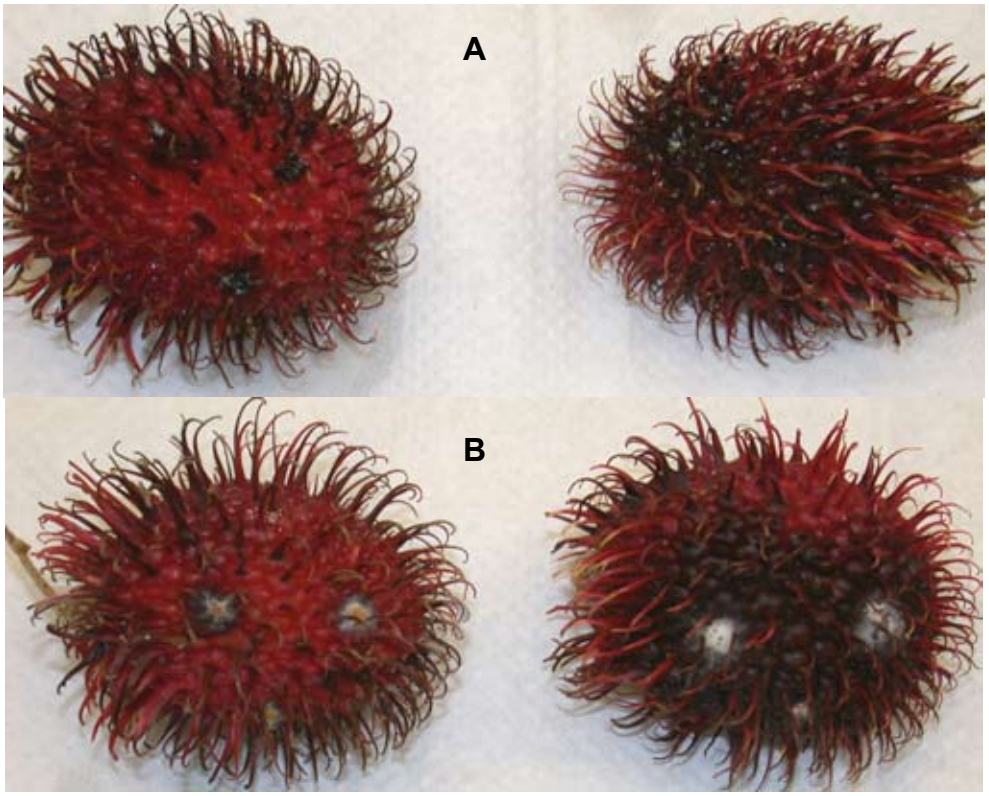

Fig.3. Symptoms of fruit disease on rambutan A, fruit cv. R134, 7 days post infection by *Pestalotiopsis* isolate 3 with wounding (right) vs. nonwounding (left). B, fruit cv. R134, 7 days post infection by *Pestalotiopsis* isolate 5 with wounding (right) vs. nonwounding (left).

Tab.4. Average percent growth inhibition by *in vitro* treatment with fungicides and resistance of *Pestalotiopsis* isolates obtained from rambutan collected in Hawaii

	Abound[a]			Trilogy[b]			Serenade[c]			
	4	6.2	15.4	1/3x	1x	3x	0.25	0.5	1	2
Isolate										
3	53 ± 1.5[d] (B)[e]	56 ± 0.9 (B)	56 ± 0.4 (B)	3 ± 1.5 (A)	28 ± 0.5 (A)	41 ± 0.6 (B)	100 ± 0.1 (C)	100 ± 0.1 (C)	100 ± 0.1 (C)	100 ± 0.1 (C)
5	47 ± 0.2 (B)	46 ± 0.7 (B)	48 ± 1.1 (B)	2 ± 1.1 (A)	24 ± 1.0 (A)	46 ± 0.6 (B)	100 ± 0.1 (C)	100 ± 0.1 (C)	100 ± 0.1 (C)	100 ± 0.1 (C)

[a] oz/gal
[b] x = 1% solution
[c] oz/100 gal
[d] % growth inhibition ± SEM
[e] Resistance level (A = highly resistant; B = moderately resistant; C = sensitive)

Molecular characterization. DNA from 4 isolates from rambutan was amplified when the PCR reaction was performed using primers ITS1 and ITS4. The corresponding PCR region amplified was the ITS rDNA sequence of region 1 and 2, which also included the 5.8S rDNA gene. The PCR product was approximately 548 to 550 bp for all isolates (data not shown). High-quality sequences of approximately 550 nucleotides of each isolate were used for analysis. The 5.8S rDNA was more highly conserved among *Pestalotiopsis* spp. Each PCR product sequence was compared by BLAST search (Altschul *et al.* 1990) and the isolates were confirmed to the species level (Table 2). Both DNA strands were sequenced for verification. Sequences of the ITS1/5.8S rDNA/ITS2 region from 4 isolates of *Pestalotiopsis* were deposited into the GenBank database with accession numbers EU047942 through EU047945. A phylogenetic tree was produced from the analysis of the aligned sequences of the ITS1/5.8S rDNA/ITS2 generated in this study (Fig. 4). The tree shows the genetic similarity of the *Pestalotiopsis* isolates.

In vitro fungicide sensitivity. Growth inhibition assays were used to determine the fungicide sensitivity of isolates within our collection. The phenotypic difference of sensitivities to azoxystrobin, clarified hydrophobic extract of Neem Oil and *B. subtilis* strain QST 713 are shown in Table 4. *Pestalotiopsis* isolates were more sensitive to Serenade and Abound than Trilogy. The *Pestalotiopsis* isolates displayed 100% growth inhibition in the presence of 0.25 to 2 oz/100 gal *B. subtilis* strain QST 713 (Serenade). In contrast, the *Pestalotiopsis* isolates tested showed <28% growth inhibition at the 1x concentration of clarified hydrophobic extract of Neem Oil. For azoxystrobin, growth of *Pestalotiopsis* isolates was inhibited between 46 and 56% for all concentrations tested. Overall, the fungal isolates obtained from rambutan showed high and moderate resistance to Trilogy depending on concentration, and moderate resistance to Abound. None showed resistance to Serenade at any level tested.

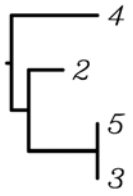

Fig.4. Phylogenetic tree of *Pestalotiopsis* isolates based on the ITS and 5.8S rDNA gene. The tree was produced using the neighbor-joining algorithm. A bootstrap analysis was performed with 100 repetitions. Scale bar indicates estimated 1.4% sequence divergence.

Discussion

Currently there is very little information known about the presence, prevalence, and etiology of fruit diseases of rambutan in Hawaii. Our disease survey revealed similar symptoms on the leaves and fruit of over 50 rambutan accessions, regardless of cultivar or location. *Pestalotiopsis* was consistently isolated and identified from diseased samples. The genus *Pestalotiopsis* Steyaert is a heterogeneous group of coelomycetous fungi consisting of 205 described species that are differentiated primarily on conidial characteristics such as size, septation, pigmentation, and presence or absence of appendages (CABI Bioscience database 2001; Nag Rag 1993; Steyaert 1953; Sutton 1980). *Pestalotiopsis* spp. were isolated from lesions on fruit at all levels of maturity and were also the causal agents of abundant lesions on leaves. According to Visarathanonth & Ilag (1987), factors that favor fungal infection of rambutan include: inadequate field spraying to reduce latent infection and surface contamination, injuries which occur during harvesting, and high humidity and temperature during packaging,

storage and transportation. Most postharvest problems of rambutan are caused by fungi which infect the fruit in the field. In studies by Farungsang *et al.* (1994), wounded rambutan fruits were infected by *Pestalotiopsis* isolates. *Pestalotiopsis* could also cause moderate infection on five kinds of tropical fruits, Banana cv. Hom-Thong, Banana cv. Nam-Wah, guava, papaya and rose apple (Farungsang *et al.* 1994). In a previous study, *Pestalotiopsis* spp. were commonly isolated from guava, waiawi and tea that were in close proximity to rambutan fields and, thus, could serve as sources of inoculum (Keith *et al.* 2006). Taguchi *et al.* (2001) provided evidence through inoculation experiments and host range studies that isolates of *Pestalotiopsis* spp. are generally not host-specific. The cross-infection potential of these fungi warrants attention.

While the majority of the *Pestalotiopsis* isolates from rambutan in our study looked similar, morphological and cultural differences did exist, and species could not be grouped based solely on colony or conidia morphology. On PDA, cultures were typically various shades of luteous and buff with pink or peach hues, which got darker as the age of the fungal culture increased. The majority of the *Pestalotiopsis* isolates produced acervuli in culture, but the amounts tended to vary. However, acervuli formed under all temperature conditions tested. Conidia were five-celled with the apical and basal cells hyaline for all isolates. The three median cells were typically olivaceous to dark brown or light brown. Temperature affected mycelial growth, with optimum growth occurring around 26°C; however, the *Pestalotiopsis* isolates were capable of growing at a wide range of temperatures (Fig. 2). Although the *in vitro* studies may not directly simulate the conditions of the natural environment, the results provide an insight to the likely behavior and growth of the pathogen in nature.

Koch's postulates were confirmed and we found that wounding prior to inoculation was necessary for disease development. According to Visarathanonth & Ilag (1987), wounding was necessary for rapid penetration and severe infection of rambutan and wounding explained why mechanically injured fruit were prone to fruit rot. Results from tests by Hopkins & McQuilken (2000) with *P. sydowiana* showed that damage to the foliage of unrooted cuttings was necessary prior to inoculation with a spore suspension of the pathogen to induce pathogenicity. Studies with other species of *Pestalotiopsis* have also found that nonwounded samples remained symptomless (Hopkins & McQuilken 2000). Consequently, growers may be able to minimize infection by avoiding physical plant damage and by growing plants to avoid stress (Hopkins & McQuilken 2000).

Cultural and molecular characterization techniques were used to compare the genetic relationship of our *Pestalotiopsis* isolates. While the isolates could be placed into groups according to color, conidial morphology, or molecular analysis of the ITS regions, it was difficult to maintain the groups when similarities were based on all of these characters simultaneously. In other words, traditional morphology-based classification schemes did not always agree with phylogenetic analysis. Even though 3 color combinations on PDA were identified, intensities of the colors varied which is why it is difficult to use them as a reliable character for taxonomic classification at the species level. However, pigmented median cells of conidia seemed to form a close relationship and appears useful for taxonomic purposes. Results of studies by Jeewon *et al.* (2003) indicated that pigmentation of median cells of conidia was a sound diagnostic character for species differentiation. A close relationship among pigmented median cells has been suggested by numerous taxonomists (Guba 1961; Sutton 1980). The mean lengths of apical and basal appendages have also been used as important taxonomic characters to delineate species; from the phylogenies generated in studies by Jeewon *et al.* (2003), it can be seen that species sharing similar apical appendage length are closely related to each other. Apical and basal appendage length of our *Pestalotiopsis* isolates showed significant variation, but conidial width seemed to agree with the molecular data (Table 3 and Fig. 4). According to Jeewon *et al.* (2003) molecular data does not tend to reveal a close relationship among species with similar conidial forms. Our results tended to support the findings of Jeewon *et al.* (2003) when overall conidial form was analyzed. This information supports the complex nature of the genus and the difficulty in classifying it at the species level.

In certain parts of the world, changes in the production methods have resulted in increasing problems with disease (Hopkins & McQuilken 2000). After known major pathogens were brought under control by improved management techniques and the use of effective but highly specific fungicides, plants became more vulnerable to attack by less competitive pathogens which had previously been considered weak or secondary (Hopkins & McQuilken 2000). *Pestalotiopsis* spp. were formerly regarded as weak, opportunistic pathogens which caused little damage (Coyier & Roane 1987; Pirone 1978); however, in the past 10 years reports of these pathogens causing widespread damage to a number of plant species including persimmon, kiwifruit, mango, blueberry, loquat, azalea, heliconia and maize (Blanco *et al.* 2008; Karakaya 2001; Ko *et al.* 2007; Luan *et al.* 2008; Perello & Larran 1999; Rivera & Wright 2000; Serra & Coelho 2007; Tagne & Mathur 2001) are increasing. This phenomenon has the potential to occur in many economically important crops because of the widespread nature of *Pestalotiopsis*.

According to Visarathanonth & Ilag (1987), proper management practices, such as a program of field spraying, postharvest fungicide applications and careful handling practices, can help to minimize the development of fruit rots in rambutan. Spraying with the appropriate fungicides must begin at flower set and continue until harvest (Visarathanonth & Ilag 1987). Farungsang *et al.* (1994) found that preharvest chemical application could slightly reduce postharvest disease incidence, with Carbendazim being the most effective fungicide. In a similar study, populations of fungi obtained from diseased rambutan fruits from the orchards with continuous fungicide application were less than those from the orchards where chemical was seldom applied; however, higher percentages of chemical resistance of isolates were obtained from the continuously applied orchards than from the others, which shows that continuous application of fungicides can enhance fungal pathogen development of resistance against chemical fungicides (Farungsang & Farungsang 1992; Griffee 1973; Ogawa *et al.* 1983). We investigated the relative effectiveness of the Abound, Trilogy and Serenade against the *Pestalotiopsis* isolates found on rambutan. In our *in vitro* studies, the fungicides Abound and Serenade showed promising results for the control of *Pestalotiopsis*. Results indicate that Serenade shows the most potential for control in the field. Field application studies will determine if these fungicides currently registered for use on rambutan in Hawaii can help to control fruit rot caused by *Pestalotiopsis*.

This study helped to identify the main species and widespread nature of *Pestalotiopsis* affecting rambutan in Hawaii (*P. virgatula* (Thuem.) Stey., *Pestalotiopsis* sp. TA-59 and *Pestalotiopsis* sp. LK23), as well as some of the morphological and environmental characteristics of selected isolates. To our knowledge, this is the first study to provide evidence that more than one species of *Pestalotiopsis* is capable of causing fruit rot of rambutan in Hawaii. This is also the first time molecular methods were used to identify *Pestalotiopsis* spp. on rambutan in Hawaii. Results showed that pathogen host range and host plant resistance are particularly important issues. Further studies are currently underway to determine if variation in resistance exists in available rambutan germplasm in Hawaii. With the knowledge gained from these and additional studies on disease epidemiology and the potential for cross-infection, effective and reliable control strategies can be developed. This is the first report in Hawaii on the resistance development of *Pestalotiopsis* spp. against chemical fungicides. This indicates that chemical fungicides must be used with caution. Due to its prevalence throughout the world and its broad host range, it appears that *Pestalotiopsis* is a potent plant pathogen that has gone largely unrecognized. Since many hosts in Hawaii are cultivated in close proximity, there is opportunity for spread of inoculum. The potential for *Pestalotiopsis* to cause yield losses in economically important crops warrants further study.

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Pacific Agriculture and Natural Resources

Theobroma Cacao L. (Malvaceae) Agroecology in Kauai: A Case Study

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Abstract: *Theobroma cacao* L. (cacao) is a widely cultivated tree of Neotropical origin and the source of cocoa beans and chocolate. Limited cocoa production is currently underway on the islands of Hawaii, but the factors that control *T. cacao*'s survival and fecundity outside of its native range remain poorly studied. Here we assess deficiencies in current knowledge of cacao ecology, and we establish research priorities for developing a profitable and renewable Hawaiian cacao farming program. We also present baseline data on fruit yield, herbivory, and insect community structure from a recently established organic cacao farm on the island of Kauai. Our observations indicate that non-native organisms, including the common agricultural pest, the Chinese rose beetle (*Adoretus sinicus*), may greatly affect the health and performance of Hawaiian cacao trees in both antagonistic and mutualistic ways.

Keyword: *Theobroma cacao, Adoretus sinicus*, Chinese Rose Beetle, pollination, herbivory.

Introduction

Theobroma cacao L. (cacao) is an evergreen tree native to Central and South America, where humans have utilized it for over 1,500 years (Motamayor et al. 2002). Cacao seeds are the source of cocoa and chocolate. Increasing global demand for these products has driven the cultivation of *T. cacao* beyond its native range. Cacao is now farmed throughout the humid tropics, and fuels a multibillion-dollar, international chocolate industry (Ploetz 2007).

Cacao trees are self-incompatible (Cope 1962), and within the Neotropics are known to be pollinated by small insects. Midges are thought to be the main pollinating agents of cacao, in addition to several species of ants, aphids, thrips, and unidentified bees (Glendinning 1972, Young 1983, Young 1994). Nonetheless, cacao pollination is still poorly characterized (Klein et al. 2007). Pollinator abundance is generally low on large plantations, and pollinator densities are not synchronized to peak flowering time (reviewed in Young 1983).

Cacao also interacts with a complex community of organisms that may adversely or positively affect its survival and fertility. Small insects including phorid flies, gall midges of the family Cecidomyiidae, microlepidoptera, and stingless or solitary bees visit cacao flowers (Entwistle 1972, Young 1994), and a diverse guild of ants associate with cacao throughout its cultivated range (Room 1971, Majer 1976, Jackson 1984). Some of these ants also tend scale insects (family Coccoidea) on *T. cacao*, from which they glean honeydew (Strickland 1951). There are over 1,500 known herbivores of cacao, however only a small number of these inflict serious damage (Dand 1999). The main insects that feed on cacao include members of the family Miridae that feed on sap (*Sahlebergella singularis* and *Distantiella theobroma* in West Africa, and *Heliopeltis* sp. in Southeast Asia), and the cacao moth (*Conopomorpha cramerella*), that lays its eggs in the cacao pods that then become food for the emerging larvae (Dand 1999). Insects can also cause damage indirectly, by transmitting fungal or viral infections (Dand 1999). Key pathogens of cacao include *Crinipellis perniciosa* (witches' broom disease) and *Moniliophthora roreri* (frosty pod rot or moniliasis disease, Ploetz 2007).

In addition to the 18-acre Dole Food Company plantation on Oahu Island, there exist a handful of small growers who cultivate cacao across the islands of Hawaii. However there is still no published research describing cacao cultivation in Hawaii. While Hawaii's climate is favorable for cacao production, insufficient knowledge of cacao's natural history makes it difficult to predict factors critical to its success or failure as a crop. Specifically, an improved understanding of potential pollinators and pests of cacao in Hawaii is required for successful cultivation. We hope to narrow this knowledge gap, with the ultimate goals of increasing the productivity of Hawaiian agriculture, and

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informing growers in Hawaii and elsewhere. Here we present findings from initial surveys and outline research priorities for the future.

Materials and Methods

Study location

Our study was conducted over three weeks in July and August of 2008, at Steelgrass Farm, which is currently one of only two plantations of cacao trees on Kauai Island capable of producing mature fruit. The farm is located near the town of Kapa'a, on the east side of Kauai, which experienced temperatures ranging from 12.2ºC to 32.9ºC (average = 22.7ºC) in 2008, as well as a total of 226.3 cm of rain. During the course of our study temperatures ranged from 18.0ºC to 87.6ºC (average = 24.2ºC) and humidity ranged from 97% to 37% (average = 70.6%).

Steelgrass Farm is an 8-acre, mixed-crop farm that specializes in the production of cacao, vanilla bean, and timber bamboo. The cacao plantation comprises approximately 300 trees, planted in three separate fields within the farm. For our study we used one field of 61 trees, which we individually numbered with metal tags and assigned to age classes (1-year-olds, 2-year-olds, 3-year-olds and >3-year-olds) based on farm records. All adult trees were surveyed exhaustively for pods. We recorded the number of pods on each tree as well as pod color. To assess overall leaf number we surveyed all trees on the farm.

Pollinator identification

To identify pollinators, ten flowers on each of three individual cacao trees were observed during the afternoon for 1 hour each on two days. Because many cacao pollinators are only active at night, we supplemented our direct visual surveys with passive flypaper trapping. We selected 32 trees and placed a strip of flypaper around one branch per tree, directly below individual flowers. After 72 hours we collected the traps and we identified all captured specimens to family in the lab.

Leaf damage survey

During our field observations we noticed damage to cacao leaves indicative of herbivory by Chinese rose beetles (*Adoretus sinicus*), a common agricultural pest in Hawaii (Furutani et al. 1995). *A. sinicus* presence is usually diagnosed by the lace-like patterns of host leaves that are generated by their feeding on non-structural leaf parts (Figure 1; Furutani et al. 1995). To assess leaf damage we surveyed a subset of 23 trees, which ranged in age from 2-6 years old. For small tress (<100 leaves) we directly counted each leaf. For larger trees we counted the number of leaves on a subset of branches that comprised 1/3 - 1/2 of the tree's canopy, and used this number to estimate the total number of leaves. We then randomly chose 10 leaves from each tree, and scored the total damage to each leaf on a scale of 0 - 4 (0 = 0% damage, 1 = 1-25% damage, 2 = 26-50% damage, 3 = 51-75% damage, and 4 = 76-100% damage), following Furutani et al. (1995). We then estimated damage to the entire tree by taking an average of these scores.

Beetle survey

To confirm the presence of *A. sinicus* at Steelgrass Farm, we conducted visual tree surveys. Rose beetles are nocturnal, and in Hawaii are active from approximately 30 minutes after sunset until shortly before dawn (Tsutsumi et al. 1993). We surveyed on four nights between 30 minutes after sunset and 10 pm. Four observers visually inspected the underside of each leaf using headlamps, and beetles were captured by hand using plastic vials.

Fig.1.*Theobroma cacao* leaf exhibiting lace-like pattern of damage caused by *Adoretus sinicus* herbivory at Steelgrass Farm, Kauai.

Results and Discussion

Characterizing cacao phenology

The flowering period of *T. cacao* ranged from mid-June through December, with several trees showing renewed blossoming vigor as late as mid-December. Only a small number of blossoms remained past January, when nighttime temperatures begin to drop. At the time of our survey in August, over two-thirds of the cacao trees (n = 43) possessed buds or flowers. Contrastingly, few of the trees in the plantation had produced pods (n = 18). Total pod number ranged from 0-13. On average, trees possessed 13.23 ±2.40 unopened buds and 5.15 ±1.18 open flowers. However, the number of pods was much lower, on average only 1.13 ± 0.32 pods per tree. Older trees produced more buds (linear regression slope = 5.98 ± 1.82, $p < 0.01$), flowers (linear regression slope = 3.80 ± 0.75, $p < 0.0001$), and pods (linear regression slope = 0.87 ± 0.24, $p < 0.001$), than younger ones (Figure 2). These positive relationships likely reflect the young ages of the trees at the farm, and increases in productivity beyond approximately 10 years are likely to be minimal, given that total tree biomass should stabilize (e.g. Zuidema et al. 2005).

Identifying pollinators and other associated insects

No pollinators were observed in our visual surveys. However, we trapped several small dipterans, potentially inclusive of the midges known to pollinate cacao in Costa Rica (Young 1983). Additionally, we observed African bigheaded ants (*Pheidole megacephala*) on both cacao and other plantation crops, during both day and night, especially on vanilla (*Vanilla planifolia*) inflorescences. *P. megacephala* is a well-known agricultural pest throughout the Pacific region (Wetterer 2007).

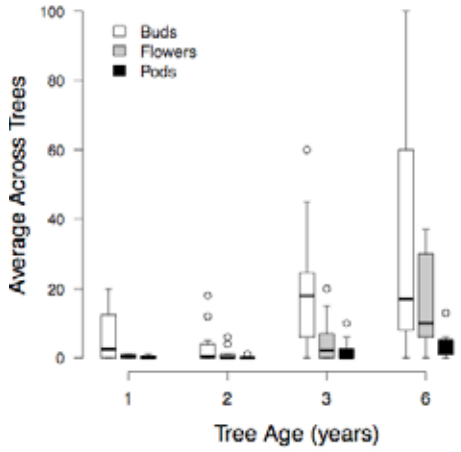

Fig.2. Reproductive output of *Theobroma cacao* trees across age classes at Steelgrass Farm, Kauai. Older tress produced more buds, flowers and pods. The number of pods is substantially lower than the number of buds and flowers, regardless of tree age.

We did not follow the progress of individual *T. cacao* infloresences, however, at the time of our survey, trees possessed far more buds and flowers than pods (Figure 2). Typically, only a small fraction of cacao flowers set fruit, which may indicate that pollen limitation constrains cacao productivity (Free 1993). Hawaiian farmers might therefore manage to increase fruit yield by enhancing pollinator abundances and activity levels. One strategy for increasing pollinator visitation rates is to provide breeding substrates for pollinating insects. Cacao's pollinating midges seem to require shaded organic substrates for development, and the addition of rotting organic material, such as banana pseudostem sections, can increase midge density (Young 1982). Visitation rates may also be affected by cacao cultivar selection, since midges are attracted to cacao by a complex suite of volatile compounds whose profile and attractiveness varies among cultivated varieties of *T. cacao* (Young and Severson 1994). At present, it remains unclear what pollinates T. cacao in Hawaii. Identifying dominant pollinator species would thus allow farmers to optimally adjust management strategies.

An alternative explanation for the low ratio of pods to flowers observed is the selective abortion of immature fruits. This is typically attributed to low nutrient supply, caused by either low photosynthetic rates or poor soil quality (Bos et al. 2006, Bos et al. 2007b). Intensive hand pollination of cacao can result in a dramatic increase in fruit abortion (Falque et al. 1996, Bos et al. 2007b, Sporn et al. 2007). Thus, while increasing visitation rates and pollinator abundances may help increase fruit set, this may not translate directly into increased pod yields, unless nutritional constraints are also addressed (e.g. Young 1983). Attempts to boost photosynthetic rates have largely relied on shade tree removal, resulting in monoculture plots, which may be more vulnerable to herbivore and disease outbreaks. Additionally, the composition of surrounding shade trees may be more important than total cover (Bos et al. 2007). Low-diversity agroecosystems may not be able to support the leguminous nitrogen-fixers needed to keep production high, or provide the diversity needed to maintain healthy pollinator communities (Beer et al. 1998, Bos et al. 2007b). Diverse shade species appear to be an important requirement for diverse bird communities as well, which in turn serve to limit agricultural pests, such as herbivorous arthropods (Van Bael et al. 2007). Increasing cacao productivity is clearly a complex process, involving multiple, potentially synergistic forces. The small scale of current cacao agroforestry on Kauai provides a unique opportunity for intensive management and study, wherein a variety of issues, ranging from pollination intensity to shade crop diversity, may be addressed at individual study locations.

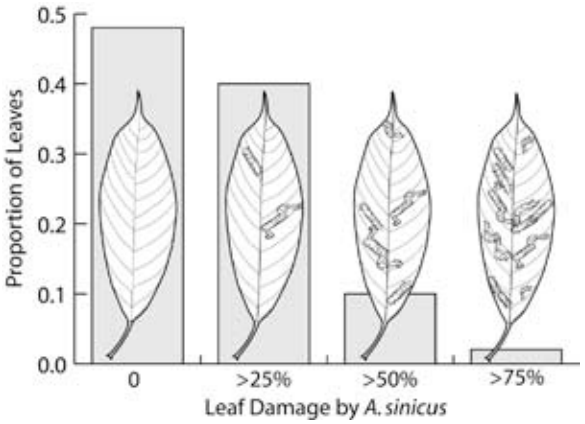

Fig.3. Distribution of damage to *Theobroma cacao* leaves caused by *Adoretus sinicus* herbivory at Steelgrass Farm, Kauai. Out of 220 leaves examined, more than half had sustained some damage.

Estimating damage caused by insect herbivory

The Chinese rose beetle, an herbivorous scarab beetle, was first documented on Oahu in 1891, and likely arrived from Japan. Within Hawaii, *A. sinicus* feeds on over 500 plant species including major crops such as taro, corn and beans (Tsutsumi et al. 1994). As adults, *A. sinicus* beetles are nocturnal feeders and are attracted to ethylene gas released by damaged leaves (Arita et al. 1988, Mau and Kessing 2002). On several nights, we directly observed *A. sinicus* individuals feeding on cacao leaves. Although some leaves were damaged heavily, most exhibited low levels of damage (Figure 3). Furthermore, the average damage for each tree was low (all ≤ 1.4, mean = 0.58). Although older trees possessed more leaves (regression slope = 118.9 ± 11.46, $p < 0.001$), herbivory did not vary across tree age classes (regression slope = 0.01 ± 0.06, p 0.82; Figure 4). Unchecked, *A. sinicus* damage can reduce the photosynthetic activity of host plants, eventually reducing fruit yield. We therefore hypothesized that highly damaged trees would exhibit reduced reproductive output. However, in our survey we found no evidence of a relationship between pod number and damage level (regression slope = 0.10 ± 0.84, $p = 0.904$). Long-term effects of herbivory on reproductive output may yet occur, and it is also possible that our measures of productivity (i.e. pod number as opposed to total biomass) were too crude. Given the damage caused by *A. sinicus* on other common Hawaiian crops, it may yet prove to be an important determinant of cacao production here.

Insecticides are currently used to control *A. sinicus* in Hawaii, but are not ideal for cacao plantations because of their negative effects on pollinators, and the lower market value of non-organic cacao. Other methods for controlling *A. sinicus*, including chemical lures and microbial agents, are under investigation (Tsutsumi et al. 1994). A fungus, *Metarrhizium anisoplia*, has been used as a biocontrol agent (Williams 1931). *A. sinicus* are phototactic (Mau and Kessing 2002) and light traps may prove useful in reducing damage on small scales. Natural enemies may also regulate Hawaiian *A. sinicus* populations. Within Kauai, anecdotal reports indicate that rose beetle damage on cacao declined following the rapid rise of feral chickens after hurricane Iniki in 1992. Rose beetles deposit their eggs into the soil below their host plants at shallow depths (Tsutsumi et al. 1994), and larvae may therefore be vulnerable to chicken predation. Introduced cane toads (*Bufo marinus*) and geckos (Gekkonidae) are abundant in Hawaii, and might also influence *A. sinicus* population dynamics.

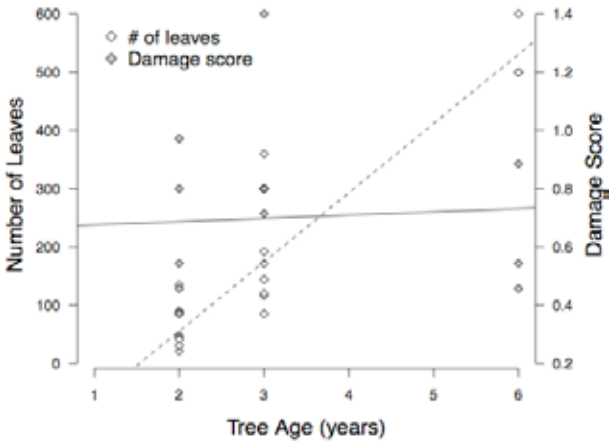

Fig.4. Relationship between age of *Theobroma cacao* trees and both leaf number and total damage from herbivores, at Steelgrass Farm, Kauai. The number of leaves increases with tree age (dashed line), but damage score does not (solid line).

Recommendations and Conclusions

We have identified three important research priorities for the sustainable farming of cacao in Hawaii. First, we recommend further work to identify pollinators and achieve a better understanding of pollinator demography and behavior. Second, there is a strong need to assess the effects of herbivores on cacao productivity and to investigate potential control strategies. Lastly, we recommend ongoing monitoring of cacao pollinators, herbivores and pathogens within Hawaii. As cacao farming increases, so too does the potential for introductions of enemies and mutualists into the archipelago. Introduced species have had cataclysmic effects on both native and cultivated plants within Hawaii (Pimentel et al. 2005). Since enemy invasions are best mitigated if detected early, monitoring efforts will facilitate a healthy expansion of cacao agriculture. Monitoring efforts will also be useful in assessing effects of land-use conversion within Hawaii on farm communities. As cacao agroforestry increases, increased host plant densities may affect the dynamics of inter-farm metacommunities. Nonagricultural land conversion can likewise impact Hawaiian farms if cacao pollinators, herbivores or mutualists disperse between farms and adjacent habitats.

We emphasize the importance of community interactions within cacao plantations in determining

plant productivity and health. These poorly understood interactions might be of great ecological importance. For example, cacao cultivation may positively impact natural resources. Organic, shade-grown cacao supports more diverse bird, beetle and ant communities than most neotropical crops (Bos et al. 2007a, Van Bael et al. 2007), and communities of arbuscular mycorrhizal fungi associated with cacao farms approach the diversity occurring in primary forest (Cuenca and Meneses 1996).

Careful monitoring of cacao-associated communities and experimental studies will help secure the domestic production of cacao. Chocolate production represents a 75-billion-dollar industry worldwide (Ploetz 2007), and while the United States is presently a major consumer of chocolate, domestic production of cacao is restricted to and handful of small-scale operations in Hawaii. The high fruit yields observed at Steelgrass Farm demonstrate the suitability of Hawaii for cacao cultivation and the potential for this industry as a source of domestic jobs and revenues.

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Second-Rotation Tropical Tree Plantation Effects on Fertility of a Hydrudand Soil in Hawaii

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Abstract: Replicated studies that examine legacy effects of first-rotation tree species on soil fertility and soil organic C in the second rotation do not appear to have been previously conducted in the tropics. This study examined the degree to which second-rotation species affected soil acidity, cation exchange properties, total C and N concentrations, extractable inorganic N, and readily mineralizable N (RMN) for the 0- to 15- and 15- to 30-cm depths of an Akaka silty clay loam (Acrudoxic Hydrudand) three and six years into the second rotation that followed an 18-year first rotation. Four first-second rotation species pairings were used: Sydney blue gum [SBG, *Eucalyptus saligna*)] to SBG, albizia [ALB, *Falcataria molluccana*] to ALB, SBG to ALB and ALB to SBG. Rotation effects on soil pH at the 0- to 15-cm depth were primarily legacy effects with lower pH for rotations first planted to ALB than SBG. The SBG-ALB rotation consistently had greater exchangeable Ca and Mg than the other rotations at both the 0- to 15- and 15- to 30-cm soil depths. By year six there was (1) greater soil organic C concentration and organic C gain since the first rotation at the 0- to 15-cm depth for ALB-ALB than SBG-SBG but ALB-ALB did not differ from SBG-ALB or ALB-SBG, (2) soil total N buildup from N_2-fixation by ALB in SBG-ALB and total N decline for SBG-SBG, and (3) no soil total N difference between ALB-SBG and SBG-ALB or between ALB-ALB and SBG-ALB.

Keyword:. soil fertility, tropical tree plantations, second rotation, *Eucalyptus saligna*, *Falcataria mollucana*

Introduction

On the Island of Hawai'i, sugarcane (*Saccharum* spp.) plantation agriculture slowly declined over a 30-yr period until operations ceased in 1996. Many of these former sugarcane lands were converted to plantations of fast-growing trees. Like other crops the productivity of forest plantations depends strongly on soil fertility, and the supply of nutrients in the surface soil can change substantially under the influence of different tree species (Sanchez et al., 1985; Hartemink, 2003; Russell et al., 2007). For example, N-fixing species can greatly enhance the soil N supply (Garcia-Montiel and Binkley, 1998; Mathews et al., 2002).

In a 18-yr Hawai'i study on an Akaka silty clay loam (Acrudoxic Hydrudand) formerly cropped to sugarcane, Mathews et al. (2002) observed a differential soil N accumulation of ~1200 kg N ha^{-1} to a 30-cm depth for first-rotation N-fixing albizia [ALB, *Falcataria molluccana* (Miquel) Barneby & Grimes] in comparison with first-rotation Sydney blue gum [SBG, *Eucalyptus saligna* (Sm.)] and a naturalized fallow of hilograss (*Paspalum conjugatum* Bergius). Differences in soil exchangeable cations, extractable P fractions, and organic C among species were few and of relatively small magnitude but ALB decreased soil pH by 0.2 units. This result for pH was most likely due to acidity generated from the nitrification of mineralized N. Pronounced tree species effects on exchangeable cations are more likely to occur on fertile soils than on acidic, nutrient poor soils like the Akaka series (Kang and Akinnifesi, 1994).

Review of the literature indicated that replicated studies examining legacy effects of first-rotation tree species on soil fertility and soil organic C in the second rotation have apparently not been previously conducted in the tropics. Determining how long first-rotation soil N enrichment by N-fixing species will last into the following rotation of a non N-fixing species, and whether N enrichment will be even greater if an N-fixing species is again used for the second rotation are important issues for sustainable forest plantation management (Weston, 2001).

The objectives of this study were to determine (1) if keeping the same or switching to a different tree species for the second rotation affects soil acidity, cation exchange properties, concentrations of total C and N, extractable inorganic N, and net N

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mineralization for the 0- to 15- and 15- to 30-cm depths of an Akaka silty clay loam following an 18-yr first rotation, and (2) if these properties change during the course of the second rotation.

Materials and Methods

Site Description

The study was conducted at two adjacent sites, locally known as Kamaeʻe, located at 480 m elevation above Hakalau town on the northeast coast of the Island of Hawaiʻi (19º52 N, 155º 9 W). The mean annual precipitation is 4600 mm, and the mean annual temperature is 21ºC. One of the sites is in a well-drained, mid-slope position while the other site is in a wetter, lower slope position with a gravelly subsoil (partially weathered cinders and aʻa clinkers) at a depth of 30 to 60 cm (Mathews et al., 2002). The lower, more poorly drained site has greater concentrations of exchangeable Ca and Mg, perhaps due in part to decreased leaching potential and some Ca and Mg release from the partially weathered materials (Mathews et al., 2002). It is also slightly lower in soil organic C than the upper site. The soil at both sites is an Akaka silty clay loam (hydrous, ferrihydritic, isothermic Acrudoxic Hydrudands) derived from weathered volcanic ash and cinders (Hue et al., 2007). Akaka soils have moderate slopes (<15%) and are usually moderately well-drained with slow runoff and high permeability. Ferrihydrite, allophone-imogolite, and goethite are the dominant minerals in Akaka soil along with ~10% micas/illite and 5% each of gibbsite and feldspars (Soil Survey Staff, 1976; Parfitt et al., 1988). Soil bulk density determined by the core method averages 0.48 and 0.45 Mg m^3 at depths of 0 to 15, and 15 to 30 cm, respectively, with no apparent effect of species (Mathews et al., 2002). These very low bulk density values are within the expected range of 0.3 to 0.6 Mg m^3 for the Akaka soil and other Hydrudands (Soil Survey Staff, 1976; Parfitt et al., 1988).

Prior to establishing the first rotation in 1982 both sites had been under continuous sugarcane (interspecific hybrids of *Saccharum* spp.) cultivation for 50 years. The first rotation consisted of four replicate monoculture plots of SBG and ALB at each site. The trees were planted at a spacing of 1.5 x 1.5 m in 12 x 18 m plots arranged in a randomized design at each site. All plots received a total of 110 kg N ha^{-1} as NH_4NO_3, 50 kg P ha^{-1} as $Ca(H_2PO_4)_2$, and 90 kg K ha^{-1} as KCl at the time of planting. Some thimbleberry (*Rubus rosifolius* Sm.) was present in all plots. For further site details the reader is referred to Mathews et al. (2002).

During late 2000, first-rotation trees from each site were subjected to an aboveground only, whole-tree harvest. The trees were felled by hand and logs along with slash residue were mechanically removed using a Timberjack 735 shovel logger. All tree biomass was removed by working from the exterior of the plots in order to minimize soil disturbance by machinery and thus preserve first-rotation species effects on soil fertility. Glyphosate herbicide (containing 2.0% active ingredient, *N*-phosphonomethylglycine) was applied (9.4 L ha^{-1}) to remaining undergrowth and tree stumps.

Second Rotation Experimental Design

In August 2001, approximately one year after the first rotation was harvested, one half the plots at each site were randomly selected for replanting with the same species used in the first rotation. The remaining plots at each site were designated for replanting with the alternate species. Tree spacing was kept at 1.5 x 1.5 m. This resulted in two replicates per site for each of the four possible rotations: SBG-SBG, ALB-ALB, SBG-ALB, and ALB-SBG. Thus, the experimental design is a generalized randomized block design (GRBD) consisting of four rotations replicated twice at each of two sites.

Table 1. Mean Akaka soil pH in water, Modified-Truog extractable P, exchangeable cations, effective cation exchange capacity (ECEC), Al saturation (Al_{sat}), organic carbon (OC), and total N (TN) for the Sydney blue gum (SBG) and albizia (ALB) plots one year prior to planting the second rotation in 2001. Standard deviations are in parentheses. Data are from Mathews et al. (2002).

Depth	pH_{water}	P	Exchangeable cations				ECEC	Al_{sat}	OC	TN
			Ca^{2+}	Mg^{2+}	K^+	Al^{3+}				
		mg kg^{-1}			$mmol_c$ kg^{-1}			%	g kg^{-1}	
0 to 15 cm										
SBG	4.6	13 (4)	4.1 (1.7)	6.7 (2.1)	1.4 (0.4)	7.0 (4.1)	19.2 (5.8)	34 (18)	120 (7)	7.4 (0.3)
ALB	4.4	12 (5)	4.4 (2.3)	7.9 (3.7)	1.6 (0.5)	8.7 (5.5)	22.6 (7.1)	37 (19)	122 (9)	8.2 (0.5)
15 to 30 cm										
SBG	4.8	9 (4)	3.5 (2.3)	5.4 (1.5)	1.2 (0.3)	4.2 (1.6)	14.4 (4.5)	30 (9)	100 (10)	5.9 (0.6)
ALB	4.6	9 (5)	2.9 (1.0)	5.4 (1.2)	1.3 (0.5)	5.2 (2.7)	14.8 (3.5)	34 (10)	102 (11)	6.8 (0.6)

Soil Sampling and Analysis

Soil sampling occurred a year prior to harvesting the first-rotation trees (Table 1; Mathews et al., 2002) and years three (May 2004) and six (May 2007) after replanting with the second rotation. Samples were obtained from 15 cores collected around the center of each plot to avoid effects from the neighboring tree species (Mathews et al., 2002). A 2.5-cm diam. corer was used to collect samples at depths of 0 to 15 (Ap horizon) and 15 to 30 cm (Bw1 horizon). Before coring, the litter layer was pushed aside. The 15 core samples per plot per depth were composited into plastic bags, placed in an ice cooler, transported to the laboratory where they were immediately sieved through a 1-cm screen to remove roots and worms, and refrigerated at 4°C prior to complete analysis within a few days.

Soil pH was measured by glass electrode in deionized water at a fresh soil-to-solution ratio of 1:1. Exchangeable cations were extracted from field-moist soil (2.5 g dry-soil equivalent) by shaking for 30 min with 50 mL of 0.2 *M* NH_4Cl followed by filtration through Whatman No. 42 filter paper (Mathews et al., 2002). Concentrations of Ca, Mg, K, and Al were measured by Inductively Coupled Plasma Emission Spectroscopy (Hue et al., 2000).

Total soil N was determined by a micro-Kjedahl method using 0.2 g oven-dry soil while available inorganic N (NH_4-N + NO_3-N) was extracted from field moist soil (10.0 g dry-soil equivalent) by shaking for 1 hr with 100 mL of 1 *M* KCl (Hue et al., 2000; Maynard et al., 2008). All N analyses were performed colorimetrically (Maynard et al., 2008). Readily mineralizable N (RMN) was determined by the anaerobic incubation procedure outlined by Sahrawat and Narteh (2003). Soil organic C was determined by a chromic acid colorimetric procedure (Hue et al., 2000). Soil bulk density was used to convert organic C and total N concentration differences among rotations to a Mg per ha gain or loss basis since the first rotation (Mathews et al., 2002).

Modified-Truog (0.01 *M* H_2SO_4 + 0.02 *M* $[NH_4]_2SO_4$) extractable P, the standard agronomic P test for acid soils in Hawai'i, was determined by the colorimetric (molybdenum blue) procedure outlined by Hue et al. (2000). Soil inorganic and total P were determined by the 0.5 *M* H_2SO_4 extraction procedure outlined by O'Halloran and Cade-Menum (2008) using non-ignited and ignited (540°C) samples, followed by colorimetric analysis.

Statistical Analyses

Data were analyzed by PROC GLM of the Statistical Analysis System (SAS Institute, 2003) using the appropriate models for a GRBD (Hinkelmann and Kempthorne, 1994). Rotation, site, and year were considered as fixed effects (Piepho et al., 2003). Year was considered a fixed sub-plot effect because of the cumulative effects of rotations in studies involving perennial crops where year effects can carry over into subsequent years. For further comparison of the first- and second-rotation species effects the data were also analyzed as a function of the first- and second-rotation species and their interactions arranged in a GRBD. All main effects and their interactions were considered significant at $P \leq 0.10$. Rotation means were separated using Fisher's F-protected LSD test at $P = 0.05$ or $P = 0.10$ depending if the overall F-test for rotation was significant at $P \leq 0.05$, or > 0.05 but ≤ 0.10 (Gomez and Gomez, 1984). The level of significance for the LSD test is noted

RESULTS AND DISCUSSION

Rotation Effects on Soil pH, Concentrations of Exchangeable Cations, Effective Cation Exchange Capacity, and Aluminum Saturation

0- to 15-cm Depth

Rotation affected pH ($P < 0.10$), exchangeable Ca and Mg ($P < 0.05$), Al saturation ($P < 0.05$), and ECEC ($P < 0.10$) in both 2004 and 2007 at the 0- to 15- cm soil depth (Table 2). Rotation effects for exchangeable K were only observed ($P < 0.05$) in 2007. Year effects were observed for pH ($P = 0.07$), exchangeable Ca, Mg, and K ($P < 0.03$), Al saturation ($P = 0.003$), and ECEC ($P = 0.10$). Rotation x site interactions were not significant ($P > 0.22$). Rotation x year interactions were observed for pH ($P = 0.10$) and exchangeable Ca ($P = 0.01$), however, the interaction for Ca was due to magnitude of response rather than direction of response.

Soil pH was greater for the SBG-ALB rotation than ALB-ALB and ALB-SBG in 2004, but did not differ from SBG-SBG (Table 2). In 2007, a similar pattern was observed but SBG-ALB only differed from ALB-ALB. Statistical analysis as a function of the first- and second-rotation species revealed that the results for pH were primarily first-rotation species legacy effects ($P < 0.02$) as there were no second-rotation species effects ($P > 0.48$) on pH. It is well known that in the long-term ALB increases soil acidity and SBG also acidifies soil but at a slower rate than ALB (Mathews et al., 2002). This is because in addition to soil base cation uptake and retention, ALB generates considerable acidity via mineralized soil N inputs which undergo the H^+ generating nitrification process (Mathews et al., 2002). *Eucalyptus* species such as SBG acidify the soil primarily by base cation retention in woody biomass coupled with leaf litter of low ash alkalinity (Noble et al., 1996; Weston, 2001). The lack of a second-rotation species effect on pH may suggest that six years is too short for ALB to acidify former SBG plots or may indicate inputs of mixed litters, exudates, and leachates can have an effect counter to the expected acidification by ALB. It is interesting to note that at initiation of the

second rotation the first-rotation SBG plots had a pH of 4.6 for the 0- to 15-cm soil depth (Table 1) and although not significant ($P > 0.10$), the pH in this layer six years later was 4.8 for SBG-ALB compared to a constant 4.6 for SBG-SBG (Table 2). After 5 yr, DeBell et al. (1985) noticed a similar trend on an Akaka soil where SBG and ALB simultaneously planted together in alternate rows in order to provide plots with a 1:1 mixture had a soil pH of 5.4 compared to a pH of 5.2 for pure SBG plots. The greater overall pH values in their study were likely due to the residual effects of lime (≈ 2000 kg ha^{-1}) applied to the last sugarcane crop.

In both years, exchangeable Ca and Mg were greater for SBG-ALB than the other rotations (Table 2). A somewhat similar pattern occurred for exchangeable K in 2007 except that ALB-ALB also had a greater K concentration than SBG-SBG. Senock (2003, unpublished data, California State Univ., Chico) found that ALB in SBG-ALB had greater leaf tissue K concentrations than the other rotations (7.4 g kg^{-1} vs 6.1± 0.5 g kg^{-1}). The factors contributing to greater exchangeable base cations with SBG-ALB are difficult to explain but may be due in part to the young ALB trees serving as a source of N and readily decomposed organic matter to accelerate microbial turnover of more recalcitrant SBG-derived organic matter from the first rotation (Briones and Ineson, 1996; Forrester et al., 2006). DeBell et al. (1985) found that surface soil exchangeable Ca and Mg concentrations were greater in their mixed plantings of SBG and ALB than in pure SBG. The overall decreases in exchangeable Ca and Mg since initiation of the second rotation for all rotations except SBG-ALB (Table 1 vs Table 2) are probably associated with both leaching and tree uptake. From an agronomic soil fertility standpoint the rotation effects on exchangeable Ca, Mg, and K were of small magnitude (Yost and Uchida, 2000) and do not indicate that ALB and SBG will induce pronounced changes in the base cation fertility status of nutrient poor soils like the Akaka series.

Table 2. Sydney blue gum to Sydney blue gum (SBG-SBG), albizia to albizia (ALB-ALB), Sydney blue gum to albizia (SBG-ALB), and albizia to Sydney blue gum (ALB-SBG) rotation effects on soil pH, concentrations of exchangeable cations, effective cation exchange capacity (ECEC) and aluminum saturation (Al_{sat}) in the topsoil (0- to 15-cm depth) in 2004 and 2007.

Rotation	pH_{water}	Exchangeable cations				ECEC	Al_{sat}
		Ca^{2+}	Mg^{2+}	K^{+}	Al^{3+}		
		$mmol_c\ kg^{-1}$					%
2004							
SBG-SBG	4.6ab†	2.6b	3.5b	0.5a	9.5a	16.1b†	56.6a
ALB-ALB	4.4b	3.6b	3.3b	0.9a	13.1a	21.0ab	60.4a
SBG-ALB	4.7a	8.5a	6.1a	0.7a	7.0a	22.4a	30.8b
ALB-SBG	4.4b	2.1b	2.8b	0.6a	10.6a	16.2b	66.9a
Mean	4.5	4.2	3.9	0.7	10.0	18.9	53.7
SE‡	-	0.7	0.7	0.2	1.9	1.8	7.7
2007							
SBG-SBG	4.6ab	3.8b	4.3b	1.2c	9.1a	18.4b	52.0a
ALB-ALB	4.4b	3.6b	4.1b	1.5ab	9.6a	18.9b	48.1a
SBG-ALB	4.8a	14.7a	8.5a	1.7a	5.3a	30.2a	17.9b
ALB-SBG	4.5ab	2.0b	3.5b	1.4bc	10.9a	17.8b	59.5a
Mean	4.5	6.1	5.1	1.4	8.7	21.3	44.4
SE ‡	-	1.4	1.0	0.1	2.0	1.6	7.9
Year effect	+	**	*	***	NS	+	**

† Rotation means in the same column and year not followed by a common letter are different at $P < 0.05$ using Fisher's F-test protected LSD test, except for pH where P is < 0.10.
‡ Standard error of a rotation mean.
+, *, **, * Significant at the 0.10, 0.05, 0.01, and 0.001 probability levels respectively, or NS (non-significant $P > 0.10$).**

Considerable rotation variation existed for exchangeable Al but there were no significant differences in either year ($P > 0.21$). The high variation in exchangeable Al may have been due in part to the relative proximity of the soil samples to decomposing tree trunks and roots from the first rotation (Baba and Okazaki, 2000). These materials can increase exchangeable Al concentrations via organic acid induced Al mobilization from allophone in Andisols (Baba and Okazaki, 2000). In 2004, ECEC was greater for SBG-ALB than SBG-SBG and ALB-SBG but did not differ from ALB-ALB. In 2007, a similar pattern occurred except ECEC for SBG-ALB was greater than all other rotations. This response can be attributed to the greater concentrations of exchangeable Ca and Mg with SBG-ALB which also contributed to a lower Al saturation of the ECEC for this rotation. Over time as ALB acidifies the soil in the SBG-ALB rotation, the base cations and ECEC are likely to decline while exchangeable Al, Al saturation, and solution Al will increase (Baba and Okazaki, 2000; Mathews et al., 2002). This is because the nitrification that occurs as N accumulates in the soil leads to a decrease in pH; concomitantly base cations are leached due a decline in ECEC.

15- to 30-cm Depth

There were rotation effects for exchangeable Ca and Mg ($P < 0.05$) in both 2004 and 2007, while Al saturation ($P < 0.10$) differed among rotations only in 2007 ($P = 0.03$ for rotation x year interaction) (Table 3). Year effects occurred for pH ($P = 0.002$), exchangeable Ca, Mg, K, and Al, and ECEC ($P < 0.03$). Rotation x site interactions were not significant ($P > 0.13$). There were no significant pH differences among rotations in either 2004 (mean = 4.9) or 2007 (mean = 4.7) (Table 3). Similar to the results for the 0- to 15- cm depth, SBG-ALB had or tended to have greater exchangeable Ca and Mg than the other rotations in both years. Also like the results found at the 0- to 15-cm depth, there was considerable variation for exchangeable Al, but no significant rotation effects ($P > 0.30$). There were no differences in ECEC in either year ($P > 0.25$). In 2004, there were no differences ($P > 0.18$) for Al saturation, however in 2007, SBG-SBG, ALB-ALB, and ALB-SBG had greater ($P < 0.10$) percentages than SBG-ALB.

Table 3. Sydney blue gum to Sydney blue gum (SBG-SBG), albizia to albizia (ALB-ALB), Sydney blue gum to albizia (SBG-ALB), and albizia to Sydney blue gum (ALB-SBG) rotation effects on soil pH, concentrations of exchangeable cations, effective cation exchange capacity (ECEC) and aluminum saturation (Al_{sat}) in the subsoil (15- to 30-cm depth) in 2004 and 2007.

Rotation	pH_{water}	Exchangeable cations				ECEC	Al_{sat}
		Ca^{2+}	Mg^{2+}	K^+	Al^{3+}		
		$mmol_c\ kg^{-1}$					%
2004							
SBG-SBG	4.8a†	0.6b	1.2b	0.2a	4.0a	6.0a	63.4a
ALB-ALB	4.8a	0.9b	1.2b	0.6a	2.8a	5.5a	49.3a
SBG-ALB	5.0a	2.3a	2.2a	0.3a	2.1a	6.9a	31.4a
ALB-SBG	4.8a	0.5b	1.0b	0.2a	3.6a	5.4a	63.9a
Mean	4.9	1.1	1.4	0.3	3.1	5.9	52.0
SE‡	-	0.3	0.2	0.2	0.8	0.6	10.6
2007							
SBG-SBG	4.7a	1.3b	1.8ab	0.8a	5.2a	9.0a	57.4a
ALB-ALB	4.7a	0.9b	1.4b	0.8a	7.2a	10.3a	63.8a
SBG-ALB	5.0a	3.9a	2.5a	0.9a	2.0a	9.4a	20.6b
ALB-SBG	4.6a	0.7b	1.2b	0.8a	6.1a	8.9a	64.6a
Mean	4.7	1.7	1.7	0.8	5.1	9.4	51.6
SE‡	-	0.5	0.3	0.1	1.9	1.6	10.4
Year effect	**	**	*	***	*	**	NS

† Rotation means in the same column and year not followed by a common letter are different at $P < 0.05$ using Fisher's F-test protected LSD test, except for Al_{sat} in 2007 where P is < 0.10.
‡ Standard error of a rotation mean.
***, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels respectively, or NS (non-significant $P > 0.10$).**

Rotation effects on Total Soil Organic C, Total N, C:N Ratio, 1M KCl Extractable NH_4-N and NO_3-N and RMN

0- to 15-cm Depth

There were rotation effects for total N, C:N ratio and RMN ($P < 0.05$) for both years whereas, there were rotation effects ($P < 0.10$) for soil organic C, organic C gain since the first rotation, total N gain/loss since the first rotation, and NO_3-N ($P < 0.05$) in 2007 only (Table 4). There were year effects for total N ($P = 0.004$), total N gain/loss ($P = 0.003$), C:N ratio ($P = 0.0004$), NH_4-N ($P = 0.08$), NO_3-N ($P = 0.001$) and RMN ($P = 0.01$). Rotation x site interactions were not significant ($P > 0.14$) but there were rotation x year interactions for total N ($P = 0.05$), C:N ratio ($P = 0.02$) and NO_3-N ($P = 0.08$).

In 2007, greater soil organic C concentration and organic C gain since the first rotation were observed for ALB-ALB than SBG-SBG but ALB-ALB did not differ from SBG-ALB or ALB-SBG. While N additions from legumes such as ALB often stimulate fresh litter decomposition rates by microbes they may also serve to enhance soil organic C because N is a substrate for humification and mineral N can suppress the ligninolytic enzymes of humus degrading soil microbes (Fog, 1998; Resh et al., 2002; Moran et al., 2005; Jandl et al., 2007). However, stabilization as amorphous Fe- and Al-oxide and clay mineral aggregates and surface complexes is the primary reason why humus has a low turnover rate and there is a limit to their ability to provide physical and chemical protection from decomposition (Six et al., 2002; Jandl et al., 2007). Thus, soils can become saturated with humus and not stabilize any more even when inputs of organic C increase (Six et al., 2002; Jandl et al., 2007). The effects of N additions on the stability and turnover of soil carbon are indeed complicated because the information available is conflicting on whether excessive N availability exacerbates soil organic C loss in the long-term (Khan et al., 2007; Coulter et al., 2009).

Table 4. Sydney blue gum to Sydney blue gum (SBG-SBG), albizia to albizia (ALB-ALB), Sydney blue gum to albizia (SBG-ALB), and albizia to Sydney blue gum (ALB-SBG) rotation effects on soil total organic carbon (OC), OC gain since the first rotation, total N, total N gain/loss since the first rotation, C:N ratio, 1*M* KCl extractable NH_4-N and NO_3-N, and readily mineralizable N (RMN) in the topsoil (0- to 15-cm depth) in 2004 and 2007.

Rotation	OC	OC gain	Total N	Total N gain/loss	C:N ratio	NH_4-N	NO_3-N	RMN
	g kg^{-1}	Mg ha^{-1}	g kg^{-1}	Mg ha^{-1}		mg kg^{-1}		
2004								
SBG-SBG	135.3a†	11.00a	8.0b	0.43a	16.9a	16a	15a	104b
ALB-ALB	147.2a	18.13a	9.8a	1.12a	15.1b	21a	28a	142a
SBG-ALB	140.9a	15.07a	8.4b	0.72a	16.8a	19a	22a	108b
ALB-SBG	148.8a	19.28a	9.7a	1.04a	15.5b	10a	29a	129a
Mean	143.0	15.87	9.0	0.83	16.1	16	24	121
SE‡	4.9	3.52	0.3	0.24	0.3	4	4	6
2007								
SBG-SBG	131.1b	7.97b	7.1c	-0.22b	18.5a	15a	25c	113b
ALB-ALB	147.5a	18.38a	9.2a	0.72a	16.1b	14a	50a	154a
SBG-ALB	142.9ab	16.51a	8.6ab	0.85a	16.7b	18a	41ab	134ab
ALB-SBG	139.3ab	12.44ab	8.2b	0b	17.0b	10a	33bc	143a
Mean	140.2	13.82	8.3	0.34	17.1	14	37	136
SE‡	4.0	2.89	0.3	0.18	0.5	3	4	8
Year effect	NS	NS	**	**	***	+	***	**

† Rotation means in the same column and year not followed by a common letter are different at $P < 0.05$ using Fisher's F-test protected LSD test, except for OC and OC gain in 2007 where P is < 0.10.

‡ Standard error of a rotation mean.

+, **, * Significant at the 0.10, 0.01, and 0.001 probability levels respectively, or NS (non-significant $P > 0.10$).**

Soil total N was greater ($P < 0.05$) in ALB-ALB and ALB-SBG than the other rotations in 2004. Statistical analysis as a function of the first- and second-rotation species revealed that this response was a first-rotation species legacy effect ($P < 0.002$) as there were no second-rotation species effect ($P > 0.47$) on soil total N in 2004. By 2007, ALB-ALB still had the greatest total N concentration but no longer significantly differed from SBG-ALB. This response was due to the emergence of a second-rotation species effect ($P < 0.001$) in 2007 in addition to a first-rotation species legacy effect ($P < 0.01$). Furthermore, ALB-SBG had a greater total N concentration than SBG-SBG. These results reflect N buildup from N_2-fixation by ALB in SBG-ALB while total N declined or tended to decline in SBG-SBG and ALB-SBG. The data also suggest that soil N accumulation may have reached the saturation point for ALB-ALB as the mean total N for 2007 was slightly smaller than observed in 2004. Accumulation of N in ALB-ALB soil may have been reduced by tree N uptake from the high existing first-rotation soil N supply coupled with a lower N-fixation rate in the second rotation induced by the high soil N status (Binkley and Giardina, 1997; Pastor and Binkley, 1998). Like organic C, the capacity of soils to stabilize additional N is also dependent on the amounts already in the soil rather than the level of input (Six et al., 2002). In 2004, SBG-SBG and SBG-ALB had wider C:N ratios compared to the other rotations. Statistical analysis as a function of the first- and second-rotation species revealed that this response for C:N ratio was a first-rotation species legacy effect ($P < 0.001$) as there was no second-rotation species effect ($P > 0.41$) on C:N ratio in 2004. In 2007, SBG-SBG had a wider C:N ratio than the other rotations which did not differ amongst each other. This result occurred because of the total N buildup in SBG-ALB and N loss from ALB-SBG indicating the emergence of a second-rotation species effect ($P < 0.02$) on C:N ratio in addition to a first-rotation species legacy effect ($P < 0.05$).

In both years there were no significant differences for NH_4-N ($P > 0.11$). In 2004, there were no significant rotation effects for NO_3-N ($P > 0.14$) however, in 2007 ALB-ALB had a greater concentration than SBG-SBG and ALB-SBG but did not differ from SBG-ALB (Table 4). In both years, RMN was greater for ALB-ALB and ALB-SBG than the other rotations with the exception that they did not differ from SBG-ALB in 2007. Overall the levels of RMN in the present study are rather high and likely reflect the high concentrations of organic C present in the Akaka soil (Mathews et al., 2002).

Table 5. Sydney blue gum to Sydney blue gum (SBG-SBG), albizia to albizia (ALB-ALB), Sydney blue gum to albizia (SBG-ALB), and albizia to Sydney blue gum (ALB-SBG) rotation effects on soil total organic carbon (OC), OC gain since the first rotation, total N, total N gain/loss since the first rotation, C:N ratio, 1*M* KCl extractable NH_4-N and NO_3-N, and readily mineralizable N (RMN) in the subsoil (15- to 30-cm depth) in 2004 and 2007.

Rotation	OC	OC gain	Total N	Total N gain/loss	C:N ratio	NH_4-N	NO_3-N	RMN
	g kg^{-1}	Mg ha^{-1}	g kg^{-1}	Mg ha^{-1}		mg kg^{-1}		
2004								
SBG-SBG	109.2b†	6.21ab	6.5a	0.39a	16.9a	9a	20a	57a
ALB-ALB	104.4b	1.64b	6.6a	-0.17b	15.9a	15a	27a	56a
SBG-ALB	117.8a	11.98a	6.6a	0.47a	17.9a	13a	15a	60a
ALB-SBG	108.6b	4.44b	6.6a	-0.15b	16.6a	11a	25a	64a
Mean	110.0	6.07	6.6	0.14	16.8	12	22	59
SE‡	2.9	1.92	0.2	0.14	0.5	2	4	5
2007								
SBG-SBG	111.6a	7.81a	6.0bc	0.07a	18.7a	7a	22a	64a
ALB-ALB	112.1a	6.83a	6.5ab	-0.24a	17.4a	7a	35a	66a
SBG-ALB	106.0a	4.05a	5.9c	0a	18.0a	9a	29a	68a
ALB-SBG	116.5a	9.79a	6.6a	-0.15a	17.8a	8a	30a	74a
Mean	111.6	7.12	6.2	-0.08	18.0	8	29	68
SE‡	2.7	1.80	0.2	0.13	0.6	2	5	5
Year effect	NS	NS	**	**	***	***	**	+

† Rotation means in the same column and year not followed by a common letter are different at $P < 0.05$ using Fisher's F-test protected LSD test, except for OC in 2004 and total N in 2007 where P is < 0.10.
‡ Standard error of a rotation mean.
+, **, * Significant at the 0.10, 0.01, and 0.001 probability levels respectively, or NS (non-significant $P > 0.10$).**

15- to 30-cm Depth

Rotation x site interactions were not significant ($P > 0.14$) but there were rotation x year interactions for soil organic C ($P = 0.002$) and total N ($P = 0.07$). In 2004, there were rotation effects for soil organic C ($P < 0.10$) and OC gain since the first rotation ($P < 0.05$) (Table 5). In 2007 there were rotation effects for total N and total N gain since the first rotation ($P < 0.10$) but there were no rotation effects in either year for C:N ratio, NH_4-N, NO_3-N, and RMN. Similar to the results found at the 0- to 15-cm depth, there were year effects for total N ($P < 0.01$), C:N ratio ($P < 0.0001$), NH_4-N ($P < 0.001$), NO_3-N ($P < 0.01$), and RMN ($P < 0.07$).

In 2004, the total soil organic C in SBG-ALB was greater than the other rotations with similar trends for organic C gain since the first rotation (Table 5). In 2007 ALB-SBG had a greater total N concentration than SBG-SBG and SBG-ALB but did not differ from ALB-ALB (Table 5).

Rotation effects on modified-Truog extractable P, inorganic P and total P

There were no differences ($P > 0.19$) for modified-Truog extractable P, inorganic P and total P at either the 0- to 15- cm or 15- to 30- cm soil depth in 2004 and 2007. The means for modified-Truog extractable P, inorganic P and total P at the 0- to 15- cm depth were 19 (SE = 4), 592 (SE = 154), and 1594 (SE = 179) mg P kg^{-1} respectively in 2007 while the means at the 15- to 30- cm depth were 17 (SE = 3), 499 (SE = 135), and 1328 (SE = 120) mg P kg^{-1}. There were no indications of foliar P deficiencies (Drechsel and Zech, 1991) at these levels of Modified-Truog (agronomic soil test) P as Senock (2003, unpublished data, California State Univ., Chico) found that SBG in SBG-SBG and ALB-SBG had leaf tissue P concentrations of 1.4 ± 0.2 g kg^{-1} while ALB in SBG-ALB and ALB-ALB had concentrations of 1.6 ± 0.1 g kg^{-1}.

Conclusions

One of the primary effects of ALB in the first six years of the second rotation was to increase surface soil exchangeable bases (Ca, Mg, and K) to a depth of 15 cm in the SBG-ALB rotation. Whether this increase can be maintained throughout the second rotation or whether it will decline due to soil acidification and subsequent base leaching induced by ALB N_2-fixation is unknown. A buildup of surface soil exchangeable bases for ALB was not present after 18 yr in the first rotation but ALB had decreased surface soil pH relative to SBG (Mathews et al., 2002; Table 1). The surface soil buildup of total N in the SBG-ALB rotation was expected as was total N decline or trends toward decline for the SBG-SBG and ALB-SBG rotations. After six years there was no difference between ALB-SBG and SBG-ALB for soil total N due to the opposite effects of ALB and SBG. It will be interesting to observe the ALB-ALB rotation to determine if soil total N accumulation has indeed reached a saturation point or a steady state condition. There were no tree species or rotation effects on soil P.

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Pacific Agriculture and Natural Resources

A Feeding Trial of Fortified Dehydrated Garbage for Growing Swine in Hawaii

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Abstract: A grower pig feeding trial was conducted in East Hawaii with processed food wastes from a demonstration dehydrating machine designed in South Korea. Three commercial swine operations were used to collect average daily gains (ADG), consumption of feed (COF) and feed conversion (FC) data in pigs fed various rations. All animals were given a daily feed allowance of 3% of body weight with the exception of animals fed wet garbage. Two farms tested a concentrate corn-soybean ration versus fortified dehydrated garbage (FDG) adjusted for dry matter content. The third farm tested FDG versus wet garbage fed ad libidum. Weekly proximate analysis of FDG showed large differences in nutrient and mineral content due to the variable moisture and food waste content of wet garbage. The FDG was deficient in calcium and lysine. Animals fed the commercial ration performed better than the FDG and wet garbage diets. At the three farms the FDG groups did not differ in ADG, COF and CF. Local producers considering using FDG should estimate the cost per pound of feed of this product versus the cost of commercial feed or wet garbage. Given the fact that the cost of imported commercialized feed continues to rise, a reasonable decrease in animal performance could be tolerated when feeding garbage.

Keywords: Swine, garbage, dehydrated, feed trial

Introduction

The increase use of corn for ethanol production has led to higher grain prices. With higher grain prices, feed prices have increased. These higher feed prices are affecting Hawaii's livestock producers since commercial feeds are shipped from the continental United States. In local landfills wet food waste disposal is generating high levels of methane gas, odor and insects. The feeding of food waste or garbage to swine is a permitted practice throughout the Hawaiian Islands as long as the product is: 1) heat treated (100°C at sea level for 30 minutes) and 2) agitated during cooking as mandated by the 1980 Swine Health Protection Act. Properly processed and nutritionally balanced cooked garbage could remedy Hawaii's dependence upon expensive imported swine feed. The purpose of this study was to examine whether the fortified dehydrated garbage (FDG) diet could deliver reasonable performance compared to other types of local diets.

Methods

A feeding trial was conducted with grower swine in East Hawaii using commercial feed, wet garbage and fortified dehydrated garbage (FDG). FDG was produced by processing local wet garbage with feed additives using a South Korean experimental cooking/dehydrating machine. Before the dehydrating process was finished, a microbial culture was added to the feed. The microbial culture was derived from the local environment. Thirty-four crossbred weanling pigs from three commercial piggeries were included in the trial. The animals were treated for internal parasites before starting the trial. Pigs were randomly assigned by sex, weight and litter to the six treatment groups. Pigs were acclimated to their respective diets for 14 days before the trial began. Groups I and II pigs were placed on a 3% body weight restricted commercial grower-finisher swine diet. Groups III, IV, and V pigs were placed on a 3% body weight restricted diet adjusted for dry matter of fortified dehydrated garbage (50% wet restaurant waste, 45% wheat mill run and 5% fish meal). Group VI pigs were fed cooked wet garbage ad libidum. On farms #1 and #2, FDG fed pigs were compared to pigs fed a commercial grower-finisher swine diet. However on farm #3, FDG fed pigs were compared to pigs fed cooked wet garbage. The FDG ration was prepared weekly and samples were collected and sent to the University of Hawaii Agricultural Diagnostic Laboratory for proximate analysis. Animals were fed twice daily and daily allowances of feed were adjusted for weight gain after each 2 week weighing. Body weight gain and feed intakes were measured. Based on the collected data, average daily gain (ADG), consumption of feed (COF) and feed conversion (FC) were calculated. After all animals within a group reached 68 kg they were weighed off of test. The Chi Square test was used for determining probability levels for the data.

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Results

Table 1 summarizes the proximate analysis of the fortified dehydrated garbage (FDG) during the growth trial. As the proximate analysis indicates FDG had a variable nutrient content which is probably related to differences in the wet garbage. Wheat mill run (WMR) was added more as a drying ingredient to the wet garbage before dehydration occurred. The WMR was analyzed to contain 17% crude protein and 75% total digestible nutrients on a dry matter basis (Yarlagadda and Lee, 2008).

The wet garbage provided minimum recommended levels (based on the National Research Council) of most minerals and amino acids except for calcium and lysine. The garbage analyzed in the study had similar low levels of calcium as reported by Barth et al, (1966) but acceptable levels of P, Mn, Cu, Fe and Mg. The low Ca levels found in wet garbage fed diets could cause skeletal problems due to lower than recommended Ca:P ratio. With a low Ca:P ratio, proper bone calcification may not occur. Sodium chloride (NaCl) is commonly deficient in most swine rations but the standard NRC recommendation of .2 to .5 percent meets the dietary Na and Cl requirements of growing and finishing pigs. Failure to supply salt to pigs causes depressed feed uptake and poorer growth rates, where as, elevated levels of Na as in FDG (.83%) could play a role in depressing intake of garbage fed pigs. Since salt can not be stored in the animals' body to any appreciable extent, excess salt is usually excreted in the urine. Thus, if wet garbage contains elevated levels of sodium, animals should have free access to fresh clean water and not be given supplemental salt. Potassium (K) and magnesium (Mg) in swine rations play important roles in body function involving electrolyte and neuromuscular function. In most cases, swine diets do not need supplemental K and Mg since commonly used feed stuffs contain adequate amounts of these two minerals. Zinc (Zn) levels were at acceptable levels in all three FDG rations. Absorption of Zn can be reduced if wet garbage contains elevated levels of Ca thus; The NRC recommends that 100ppm of Zn be supplemented for each 1 percent of Ca in the diet. Iron (Fe) and copper (Cu) concentrations were analyzed to be higher than NRC recommended levels for swine diets. Cu deficiencies are seldom seen in pigs but reduced dietary concentrations of Zn and Fe or high Ca levels could accentuate copper toxicity.

Tab.1. Average composition of fortified dehydrated garbage fed on sample farms (n=15).

Item	NRC recommendations	Mean	Standard error	Range
Dry Matter,%		72.07	3.98	65.61 to 79.57
Ash ,%		5	0.82	3.81 to 6.61
Crude Protein,%	16	19.22	1.65	15.38 to 22.28
EE% Crude Fat,%		8	1.43	5.02 to 9.54
NDF% Neutral Detergent Fiber		36.51	1.75	35.45 to 41.53
ADF % Acid Detergent Fiber		11.96	1.65	10.23 to 16.19
PMF % Lignin		7.86	0.76	7.15 to 9.54
Cellulose %		3.81	0.54	3.01 to 4.08
P,% (Phosphorous)	0.55	0.59	0.17	0.36 to 0.83
K,% (Potassium)	0.2	1.12	3.98	1.0 to 1.22
Ca,% (Calcium)	0.65	0.25	0.82	0.1 to 1.11
Mg,% (Magnesium)	0.04	0.46	1.65	0.25 to 0.54
Na,% (Sodium)	0.35	0.27	1.43	0.18 to 0.83
B ppm (Boron)		10.3	1.75	4 to 42
Cu ppm (Copper)	10	13	1.65	10 to 20
Fe ppm (Iron)	100	1442.5	0.76	299 to 3225
Mn ppm (Manganese)	10	178.2	0.54	147 to 261
Zn ppm (Zinc)	100	136.7	0.17	77 to 171
Lysine% a	0.7	0.63	0.03	0.48 to 0.82

*a: only three samples collected for analysis

Tab.2. Average daily gains, feed consumption and feed conversion of grower pigs on feed trial. Pigs feeding program: Groups I and II received a restricted 3% body weight concentrate corn and soybean-meal diet. Groups III, IV, and V received a restricted 3% body weight diet of dehydrated garbage adjusted for dry matter. Group VI, received ad libitum wet garbage.

Group	Grower ADG lb/kg*	Grower Total lb/kg gain	Feed Consumption Total lb/kg	Feed Conversion	Extended days to 150 lbs
I	1.21#/.55kg	119#/53.98kg	440#/199.58kg	3.7:1	0
II	1.02#/.46kg	102#/46.27kg	326#/147.87kg	3.2:1	19
III	0.72#/.33kg	71#/32.21kg	520#/235.87kg	7.3:1	30
IV	0.72#/.33kg	71#/32.21kg	406#/184.16kg	5.7:1	55
V	0.70#/.32kg	69#/31.30kg	458#/207.45kg	6.6:1	50
VI	0.58#/.26kg	58#/26.03kg	792#/359.25kg	13.0:1	158

For good performance, diets require a proper balance of essential amino acids for normal bodily functions in swine. The most limiting amino acid in most swine diets is lysine and most swine diets require lysine additions. In the wet garbage and FDG diets lysine levels fluctuated below and above the NRC recommended levels. Because of this, lysine supplementation would be recommended. Lysine deficiencies result in slower, less efficient gains and more fat deposition (Luce, et al, 1998). In this study, vitamin levels were not analyzed. It is possible that the wet garbage and FDG were deficient in certain vitamins. Further studies need to examine vitamin levels in garbage rations.

Research has shown that pigs have the ability to convert concentrate feed into lean meat more efficiently than ruminant animals. The amount of feed required per unit of gain is lower in younger pigs and increases steadily as they mature. NRC has shown the rate of gain begins to decrease rapidly at about 90.72 kg (200#). In our study the animals fed the commercial concentrate diet had the most rapid rate of gain, 0.46 and 0.55kg/day, followed by the fortified dehydrated garbage (FDG) group which averaged 0.32kg/day followed by wet garbage pigs at 0.26 kg/day. The FDG fed groups (III, IV and V) had similar average daily gain (0.33, 0.33, and 0.32 kg/day) and total weight gained on trial (32.31, 32.31, and 31.30 kg). Feed conversion ratios were less than a 4:1 value for the pigs fed the commercial feed. Feed conversion (feed/gain) ratios were above 5:1 value and differed substantially amongst the FDG groups. These differences could be due to a number of factors including the accuracy of feed weighing. Feed conversion ratios were high for the pigs fed the wet garbage. This could be expected due to the high water content in wet garbage. The differences in the total number of extended feeding days between the commercial feed, FDG and wet garbage fed pigs are shown in Table 2. When comparing the commercial diet groups, Group II started the trial at a lighter weight and thus required 19 more days of feeding. Pigs on FDG rations would be expected to require about 45 to 55 additional days of feeding while wet garbage fed pigs would require 150 additional days of feeding to reach the 68 kg body weight. Both the feed efficiency and the cost per unit of feed need to be considered in comparing the economic differences between feeding commercial feed, FDG and wet garbage. In this study, a microbial culture was added to FDG diet to start a fermentation process. The effect of the microbial culture is unknown. Future studies should examine the differences in animal performance and microbial populations between pigs fed diets with and without having microbe cultures added to the feed.

Discussion

Feed accounts for 70 to 75% of the total costs of swine production in Hawaii. Because of this, it is important to select cost effective diets. Food waste has been reported to makeup about 8.9% of the U.S. municipal solid waste stream; therefore, recycling of food waste into swine feeds can be a viable alternative to land filling (NRC, 1983). Recycling organic wastes for commercial pig production is not a new idea and the utilization of kitchen wastes from institutions such as hospitals, schools or hotels, would help to reduce the increasingly problematic question of environmental pollution. Garbage wastes are subject to rapid deterioration and contamination by microorganisms, some of which can be pathogenic in nature. The use of ensiling, or heat treatment can be an effective way of making garbage safe for swine feeding. It is difficult to predict intake when pigs are fed wet garbage since the water and nutrient content varies. Extensive research conducted over the last 40 years has documented the effectiveness of recycling garbage into swine diets (Westendorf, et al, 1998). However, commercial use of garbage in swine feeding has not become a wide spread practice in the U.S.

Garbage as a feedstuff can be a nutritious food source for swine with respect to protein and energy, however, its high water content tends to affect animal growth due to a reduction in total dry matter intake, principally in younger growing animals, fed ad libidum (Kornegay, et al., 1955). It is also likely that garbage is deficient in

certain minerals and vitamins. Most swine fed exclusively on garbage based diets may attain maximum gains of about 0.5 kg (or one pound) per day, but to achieve this, swine must consume garbage in larger quantities.

To examine this question one should look at the results of garbage studies. Most garbage feed trials have involved grower pigs weighing between 22 to 68 kg (50# to 150#). Our estimates of intake range from about 3.18 to 4.54 kg (7 to 10# as-fed) per pig per day for hogs under 45.36 kg (100#) and 9.07 kg (20#) or more for animals weighing 90.72 kg (200#) or more. These values were similar to the work done by Westendorf et al. (1998) with 25% dry matter garbage.

The feeding of a small amount of supplemental grain (e.g. ground corn, soybean meal) may be desirable to achieve better daily weight gains. Research conducted in New Jersey and Florida, (Myer et al., 1999), indicated that it may be possible to obtain growth rates similar to pigs fed traditional commercial dry diets when garbage is supplemented with ground corn. In this trial wheat mill run was used to fortify wet garbage. Due to the variability of composition and poor shelf life of wet garbage in Hawaii, it would be difficult to make this into a commercial feed. Mineral and vitamin supplementation of garbage may be beneficial.

New processing practices that reduce moisture content of the feed may help extend the shelf life and make the product easier to feed (Watanabe, et al 2001). Processes such as pelletizing, the use of extrusion or dehydration can result in a more uniform and stable product. These issues that influence new processing techniques will probably be regulatory in nature, such as whether processed garbage will meet the requirements of the Swine Health Protection Act (1980), or whether the Food and Drug Administration or Department of Agriculture will control commercialization.

Conclusion

The results of this feeding trial suggest that FDG could be used in grower-finisher diets. Since this was a preliminary study in examining the effect of using FDG, further studies are warranted. However, it may be beneficial to add supplemental minerals (such as calcium) and vitamins to this feed. Further studies need to examine the effects of using mineral and vitamin supplementation in garbage based diets. Possible differences in performance need to be examined for diets that include and do not include bacterial cultures. The swine industry should consider the development of a swine feed cooperative that helps to consolidate food waste. The charging of tipping fees for one stop garbage collection and the possibility of multi-located dehydrating machines should also be considered. More research in nutrient supplementation, shelf life storage and pelleting for the ease of handling of FDG is recommended for the development of a uniform, yet cost efficient commercialized feed for Hawaii.

There are several possible economic measures of a successful feeding program. Cost per metric ton of feed is probably the least accurate way of comparing feed since it gives no consideration to pig performance or revenue. Better economic measures should include feed cost per kilogram of gain; feed cost per pig marketed, profit per pig and the best being returns on space per pig. When traditional feedstuffs are expensive, hog producers become more interested in using less expensive alternatives. However, better management and more effort to successfully use these substitutes need to be considered.

Recycling of garbage by direct feeding to swine continues to be a niche which could work economically by providing producers with a cheaper source of feed; however, with the increase fuel prices, farmers will have to charge a tipping fee to cover collection and other costs associated with processing garbage. Recently, the practice of feeding garbage has been questioned due to the possibility of transmission of communicable disease, vector and fly nuisances and odors. There are nationwide efforts underway to determine whether changes should be made to the federal regulations which govern the feeding of garbage to animals (27 states have banned feeding garbage to swine). This research trial has shown that FDG can be produced economically without any negative effects to the environment, while providing satisfactory weight gains for grower pigs. Given the fact that the cost of imported commercialized feed continues to rise in Hawaii, a reasonable decrease in animal performance could be tolerated when feeding garbage. With more research on supplementation, dehydration and pelleting FDG, we may be able to produce a nutritious, lower priced feedstuff for pigs while offering a viable option to solid waste disposal at landfills. Producers who are currently feeding wet garbage should examine and re-evaluate feeding of FDG as an alternative in making their herd more profitable.

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Pacific Agriculture and Natural Resources

Genetic Diversity Analysis of Tianfu Goats and Three Relative Breeds Using Microsatellite DNA Markers

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Abstract: Genetic variability and relationships among three subpopulations of Tianfu goat (SAU-B, SAU-R and SAU-W) and three related breeds (Boer, Chengdu Ma, and Nanjiang Yellow goats) were investigated using microsatellite DNA markers at 11 loci. A total of 262 animals were tested; 92 alleles were detected across 11 loci with an average of 8.36 alleles per loci. Effective allelic numbers of all loci were between 4.13 and 7.35. The polymorphic information contents (PIC) values of 11 microsatellite loci varied from 0.64 to 0.80 with an average of 0.71. Observed heterozygosity varied between 0.271 and 0.561 with an average of 0.418 across 11 loci, lower than expected heterozygosity with an overall mean of 0.805. Shannon information index was 1.80±0.20 with a range of 1.52 to 2.21. All the 6 detected subpopulations or breeds showed a high level of gene diversity and gene flow. Overall F-Statistics (Fst) was 0.077; indicating that 7.7% of total genetic diversity was partitioned among populations or breeds and showing that most of the variability is within populations or breeds with a percentage of 92.3%. Genetic distance Nei's Standard Genetic Distance (D_S) and Modified Cavalli-Sforza Genetic Distance (D_A) were estimated for each pair of subpopulations or breeds. Phylogenetic trees constructed from D_A by UPGMAM and N-J method, revealed a pattern consistent with the breeding history and geographic origin. All three subpopulations of Tianfu goats have strong genetic similarities, and should be considered as belonging to the same population with a distinct differentiation from the blood-related breeds. A high level of gene flow and a close genetic distance among subpopulations and breeds confirmed that the breeding objectives over twenty years have been met. Abundant genetic polymorphism existed in the three subpopulations suggested that Tianfu goats have great potentialities for further selection.

Keywords: Tianfu goats, genetic diversity, microsatellite, DNA, Boer goats, subpopulations

Introduction

China has approximately two hundred million goats and stands first in the number of goats among the countries of the world (Zhang et al., 2006); however most of them are indigenous goats (Luo et al., 2005). Mean carcass weight is about 12.6 kg, and lower than the world average of 15.0 kg (Pu and Xu, 2006). Thus it has been an important objective to improve productive performance of goats in China.

Sichuan Province is one of the main regions of goat production in China, and has approximately 1.8 million goats with 11 goat breeds that are nationally and provincially recognized (Xiong, 2004). Begginig in 1980, a provincial project was assumed by Sichuan Agricultural University (SAU) for rearing a new meat goat breed using Chengdu Ma goats (SCM), a local breed, crossbreeding with introduced goat breeds (Nubian, Saanen, Toggenburg and Boer goats). The reared new goats were named Tianfu meat goats. The emerging goat population was divided into three subpopulations according to the hair color in the head and neck region: black (SAU-B), red (SAU-R) and white (SAU-W), and it has been reported that no significant differences were observed on productive performance among the three goat subpopulations (Xu et al., 2005). The genetic diversity among the 3 subpopulations remains unknown.

Out of many genetic markers now available, microsatellites are best suited for genetic diversity analysis because of their high variability, high mutation rate, abundance, distribution throughout the genome,

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co-dominant inheritance and neutrality (Gour et al., 2006). The usefulness of microsatellite markers for the estimation of genetic diversity and relationships among livestock breeds has been documented in numerous studies; they have been used as good tools to analyze genetic variations in cattle (Martin-Burriel et al., 1999; Maudet et al., 2002), sheep (Arranz et al., 2001), pig (Martinez et al., 2000), water deer (Hu et al., 2007), chicken (Zhou and Lamont, 1999; Bao et al., 2008), goat (Saitbekova et al., 1999; Iamartino et al., 2005; Rout et al., 2008) and other animals.

The objective of this study was to analyze genetic diversity of the three subpopulations of Tianfu goats and three related breeds (Boer, Chengdu Ma and Nanjiang Yellow goats) by Microsatellite DNA Markers method. Nanjiang Yellow goat (SNY, a goat breed certificated by Chinese Agricultural Ministry in 1998) was included in this study because of its breeding history with SCM and Nubian (Zhang et al., 2006).

Materials and Methods

Animals

One hundred and thirty-six Tianfu goats (48 SAU-B, 44 SAU-R and 44 SAU-W) were selected from the two breeding farms of Tianfu goats, SAU in Yaan City and HuiYan Livestock Company in Yanting County, and the adjacent regions. All the goats were confirmed to be genetically unrelated at least for two generations and to have an extensive distribution in the subpopulations according to the pedigree information. Forty-one Boer goats (Boer) were provided by SAU; and 42 Chengdu Ma goats (SCM) and 43 Nanjiang Yellow goats (SNY) were from Shuangliu and Nanjiang Counties in Sichuan Province, respectively. A random stratified technique was used to select the villages in the breeding region of Chengdu Ma and Nanjiang Yellow goats; only one sample from small flocks (< 15) and no more than 4 samples from large flocks (> 50). All the blood samples were obtained from jugular aseptic venipuncture into sterile tubes with sodium EDTA as anticoagulant, and were frozen at -20 °C until analysis.

Microsatellite election, PCR conditions and fragment analysis

Genomic DNA was isolated from whole blood by the method described by Sambrook et al. (2001). Eleven selected microsatellites are listed in Table 1. The 11 microsatellite markers were selected based on the guidelines of the International Society for Animal Genetics (ISAG) & FAO's Domestic Animal Diversity Information System (Measurement of Domestic Animal Diversity Program) to generate data from a panel of 50 animals.

Polymerase chain reaction (PCR) was carried out in Tgradient (Whatman Biometra, Goettingen, Germany). Total reaction volume was 25 □L, and the reaction mixture consisted of 2□L (50-100 ng) genomic DNA templates, 12.5 □L 2×Taq PCR MasterMix (QIAGEN, Hilden, Germany), 1.0 □L (100ng/□L) of each primer (Invitrogen, Carlsbad, USA) and 8.5□L ddH_2O. The "touchdown" PCR protocol was used in this study: initial denaturation at 94 °C for 5 min, 6 cycles (94°C, 50s: 60°C, 45s; 72°C, 1 min), 6 cycles (94°C, 50s: 58°C, 45s; 72°C, 1 min), 4 cycles (94°C, 50s: 56°C, 45s: 72°C, 1 min), 20 cycles (94°C, 50s: 54°C, 45s; 72°C, 1min), and an extension for 8 min at the end of the reaction.

Tab.1. Characters of 11 selected microsatellite DNA markers.

Locus	Primer sequence	Reference
SR-CRSP-1	F: 5□-TGCAAGAAGTTTTTTCCAGAGC-3□ R: 5□-GTACCCTGGTTTCACAAAAGG-3□	Arevalo et al., 1994
SR-CRSP-5	F: 5□-GGACTCTACCAACTGAGCTACAAG-3□ R: 5□-TTATGAAATGAAGCTAAAGCAATGC-3□	Arevalo et al., 1994
SR-CRSP-9	F: 5□-CTAGAGGATCTGGAAATGGAATC-3□ R: 5□-GCACTCTTTTCAGCCCTAATG-3□	Bhebhe et al., 1994
ILSTS030	F: 5□-CTGCAGTTCTGCATATGTGG-3□ R: 5□-CTTAGACAACAGGGGTTTGG-3□	Kumar et al., 2005; Gour et al., 2006
ILSTS008	F: 5□-GAATCATGGATTTTCTGGGG-3□ R: 5□-TAGCAGTGAGTGAGGTTGGC-3□	Kumar et al., 2005; Gour et al., 2006
INRABERN175	F: 5□-TGATGAGGATGGATGCTAAACT-3□ R: 5□-CTGCAAATAAGAAAACTGAATAAA-3□	Saitbekova et al., 1999
INRABERN192	F: 5□-AGACCTTTACAGCCACCTCTTC-3□ R: 5□-GTCCCAGAAACTGACCATTTTA-3□	Saitbekova et al., 1999
ILSTS087	F: 5□-AGCAGACATGATGACTCAGC-3□ R: 5□-CTGCCTCTTTTCTTGAGAGC-3□	Kumar et al., 2005; Gour et al., 2006
SR-CRSP-8	F: 5□-CGGTCTGGTTCTGATTTCAC-3□ R: 5□-GCATGAGAAAGTCGATGCTTAG-3□	Bhebhe et al., 1994
OarFCB48	F: 5□-GAGTTAGTACAAGGATGACAAGAGGCAC-3□ R: 5□-GACTCTAGAGGTACGCAAAGAACCAG-3□	Gour et al., 2006
OarFCB304	F: 5□-CCCTAGGAGCTTTCAATAAAGAATCGG-3□ R: 5□-CGCTGCTGTCAACTGGGTCAGGG-3□	Gour et al., 2006

Tab.2. Measures of genetic variation at 11 microsatellite loci.

SN	Loci	Gt[1]	Na	Ne[2]	I[3]	Heterozygosity			Heterozygote Deficiency	PIC
						Ho	He1[4]	He2[5]		
1	SR-CRSP-8	46	14	7.3499	2.2063	0.3664	0.8656	0.8639	- 0.5767	0.8497
2	SR-CRSP-1	30	8	5.7903	1.8829	0.3779	0.8289	0.8273	- 0.5441	0.8070
3	SR-CRSP-5	28	8	4.8373	1.7394	0.5611	0.7948	0.7933	- 0.2940	0.7645
4	SR-CRSP-9	27	8	5.1181	1.7929	0.3855	0.8062	0.8046	- 0.5218	0.7780
5	ILSTS030	34	11	6.2872	1.9826	0.5344	0.8426	0.8409	- 0.3658	0.8215
6	ILSTS008	17	6	4.1307	1.5242	0.3435	0.7594	0.7579	- 0.5477	0.7196
7	ILSTS304	32	9	4.9480	1.7960	0.4466	0.7994	0.7979	- 0.4413	0.7710
8	OarFCB48	29	8	6.2620	1.9517	0.5000	0.8419	0.8403	- 0.4061	0.8216
9	INRABERN192	18	7	4.2374	1.6005	0.3359	0.7655	0.7640	- 0.5612	0.7275
10	ILSTS087	17	6	4.4045	1.5826	0.2710	0.7744	0.7730	- 0.6501	0.7369
11	INRABERN175	26	7	4.6981	1.7257	0.4771	0.7887	0.7871	- 0.3951	0.7608
	Mean	27	8.36	5.2785	1.7986	0.4181	0.8061	0.8046		0.7780
	SD		2.34	1.0183	0.2002	0.0918	0.0346	0.0346		0.0424

[1]Gt: Genotype number; Na: Observed number of alleles; [2]Ne: Effective number of alleles; [3]I: Shannon's Information index; [4]He[1]: Levene's (1949) expected heterozygosity;[5]He[2]: Nei's (1973) expected heterozygosity; [5]PIC: polymorphic information content.

The PCR products were electrophoresed on agarose gels (2%) and then on 8% non-denaturing polyacrylamide gels using Biorad Protean II xi Cell (Bio-Rad, Calif., USA) to separate PCR products with different sizes, DNA marker pBR322 DNA/MspI (QIAGEN, Hilden, Germany) was used as a size standard for sizing PCR products. To visualize the PCR products, gels were silver-stained (Sambrook and Russell, 2001), and the image acquisition and analysis system GelDoc XR (Bio-Rad, Calif., USA) was used to obtain the electropherogram. Genotypes were scored manually by four persons divided into two independent groups and with the help of the Quantity One 4.3.1 software (Bio-Rad, Calif., USA).

Since microsatellite markers were co-dominant (the 262 samples represent 524 alleles for a single locus), a combination of 11 co-dominant loci and 262 samples were expected to generate 5764 allelic data for the populations under study.

Statistical analyses

For the 11 microsatellite loci analyzed, effective numbers of alleles (Kimura and Crow, 1964) were calculated using POPGENE software (Yeh et al., 1999). Observed heterozygosity and expected heterozygosity (Levene, 1949; Nei, 1973) estimates and Shannon's information index (Lewontin, 1972) were computed, as implemented in POPGENE software. Heterozygosity deficiencies were expressed as D = (Ho - He) / He.

Cervus 2.0 software (Marshall et al., 1998) was used to calculate polymorphic information content (PIC) which was estimated according to Botstein et al. (1980). F-Statistics for all loci were computed (Nei, 1987), gene flow were estimated from Fst, Nm= 0.25(1 - Fst)/Fst (Nei, 1987), both analyzed with POPGENE. Genetic distance of D_A (Nei and Takezaki, 1994) and D_S (Nei, 1972) were calculated by PowerMarker V3.25 software (Liu and Muse, 2005). Phylogenetic tree was constructed from D_A by the Unweighted Pair-group Method using Arithmetic Mean (UPGMAM) and Neighbor-joining (N-J) method, also using PowerMarker V3.0 and viewed in TreeView software (Page, 1996).

Results

Allele frequency and distributions

Eleven microsatellite loci were amplified in the 3 subpopulations of Tianfu goats and their 3 relative breeds, generating specific genetic information in the present study. The allele frequencies and their distributions at 11 microsatellite loci in various subpopulations or breeds are shown in Fig. 1. A total of 92 alleles were detected across the 11 loci with a range from 6 (ILSTS008 and ILSTS087) to 14 (SR-CRSP-8) and an overall mean of 8.36 ± 2.34. The generated genotype ranged from 17 (ILSTS008 and ILSTS087) to 46 (SR-CRSP-8) with an average of 27 in all the tested samples. The major allele frequencies ranged from 0.212 (SR-CRSP-8) to 0.363 (INRABERN175) with an overall mean of 0.299. Two private alleles were found at ILSTS030, 1 at ILSTS008 in SAU-B, 2 at SR-CRSP-8 (0.0244) and SR-CRSP-1 (0.0488) in Boer goat, and 1 at ILSTS304 in SNY (0.0119). The result indicated that the marker panel used in this research was useful for the planned objective.

Genetic variation

Various measures of genetic variation across 11 loci are in Table 2. The numbers of effective alleles expected ranged from 4.13 to 7.35 with an overall mean of 5.28 ± 1.02. All the loci were not in Hardy-Weinberg equilibrium. Observed heterozygosity which varied between 0.271 (ILSTS087) and 0.561 (SR-CRSP-5) with an average of 0.418.

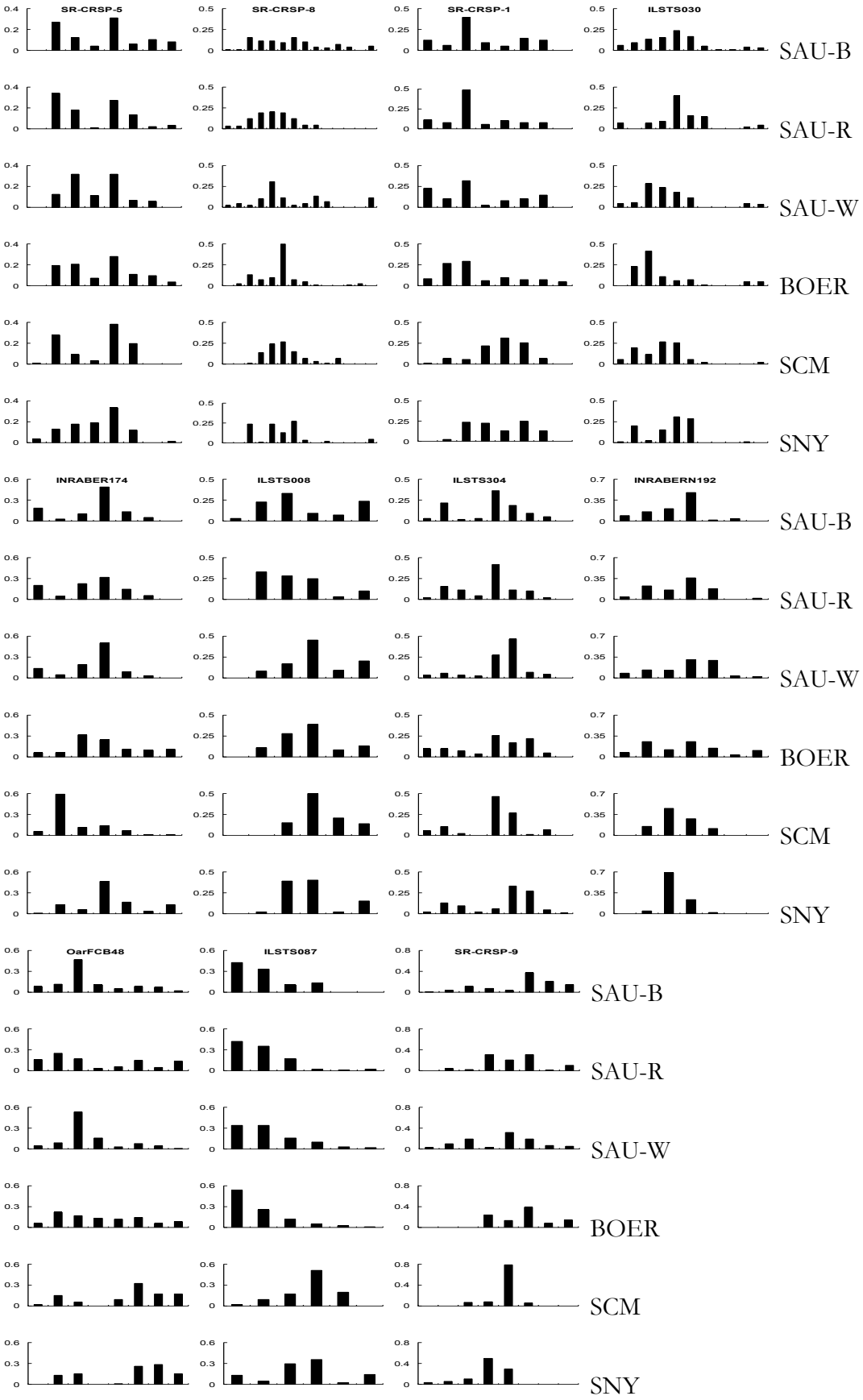

Fig.1. Allele frequencies and their distributions at 11 microsatellite loci in 3 subpopulations of Tianfu meat goat and their 3 relative breeds (SAU-B: B-subpopulation, SAU-R: R-subpopulation, SAU-W: W-subpopulation, BOER: Boer goats, SCM: Chengdu-Ma goats, SNY: Nanjiang Yellow goats).

Tab.3. Genetic variation of 3 subpopulations of Tianfu goats and 3 relative goat breeds[1].

Subpopulations/ /breeds	Sample Size	Genotype No.	Allele No.	MAF[2]	Expected Heterozygosity	Heterozygosity	PIC[3]
BOER	41	14.8182	7.1818	0.3503	0.7704	0.4479	0.7409
SAU-B[3]	48	14.7273	7.6364	0.3674	0.7649	0.4981	0.7347
SAU-R	44	13.4545	7.0000	0.3492	0.7620	0.4174	0.7281
SAU-W	44	13.1818	7.2727	0.3771	0.7521	0.3657	0.7201
SCM	43	10.6364	6.1818	0.4429	0.6889	0.3425	0.6528
SNY	42	10.1818	6.3636	0.3820	0.7201	0.4307	0.6793

[1] SAU-B: B-subpopulation, SAU-R: R-subpopulation, SAU-W: W-subpopulation, BOER: Boer goats, SCM: Chengdu-Ma goats, SNY: Nanjiang Yellow goats. [2]MAF: Major Allele Frequency; [3]PIC: Polymorphic information contents;

It was less than the expected heterozygosity calculated by Lewontin (1949) or Nei's (1973), with an overall mean of 0.806 and 0.805, respectively, across the 11 loci (all the loci showed heterozygote deficiency). The PIC varied from 0.720 (ILSTS008) to 0.850 (SR-CRSP-8) with an average of 0.778 ± 0.04; all the selected loci in this study were recognized to be high polymorphic loci (PIC > 0.5). Shannon information indexes were 1.80 ± 0.20 with a range of 1.52 (ILSTS008) to 2.21 (SR-CRSP-8). The PIC and Shannon information index (I > 1.5) showed that all the loci were highly informative with abundant polymorphism. The observed heterozygosities (Ho) were significantly lower than the expected heterozygosities (He), indicating that a high frequency of homozygote existed in the populations or breeds.

Lower observed heterozygosity was detected across subpopulations or breeds than expected heterozygosity or gene diversity (Table 3). The PIC varied from 0.653 to 0.741 across populations or breeds, and all above 0.5 (Table 3). F-statistics (Nei, 1987) and gene flow for all loci are shown in Table 4. Overall Fst was 0.077, indicating that only 7.7% of total genetic diversity was partitioned among populations or breeds and most of the variability is within populations or breeds with a percentage of 92.3%. All loci showed a high level of gene flow.

Tab.4. Summary of F-statistics (Nei, 1987) and gene flow for all loci.

Locus	Sample size	Fis[1]	Fit[2]	Fst[3]	Nm[4]
SR-CRSP-8	262	0.5475	0.5721	0.0543	4.358
SR-CRSP-1	262	0.5128	0.5426	0.0612	3.8323
SR-CRSP-5	262	0.2776	0.297	0.0268	9.0933
SR-CRSP-9	262	0.4424	0.5275	0.1526	1.3886
ILSTS030	262	0.3285	0.3685	0.0595	3.9515
ILSTS008	262	0.5207	0.5496	0.0603	3.8958
ILSTS304	262	0.4084	0.4414	0.0558	4.2312
OarFCB48	262	0.3537	0.4016	0.0741	3.1241
INRABERN192	262	0.5188	0.5614	0.0886	2.5715
ILSTS087	262	0.6037	0.6496	0.1158	1.9095
INRABERN175	262	0.3336	0.3979	0.0964	2.3422
Mean		0.4388	0.4817	0.0765	3.0176

[1]Fis: fixation index within subpopulations; [2]Fit: fixation index of total population; [3]Fst: Fixation index resulting from comparing subpopulations to total population; [4]Nm = Gene flow.

Tab.5. Genetic distance among the 3 subpopulations of Tianfu goat and 3 relative breeds[1, 2]

Subpopulations/breeds	BOER	SAU-B	SAU-R	SAU-W	SCM	SNY
BOER		0.2257	0.1770	0.2322	0.5076	0.4454
SAU-B	0.0970		0.1243	0.1713	0.5909	0.4027
SAU-R	0.0807	0.0713		0.2244	0.4690	0.3779
SAU-W	0.1002	0.0765	0.0937		0.4329	0.3946
SCM	0.1883	0.2106	0.1791	0.1749		0.2943
SNY	0.1903	0.1893	0.1789	0.1819	0.1296	

[1]Above the diagonal is Nei's standard distance (Nei, 1972) D_S and below the diagonal is the modified [2]Cavalli-Sforza genetic distance, Nei *et al.*'s (1983) D_A.
SAU-B: B-subpopulation, SAU-R: R-subpopulation, SAU-W: W-subpopulation, BOER: Boer goats, SCM: Chengdu-Ma goats, SNY: Nanjiang Yellow goats.

Genetic distance

Nei's Standard Genetic Distance (D_S) and the modified Cavalli-Sforza Genetic Distance (D_A) were estimated for each pair of subpopulations or breeds (Table 5). The closest distances were observed among the SAU-R, SAU-B and SAU-W of Tianfu goat with approximately equivalent distances, ranging from 0.071 to 0.094 based on the genetic distance of D_A. Furthermore, Boer goat had approximately equivalent distances with each of the 3 subpopulations of Tianfu goat with a range from 0.081 (SAU-R) to 0.100 (SAU-W), and SAU-R seemed to be closer to Boer than SAU-B and SAU-W. The genetic distance between SCM and SNY was 0.1296. SCM and SNY seemed to be distinct from Tianfu and Boer goat with approximately equivalent far distances ranging from 0.175 to 0.211. Genetic distance D_S among the studied subpopulations and breeds showed the same trend.

The phylogenetic trees constructed from D_A by the unweighted pair-group method using arithmetic mean (UPGMAM) and neighbor-joining method (N-J) are presented in Fig. 2 and 3. Both in the UPGMAM and N-J phylogenetic trees, SCM and SNY goats were clustered together and were separated from Tianfu and Boer goat. In the UPGMAM phylogenetic tree, 3 subpopulations of Tianfu were clustered together, separated from Boer and SAU-R were clustered on the node with Boer goat in the N-J phylogenetic tree, indicating that Boer goat had a closer phylogenetic relationship with Tianfu goat.

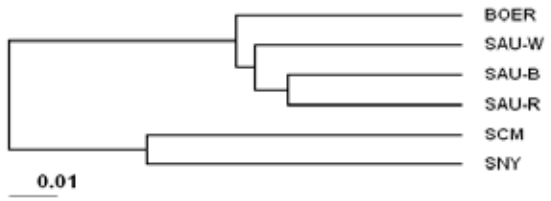

Fig.2. Phylogenetic tree constructed from DA by the UPGMAM method (SAU-B: B-subpopulation, SAU-R: R-subpopulation, SAU-W: W-subpopulation, BOER: Boer goats, SCM: Chengdu-Ma goats, SNY: Nanjiang Yellow goats).

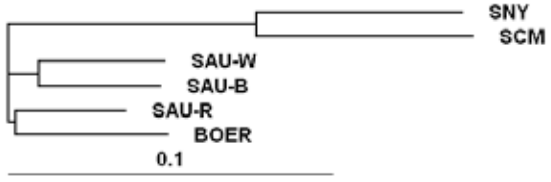

Fig.3. Phylogenetic tree constructed from DA by the Neighbor-joining method. (SAU-B: B-subpopulation, SAU-R: R-subpopulation, SAU-W: W-subpopulation, BOER: Boer goats, SCM: Chengdu-Ma goats, SNY: Nanjiang Yellow goats).

Discussion

This paper provides the first description of genetic diversity using molecular markers on subpopulations of Tianfu goat and its relative breeds. It contributed to a better genetic characterization and made it possible to gain an insight into the phylogenetic relationships of the six subpopulations or breeds.

With respect to genetic diversity, both SCM and SNY goats had the lowest average allele numbers of 6.18 and 6.36, respectively (Table 3). These can be considered as native populations and attributed to generations' selection, although SNY goat emerged from 2 local varieties crossbreed with the male hybrid progenies of SCM and introduced Nubian goats (Zhang et al., 2006). SCM and SNY contained less allochthonous genes compared to local varieties. The three subpopulations of Tianfu goats had the highest average allele number, and it could be attributed to complex hybridization of multi-varieties. The average allele number for Boer goats (7.18) was higher than that of SCM and SNY, probably because the tested individuals were sampled from the population introduced from different countries.

The average heterozygosity values of 6 subpopulations or breeds were between 0.343 (SCM) and 0.498 (SAU-B) with a high expected heterozygosity ranging from 0.689 (SCM) to 0.770 (Boer). These are similar to those observed in nine black goat breeds or populations in Sichuan Province (0.38 to 0.43). Furthermore, 6 populations or breeds studied in this research exhibited slightly lower PIC values than that of nine black goat breeds or populations (Wang et al., 2006). Populations or breeds studied in both, this studhy and that of Wang et al. (2006) had abundant polymorphism. In this study, only 7.7% of total diversity was partitioned among populations or breeds, partly because the 3 populations of Tianfu goat had a low genetic diversity and occupied a fairly large proportion in the tested samples. The 6 detected subpopulations or breeds that showed a high level of gene flow, higher than that of seven indigenous goat populations in Southern China (Yang et al., 2008), demonstrate that the rearing populations had a large gene flow with their relative breeds, more than with indigenous populations; indicating that the genetic background of Tianfu goat was complex as a filial generation of multi-breed crossbreeding.

The phylogenetic tree revealed a pattern consistent with the history and the geographic origin of populations or breeds. To a certain extent, the close distance among SAU-R, SAU-B and SAU-W was obviously due to their common genetic background. The observation of close relationship between SNY and SCM is consistent with the geographic origin and the history of the SNY breed. For the phylogenetic tree constructed from D_A by the neighbor-joining method, it is unclear why Boer first clustered with SAU-R; which may be related to the use of Boer goats for upgrading flocks in generations in SAU-R. The differentiation of the hair coat color probably resulted from different schemes of hybridization or various degrees of hybridization.

Conclusion

The results of this study agree with those obtained from morphological data and history of breeding. The study suggested that the three subpopulations of Tianfu goats have strong genetic similarities. A low level of intra-specific genetic diversity observed makes population differentiation difficult. All three should be considered belonging to the same population, although the three populations are phenotypically different. Genetic microsatellite analysis revealed that an abundant genetic polymorphism existed in the three subpopulations and suggested that Tianfu goats have great potentialities for further selection.

With great advantages of its genetic background, Tianfu goats have emerged as a new goat breed with excellent performance for meat production, reproduction efficiency, and easily adapted to the local environment. Consistent with the breeding history, the study suggested that Boer goat has a closer genetic distance with Tianfu. The genetic diversity among Tianfu, SCM and SNY goats revealed that Tianfu goats have a significant varietal differentiation. Microsatellite typing could be used as a reliable method to resolve perplexities for breed identification in the goat populations.

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Pacific Agriculture and Natural Resources

Treating Footrot in Goats Using a Zinc Sulfate Solution

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Abstract: A study was conducted to determine if a weekly footbath could reduce the severity of footrot in goats kept on pasture in a wet, subtropical environment. Thirty goats under one year of age were randomly assigned to two experimental groups. Fifteen goats received five-minute footbaths once a week, using a 10% zinc sulfate solution. The remaining 15 goats did not receive the weekly footbath. A scoring system was used to assess the severity of footrot damage in the goats during the study. This treatment regime proved ineffective in reducing the severity of footrot.

Keywords: Goats, footrot, treatment, zinc sulfate, foot bath.

Introduction

Footrot is a disease of goats and sheep that can lead to lameness and lower productivity. This disease is caused by different strains of the bacterium, Dichelobacter nodosus, which varies in its ability to digest the connective tissue of the hoof (Glynn, 2009).

Leite-Browning (2007) described several factors that can increase susceptibility to footrot in goats and sheep. Footrot is more common during periods of high rainfall when animals have to spend time in wet environments. Overgrown hooves can predispose animals to footrot.

Smith and Sherman (1994) recommended several practices to help control footrot in goats. One of those practices is the regular trimming of hooves to prevent hoof walls from overgrowing. During periods of wet weather, they recommended the use of footbaths for soaking hooves but they did not discuss the amount of time needed for this treatment.

Leite-Browning (2007) recommended using a 10% solution of copper sulfate or zinc sulfate to treat footrot. Farmers commonly use the 10% zinc sulfate solution in a footbath when treating animals. Leite-Browning (2007) did not explain why the 10% solution was recommended. However, it is theorized that copper sulfate and zinc sulfate solutions have bactericidal properties which are useful in killing the organism that cause footrot.

Treating a flock of goats with the footbath method is a time consuming process. Many farmers are very busy with day to day activities that would prevent them from devoting large amounts of time to soaking the hooves of goats. Any treatment program that would be adopted by farmers would have to be practical and not require large amounts of time. This question beckons to be answered how much time is needed, at a minimum, to treat footrot infected hooves.

From our literature search, we found one study (Christiansen and Cleveland, 1993) that tried to answer this question. In this study, a one-minute footbath was used every two weeks for treating footrot. This treatment protocol proved to be ineffective.

The objective of our study was to examine the effectiveness of a different footbath treatment protocol, of a longer and greater frequency. In this study, we wanted to mimic a treatment protocol that might be used at goat farms. It was decided to assess the effectiveness of a weekly, five-minute footbath using a 10% zinc sulfate solution.

Materials and Methods

Goats under one year of age were used for this study. These goats were a part of the University of Hawaii at Hilo flock which had experienced footrot during times of wet weather. Footrot causes a breakdown of the hoof pad which causes lesions. The sizes of the lesions are a measure of the severity of the disease.

Twenty-two wethers and eight doe kids (30 goats) were weaned off their mothers in late spring and were being grown out for meat production in the fall of 1999. A week before the study started, the hooves of all the kids were trimmed uniformly. Hooves were not trimmed during the experimental period.

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The 30 kids were randomly assigned to two experimental groups. The control group was not treated for footrot while the treatment group received a five-minute footbath once a week during the eight-week study. The treatment animals were treated in a random order each week. Because of time constraints, sets of about four to five goats were moved into a chute with the footbath. After entering the chute, the soaking of the hooves began. After standing in the treatment solution for five minutes, the goats were released from the chute and placed on pasture.

To obtain the 10% zinc zulfate solution for the footbath, 8 pounds of zinc sulfate was mixed with every 10 gallons of water. After adding the zinc sulfate to the water, the solution was stirred for about two to three minutes. During the study, the footbath was drained and flushed out every two weeks. At that time, a newly mixed batch of 10% zinc sulfate solution was added to the footbath.

The experiment started on October 15, 1999 and ended on December 3, 1999. Each week during the study the feet of all the goats were examined and scored for severity of footrot damage. Scores on each animal were determined by the same two scorers for weeks 1, 2, 3, 4, 5, 6, and 8 of the study. On week 7, one of the scorers was absent so all scores during that week were determined by the second scorer.

A system was developed where each claw of the hoof was scored for both depth and size of footrot lesions. A given claw could have multiple lesions. The depth of the lesion was scored as follows:

0 = no lesion

1 = a lesion of slight depth (less than 8% of plantar hoof length)

2 = a lesion of moderate depth (8% to 16% of plantar hoof length)

3 = a lesion of great depth (>16% of plantar hoof length)

The area of the foot rot lesion was scored as follows:

0 = no lesion

1 = lesion covering a small area of hoof pad (less than 20% of area)

2 = lesion covering a moderate area of hoof pad (20% to 40% of area)

3 = lesion covering a large area of the hoof pad (> 40% of area)

The score for the depth of lesion was multiplied by the score for the lesion area. Every lesion on a claw was scored in this manner. The overall score for a claw was calculated as the sum of these lesion scores (score for depth of lesion multiplied by score for lesion area). The total score for the four feet of an animal was calculated as the sum of the overall scores for the eight claws. The average score per claw was calculated by dividing the total score (for the four feet) by eight. The average score per claw were used in the analysis of variance.

During the experiment, the goats were kept together in one group and allowed to graze various paddocks on the University of Hawaii Farm near Hilo. The paddocks contained tropical grass, weeds, shrubs, and trees on land with soil and rock substrates. Goats were also given access to a salt and trace mineral supplement.

For analysis of the data, GLM procedures (SAS ver. 9.1.3, 2002-2003) were used. For each animal, repeated measurements (average score per claw) were made during the study. The GLM procedure for between subject effects included gender and treatment in the model. For the univariate analysis for within subject effects, the GLM procedure was used with time, time*gender, and time* treatment being included in the model.

Results and Discussion

The mean claw scores for each group are presented in Table 1. The two genders did not differ ($P = 0.30$) in claw scores. We would not expect wethers and doe kids to differ in the severity of footrot damage. Previous footrot studies did not included different genders in their trials (Thompson et al.1995 and Vandyke et al. 1999).

Tab.1. Means claw scores for goats in the control and treated group on different dates.

Date of Scoring	Control	Treatment
October 15	3.2 ± 1.2	3.1 ± 0.8
October 22	4.0 ± 1.2	4.2 ± 1.0
October 29	5.6 ± 1.2	5.7 ± 1.3
November 5	5.4 ± 1.1	5.5 ± 0.9
November 12	5.7 ± 1.5	5.8 ± 0.8
November 19	5.0 ± 1.4	4.8 ± 1.6
November 26	2.9 ± 1.2	2.3 ± 0.9
December 3	5.0 ± 2.2	4.7 ± 1.3
Overall Mean	4.6	4.5

Both treatment and control goats showed signs of footrot damage throughout the study. However, the control and treatment goats did not differ ($P = 0.81$) in mean claw scores. Weekly and the overall mean claw scores were very similar for the treatment and control goats (Table 1). These results indicate that a once a week, five-minute footbath using a 10% zinc sulfate solution is ineffective in reducing the severity of footrot damage. These results are very similar to those

of Christiansen & Cleveland (1993) who used a one-minute foot bath every other week to treat goats. Future research trials should examine the effectiveness of using footbaths of a greater length and frequency.

Time had a significant effect (P <0.0001) on digital foot rot scores but time*gender and time*treatment effects were not significant. Mean claw scores increased from October 15 to November 12 but declined from November 12 to December 3 (Table 1). Christiansen & Cleveland (1993) also reported a significant time effect in their study which they attributed to changes in rainfall. Wet conditions could be expected to exacerbate footrot in goats.

Our study was conducted during the months of October, November and December of 1999. During those months the respective rainfall amounts were 3.61 inches, 7.74 inches and 14.41 inches (U.S. National Climatic Data Center, 1999). The change in rainfall may have had some effect on our results.

Scorer errors or other factors may have also contributed to the time effect. In our study the scorers tried to be consistent scoring the goats. However, there can be errors in any footrot scoring system.

Acknowledgements

We greatly appreciated the help that Ken Smith, Lani B. T. Tran and other students provided during the study. Without their help, we could not have conducted this study. These students helped with handling, scoring and treating the goats. Ken Smith also helped in summarizing the data. Thanks are also due to Arnold Miyasaki who helped move the goats to and from the facility each week.

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First Annual TCBES Symposium

ABSTRACTS

1st Annual Tropical Conservation Biology and Environmental Science Symposium

TCBES Symposium Organizing Committee: Donald Price, Misaki Takabayashi, Patrick Hart, Nancy Chaney. Layout and original art by Brittini Kimura

The first annual TCBES Symposium includes papers reporting original research by graduate students in the TCBES Master of Science Program at University of Hawai'i at Hilo. Graduate students currently enrolled in, or recently graduated from, the program are invited to present their research in oral or poster form. This first annual TCBES Symposium includes more than 20 presentations.

The primary purpose of the M.S. Program in TCBES is to provide graduate training in conservation biology and environmental science to people with baccalaureate degrees and others currently working in the field. The program draws on the extraordinary biological, physical and cultural complexity of Hawai'i Island as a focus of investigation. TCBES prepares students for technical positions and for entry into Ph.D. programs in related fields. The program admitted its first cohort in fall 2004, and in maintains an average enrollment of more than 40 students per year. The first nine graduates received their degrees in May 2007, and an additional 14 students graduated in 2008. Recent graduates are working in the fields of conservation and education as well as pursuing Ph.D. degrees.

TCBES unites 31 faculty at UH Hilo with 51 affiliated faculty from federal and state agencies and other universities. Participating UH Hilo faculty reside in the Anthropology, Biology, Chemistry, Geography, Geology, and Marine Science Departments of the College of Arts and Sciences, and in the College of Agriculture, Forestry and Natural Resource Management. As a multidisciplinary, multi-college program, TCBES encourages and facilitates sustained collaboration across the research areas of ecological and evolutionary genetics, ecosystems analyses and responses to environmental change, cyberinfrastructure for environmental research, and geospatial analyses. Faculty and students engage in projects in environments that range from marine coastal habitats to tropical rain forests, mesic and dry forests and shrublands, and rivers. At the core of the TCBES Program are the dynamic research collaborations between faculty/students and federal and state agencies on Hawai'i Island.

TCBES is supported by the College of Arts and Sciences and Natural Science Division at the UH Hilo, the National Science Foundation (NSF) Experimental Program to Stimulate Competitive Research (EPSCoR), the NSF GK-12 Partnership for Reform through Investigative Science and Math (PRISM), the NSF Centers for Research Excellence in Science and Technology (CREST), and grants to TCBES faculty.

A Survey of The Genetic Diversity of Free-Living Symbiodinium: The Foundation of A Mechanism of Adaptation in Reef Corals

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Coral bleaching is one of the most imminent threats to coral reefs worldwide and is primarily caused by severe thermal stress, which disrupts the coral's symbiotic association with the unicellular algae Symbiodinium. Thermal sensitivity, in some coral species is determined by the type of Symbiodinium the host associates with. One predominating theoretical mechanism of coral adaptation is that some species of host can adapt or acclimatize by switching the dominant type of Symbiodinium. Free-living Symbiodinium are the source of symbionts for many hosts, and could potentially offer a great deal of diversity and thus opportunity to coral hosts. Our research is one of the first to survey and compare the genetic diversity of free-living Symbiodinium in the water column and sediments of coral reefs of Hawai'i, the Florida Keys, and Okinawa Japan, using direct detection methods. In preliminary DNA analysis, we have detected 43 different Symbiodinium types that represented 6 of the 8 clades of Symbiodinium. Of these types, 15 have been previously identified in symbiotic hosts and 28 types are novel to field. Trends in diversity between habitat type and region may also be found in further analysis. This research will likely serve as a platform for research investigating the response of corals to warming sea temperatures and other effects of climate change.

Effects of Hydrological Forcing on the Structure of A Tropical Estuarine Food Web

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The impact of river flow on organic matter (OM) source utilization of a tropical estuarine food web was examined using stable carbon (C) and nitrogen (N) isotope signature analyses of OM sources (terrestrial, estuarine, and marine), zooplankton, and juvenile fish. Over one year, dominate zooplankton taxonomic groups, 150-500 □m and >500 □m bulk zooplankton samples, and fish of recreational, economical, and cultural importance were collected from three stations within Hilo Bay, Hawaii, USA, during both periods of high and low river flow conditions. Results show that there was significant hydrological and spatial variability in OM source utilization for consumers collected within Hilo Bay. During increased river flow in Hilo Bay, contribution of estuarine OM to consumers' 13C and □15N signatures was generally reduced by three to nine times, and the contribution of terrestrial OM to consumers' signatures was reduced by ~10%. In contrast, marine OM contributions to consumers' 13C and 15N signatures generally increased by about three times. Our results suggest that the hydrological variability in inorganic nutrients and turbidity of exported river water, biological availability of exported terrestrial OM, and estuarine bacteria biomass were the main factors driving OM source utilization by consumers in Hilo Bay. If Hilo Bay is representative of tropical estuaries, then our results suggest that food web structures within these systems are more vulnerable to intra- and interannual climatic variables as well as changes to upland watersheds.

Connectivity Patterns of Two Hawaiian Marine Gastropods Possessing Nonpelagic Development

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Connectivity of benthic marine gastropods in Hawaii is poorly understood due to the lack of extensive studies. In species lacking a pelagic larval phase, connectivity between populations would be expected to be low because of limited dispersal ability. To test if geographic distance is related to genetic distance, we sampled two nonpelagic developers from the Superfamily Buccinoidea, Mitrela fusiformis and Peristernia chlorostoma. These two species both occur in the subtidal marine environment within shallow, protected inlets along the coast of the Hawaiian Islands. Samples of Mitrella fusiformis were collected from locations on the islands of Hawaii, Kauai, and Oahu. Samsples of Peristernia chlorostoma were collected from numerous sites across the Hawaiian archipelago. Overall, the neighbor joining tree analysis of both species places geographically nearer populations closer together on the tree. The resulting DNA sequences from Mitrella fusiformis, spanning a 360 base pair (b.p.) section of the mitochondrial COI gene, show evidence that there is clustering in genetic variability of within island populations. The 656 b.p. COI sequences from Peristernia chlorostoma also exhibit this clustering of within island populations. In addition, there are differences in sequence between populations that are located less than 1 km apart from each other. These data support the concept that some nonpelagic developers from distinct, localized populations and that connectivity between populations may be very low. Understanding these gastropods' connectivity may provide information important to other nonpelagic species, and help to associate and utilize these patterns within the context of marine conservation.

Breeding Phenology of Hawaiian Petrels and Newell's Shearwaters on Kaua'i, Hawai'i: Insights From Radar, Auditory, and Visual Surveys

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Hawaiian petrels (Pterodroma sandwichensis) and Newell's shearwaters (Puffinus auricularis newelli) are endemic to the main Hawaiian Islands, and considered federally endangered ant threatened, respectively. For both species, regular monitoring is required by management to determine population trends. Since direct measures of population size and breeding success have proven impractical on Kaua'i Island at many of the steep and densely vegetated breeding sites, monitoring for both species to date has relied upon indirect measures of activity, primary movement rates during June (incubation) using ornithological radar. Interpretation of these results and informed decision making about survey design can be improved with an understanding of how indirect measures such as movement and calling rates vary with major breeding tasks. From March – December 2008, we undertook weekly ornithological radar (both species), auditory (Newell's shearwaters only) and visual surveys (Hawaiian petrels only) to monitor intra-annual variation in movement and calling rates throughout an entire breeding season. Results were compared against a comprehensive literature review of predicted breeding phenology. All three measures showed correlation with major breeding tasks, including decreased activity with pre-laying exodus and peak activity during chick-rearing and presence of non-breeders. Ornithological radar data consistently showed the lowest coefficient of variation, suggesting this method would have the greatest statistical strength as an annual monitoring tool, with incubation and guard surveys likely to yield reliable annual monitoring data. The combination of radar, auditory and visual surveys

provided most information when determining the timing of breeding tasks for each species separately.

The Effects of Skin and Body Hydration on the Susceptibility of the Frog, Eleutherodactylus Coqui, to Citric Acid as A Control

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Since 2002, 16% citric acid solution has been widely used for controlling the rapid spread of the invasive Caribbean tree frog, Eleutherodactylus coqui, in the Hawaiian Islands. Subsequently, laboratory studies have shown a large variation in mortality from citric acid, possibly related to frog acclimatization to drought conditions. I evaluated the efficacy of citric acid treatments under controlled laboratory climatic conditions while manipulating frog skin and body hydration status. Frogs were exposed to high (95-99%) or low (20-23%) relative humidity while in ventral contact with liquid water, so that low humidity would dehydrate the dorsal skin without affecting whole body hydration. Eight percent citric acid solutions produced 100% mortality in both low and high relative humidity groups, but at 4% citric acid, there were significant differences in survival rates. Frogs exposed to high humidity with moist skin had mortality rates of 60-65%, while frogs exposed to low humidity air with dehydrated skin had mortality rates of 90-100%. At 2% citric acid exposure, frog mortality showed a similar pattern with a further mortality reduction: frogs exposed to high humidity with moist skin had mortality rates of 0% while frogs exposed to low humidity with dry skin had mortality rates of 10-40%. Frog plasma osmolality was analyzed to verify that humidity treatment did not affect body hydration as a possible mechanism of toxicity. There was a significant increase in plasma osmolality when the frogs were exposed to 4 or 8% citric acid versus distilled water, but there was no significant difference in the plasma osmolality between frogs that lived or died when exposed to 4% citric acid. Hydration status of the coqui frog skin and body does not appear to affect the efficacy of citric acid exposure unless the concentration drops below 8%. Dry skin seems to have a greater effect increasing susceptibility to citric acid exposure. Environmental drought conditions, with lower relative humidity that dehydrates frog skin, is expected to make frogs more susceptible to citric acid exposure. However, dry conditions are likely to induce frogs to seek cover and thus avoid contact with citric acid sprays.

Population Differentation and Local Adaptation Within the Picture-winged Drosophila, D. Sproati

Jon Eldon
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(Advisor: Dr. Donald Price)

The Big Island oh Hawai'i, a conglomeration of five volcanoes that range from active to 700,000 years dormant and reach up to nearly 14000' above sea level, contains nearly all of the world's major ecotypes. Within this relatively small land area there are arid deserts and thick rainforests, barren lava flows and deep fertile soil, continuous tracts of forest and naturally fragmented islands of habitat. In many cases these disparate environments are found within a few miles or even a few feet of each other, though both between and within them there are clear and often continuous gradients of temperature, humidity, soil quality, and even biomass. These conditions make the Big Island an ideal location to study the mechanisms that drive the differentiation of populations, a precursor for speciation and a fundamental part of our understanding of the generation and maintenance of biodiversity. The Hawaiian Drosophila flies are an ideal group of species to study local adaptation, population genetics, and speciation. Though to have derived from a single wind-blown founder over 10 million years ago, these flies have radiated tremendously and there are estimated to now be at least 700 endemic species. Using the natural laboratory of the Big Island, I am investigating the role of distance, fragmentation, and environmental gradients in producing population differentiation within a nearly unknown species, D. sproati. Now the most common and widespread species among the well studied "picture-wing" group on the island, D. sproati is found on all 5 volcanoes and within a 2000' range. I am currently assessing variation between D. sproati populations using mitochondrial and nuclear neutral genetic markers and through multiple temperature-tolerance measures of local adaptation.

Plumage Coloration of A Hawaiian Honeycreeper(Je,ogmatjis Virens) Across Gradients of Biogeography

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(Advisor: Patrick Hart)

Avian vision is unlike human vision in that it encompasses both the ultraviolet and visible range of the light spectrum. Therefore, studies on avian plumage require an objective method for measuring coloration such as spectrometry, where the reflectance of the feathers is measured across the entire spectrum 200-700 nm. The sites of this study were comprised of three elevation categories (low, middle, and high) and two rainfall categories (mesic /wet and xeric/ dry), to give 6 biogeographical categories with two replicates each. At each site all adult 'amakihi were measured for morphometrics, banded, sex and age was determined by key, and feather samples (7-10 feathers) were taken

each from the breast and rump of each bird. Feathers were affixed to non-reflective black paper, mimicking the way they lay on the bird and scanned in triplicate for percent reflectance. All spectra were summarized as color variables of chroma (S3, S9, S1B), hue (H1), and brightness (B1) using a color analysis program (CLR, Montgomerie). I used a two-way ANOVA and found elevation and rainfall as well as the interaction between elevation and rainfall to be significant factors in the prediction of breast and rump chroma (S3, S1B). A nested ANOVA (nested factors: rainfall/ elevation/ site) model was significant in predicting carotenoid chroma (S1B) for breast and rump plumage. Our finding that 'amakihi coloration varies with elevation and rainfall may imply that present plumage is a result of natural selection across these environmental gradients, or may be indicative of carotenoid-rich resource availability at each site, as carotenoid pigments cannot be synthesized. In the case of support for the former, these results may be considered a phenotypic compliment to recent molecular studies which found 'amakihi populations to be genetically distinct and spatially structured according to elevation on the Island of Hawai'i.

Feeding Ecology of Hawksbill Sea Turtles (Eretmochelys Imbricata) in Hawai'i

Shannon Graham
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(Advisor: Dr. Jason Turner)

Foraging studies on hawksbill sea turtles (Eretmochelys imbricate) in Hawai'i are limited. Previous tracking investigations have indicated the location of hawksbill foraging habitats, however, the only known data on pretty species (sponges), comes from the stomach of a single deceased turtle. Here I incorporated satellite telemetry with dive depth information on post-nesting females (n-3) from Hawai'i Island to ascertain feeding depths. Further, benthic surveys at the chief foraging habitat were conducted to determine sponge abundance, and stable isotope analysis was used to retrospectively verify the proximity of dominant prey species to hawksbill sea turtles. Shallower dives (0-10m) took place >90% of the time at the foraging habitat where benthic surveys were conducted. Sponges accounted for 0.46% of the survey areas, the majority residing in areas with higher topographical relief. Carbon stable isotope values for mussels (Isognomon sp.), sea cucumbers (Holothuria sp.), fireworms (Pherecardia sp.), and a variety of sponges including Tetrapocillon sp., Spongia sp., Cacospongia sp., and Spirastrella sp. were significantly distinguished from brittlestars (Ophiocoma sp.), urchins (Tripneustes gratilla), and zoanthids (Palythoa caesia) (p value < 0.001, $r^2 = 0.92$). Adult turtles had a mean $\delta^{13}C$ value of -15.6 ± 0.8 ‰ and $\delta 15N$ value of 8.1 ± 1.2‰. Dominant prey organisms can be inferred by comparing the proximity of carbon and nitrogen isotopes. Further statistical analysis is currently in process to confirm preferred prey species. Results from this study will contribute to the baseline database for feeding behavior of local hawksbill turtles and identify potential hotspots upon which to focus future conservation efforts.

Transfer if Maternal Antibodies in Hawai'I 'Amakihi (Hemignathus Virens)

Bobby Hsu
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(Advisor: Patrick Hart)

In many bird species, breeding females may provide additional protections against diseases in form of maternal antibodies in order to improve the survival of the nestling at early fledgling stage. If passive transfer of maternal antibodies against avian malaria (Plasmodium relictum) occurred in Hawai'i 'amakihi (Hemignathus virens virens), the effects of maternal antibodies would be observed in a wild population at low elevations in Hawai'i where avian malaria is prevalent. I conducted a three-part investigation to test this prediction. First, using Enzyme-Linked ImmunoSorbent Assay (ELISA), I analyzed the plasma and eggs of breeding female 'amakihi for anti-Plasmodium antibodies at a combination of low and high elevation sites on the Island of Hawai'i. Analyses showed significantly higher anti-Plasmodium antibody levels in 'amakihi eggs at low elevation. Second, I used captive canaries (Serinus canaria) as a model to investigate the effects of maternal antibodies in the neonates of passerine birds. I quantified parasitemia and anti-Plasmodium antibody levels in eggs and plasma of the offspring in a treatment group (hens with positive infection) and a control group (hens with negative infection). Canary eggs from the treatment group showed a higher anti-Plasmodium antibody level compared to eggs from the control group. However, I found no significant effect of maternal antibodies on the immunity of canary offspring, possibly due to insufficient sample size. Third, using data available from the Biocomplexity of Introduced Avian Diseases in Hawai'i research project, I examined evidence for the presence and effects of maternal antibodies in hatch-year 'amakihi. Hatch-year 'amakihi exhibited suppressed immunity responses during earlier months of the year at low elevation forests. This phenomenon could be caused by the "blocking effect" from maternal antibodies.

Factors Influencing Success of A Marine Protected Area Network in Hawai'i

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Jim Beets, Brian Tissot, Mark Hixon, Stosh Thompson
(Advisor: Dr. James Beets)

At Marine Protected Area (MPA) Networks gain management support, the importance of documenting factors that affect their effectiveness increases. Previous work showed greater abundance of juvenile and adult fishes harvested for the aquarium trade in individual MPAs compared to adjacent control sites within the MPA Network on the western coast of the island of Hawai'i (Tissot et al. 2004). The present study evaluated the relative contribution of habitat characteristics, adult abundance, and current exposure to the greater juvenile abundances observed within MPAs. Oceanographic, substrate and habitat characteristics were sampled with juvenile and adult fish abundances at three paired sites (MPA vs. control) along the western coast of the island of Hawai'i. All three factors contributed to greater juvenile abundance of the study species, but differed in relative contribution among sites. We demonstrated the importance of selected factors to the effectiveness of a MPA network. Based on or results, we emphasize the value of consideration of several factors that potentially influence success of the defined goals for specific MPAs or networks.

Strategic Light Manipulation Can be A Sistaomab;e Restoratopm Strategy to Suppress Alien Grasses and Encourage Native Regeneration in Hawaiian Mesic Forest

Sierra McDaniel
TCBES Program, UH Hilo, & Natural Resources Management, Hawaii Volcanoes National Park
(Advisor: Dr. Rebecca Ostertag)

Strategic canopy development may reduce the presence of alien grasses and foster native, self-sustaining communities in abandoned pastures. In a two-part study we examined the effect of light availability on common native woody and alien grass species found in secondary forests in Hawaii dominated by Acacia koa. We first conducted a field survey to examine the relationship between light availability and canopy type (open pasture, planted canopy, and secondary forest) on understory grass biomass and litter accumulation. Alien grass biomass was greatest under the open pasture and lowest in the secondary forest. There was a positive relationship between understory light availability and alien grass biomass, with no grass when light was less than 4%. Secondly, we experimentally manipulated light levels to determine the effect of light availability on growth and survival of six native woody species and three alien grasses. At three sites in secondary A. koa forest, three different light treatments, low light (5% transmittance), medium light (10% transmittance), and high light (20%-30% transmittance), were created using shade structures erected beneath the existing koa canopy. Across sites, relative growth rate (RGR) decreased as light availability decreased for all of the grass species and four of the six woody species. Although growth is substantially reduced, survival is still high (84-100%), indicating these species will persist under closed canopy. Establishment of sufficient canopy cover to suppress alien grasses and improve conditions for native species is a promising approach to create native self-sustaining communities through restoration of native structure and diversity.

Prism Fellows: Communicating Science Thorugh Culture, Connections & Conservation

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(Advisors: Dr. Elizabeth Stacy, Dr. Jan Zulich and Dr. Donald Price)

PRISM Fellows at UH Hilo are broadening science literacy in Hawai'i by developing culture-infused, place-based science curricula, forging connections between K-8 students and local scientists, and promoting environmental awareness and action. Over three years, 28 PRISM Fellows have partnered with over 50 elementary and middle school teachers to develop 19 standards-based curricula in marine and terrestrial environments. PRISM curricula incorporate math, art, language arts, and technology and have been implemented in over 60 classrooms, teaching over 1,400 students. PRISM Fellows bridge modern scientific practices, including the important Hawaiian value, "Malama ka 'Aina," or "Caring for the Land." Fellows further enrich these lessons by recruiting scientists from Hawaii's federal, state, and nongovernmental agencies into the PRISM 'ohana (family). These scientists, many of whom are affiliate faculty of UH Hilo's TCBES M.S. Program, are broadening hands-on opportunities for students in he conservation and restoration of native species and habitats. Through such collaborations, PRISM is developing a community of practice in science education and empowering students to make a difference in their communities. Fellows further develop their communication skills and promote scientific literacy through outreach to non-partner teachers, integration of PRISM curricula into teacher workshops, coordination of Science Nights at PRISM schools, and participation in community events. Fellows also communicate science to a broad audience through the development of PRISM website, newspaper and newsletter articles, a PRISM brochure, individual project posters, and local TV and radio segments.

Effects of Light Availability on Biomass and Reproductive Organ Production of the Invasive Rangeland Shrub Ulex Europaeus L. on Mauna Kea, Hawaii

Cheyenne Perry
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(Advisor: Dr. Rebecca Ostertag)

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I surveyed forests and plantations on the eastern slopes of Mauna Kea to determine light availability under different species' canopies and then tested whether artificial shading (shade cloth structures) that mimics these forests would limit gorse growth and reproduction. I created and applied five light treatments to gorse individuals/cohorts: ambient light (control), 73%, 80%, 90%, and 98% shade and measured basal diameter, height and volume of plants for 12 months. Following the final measurement all plants were harvested to determine dry mass. I used allometric modeling to determine relative growth rates and reproduction for gorse. Preliminary results show that the 73% shade treatment decreased biomass accumulation to 1/3 that of the control and that this trend continued up to 98 % where gorse individuals/cohorts died and biomass was reduced to just 1/10 that of the control. These early results suggest that planting native forests and/or plantations in gorse stands will significantly reduce biomass accumulation.

Ulex europaeis L. (gorse) are thorny shrubs commonly associated with degraded pasturelands, an extreme fire hazard, and classified as noxious weeds in the State of Hawaii. They can form monotypic stands that infest thousands of acres that alter native ecosystems, produce highly acidic soils, and suppress native plant germination, such as at Mauna Kea, Hawaii. However, gorse is light demanding so the addition of forest canopy over gorse stands may be an effective bio-control. I seek to isolate the percentage light availability provided by forests and plantations that produce significant decreases in gorse biomass accumulation and reproductive organ production.

Evolution of Metrosideros Polymorpha Across an Island-age Gradient in Hawai'i

Doug Powless
TCBES Program, UH Hilo
(Advisor: Dr. Elizabeth Stacy)

Island ecosystems, especially in Hawai'i, are famous for their diversity of endemic species. Understanding the basic processes that produce theses adaptive radiations is important for conservation of biodiversity. The highly variable Hawaiian tree species, 'ohi'a (Metrosideros polymorpha), is a dominant and foundational species across most natural communities of the main islands of Hawai'i. In this study, we focus on the divergence of the three most common 'ohi'a varieties (polymorpha, incana, and glaberimma) across an island-age gradient. We examined the divergence of morphological and phenological traits and the relative fitness (reproductive output) of varieties and apparent hybrids at six sites representing different ages in the Ko'olau Mountains of O'ahu (~2.5 million years old), the Kohala Mountains of northwestern Hawai'i Island (~1 million years old), and young lava flows of eastern Hawai'i Island(<200 years old). Under the hypothesis that disturbance (primary volcanism) promotes introgression among varieties, and that lack of disturbance promotes divergence and potential speciation of varieties, we expect to find more reproductive isolation (e.g., different peaks in flowering time) and more morphological variation between varieties at older sites than at younger sites. Analyses of morphometrics suggest that divergence is occurring within this species and that age of site (and hence age of population) may influence differentiation between varieties. This is consistent with greater introgression among varieties in eastern Hawai'i Island, where populations are regularly disturbed by volcanism. However, preliminary analyses of phenology data do not support our second prediction that varieties at older sites will be more reproductively isolated from one another through flowering phenology than at younger sites.

Host Specificity and Biology of Syphraea Uberabensis (Coleoptera; Chrysomelidae) for the Potential Biological Control of Tibouchina Herbacea (Melastomataceae) in Hawaii

Steven Kazunori Souder
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(Advisor: Dr. Donald Price)

The South American flea beetle Syphraea uberabensis was evaluated as a potential biological control agent for the invasive weed, Tibouchina herbacea (Melastomataceae), in Hawaii. Establishment and distribution of S. uberabensis in Hawaii appeared unlikely to be limited by temperature. Beetles had a minimum temperature threshold near 13 ℃. Egg eclosion required 107 degree day units, and egg to adult eclosion required 348 degree day units. The potential host range of S. uberabensis was assessed on the basis of behavior, feeding damage, survivorship, oviposition, and fecundity using simultaneous no-choice testing on 35 plant species in 11 families. S. uberabensis was not mono-specific to the target weed, T. herbacea, but oligophagous towards several species in the family Melastomataceae. Its host range appeared to be acceptable for Hawaii, where there are no native species of Melastomataceae and many are listed as noxious weeds. S. uberabensis oviposition and fecundity revealed a strong host preference for T. herbacea, Tibouchina longifolia, Pterolepis glomerata, and Melastoma candidum. K-means cluster analysis using 8 test variables and predetermined partitioning, clustered T. herbacea, T. longifolia, P. glomerata, and M. candidum as strong host plant candidates. In Hawaii, S. uberabensis may prove to be an effective control against these weedy melastome species.

Field Control of the Invasive Little Fire Ant, Wasmanna Auropunctata (Roger) (Hymenoptera: Formicidae) in Tropical Fruit Orchards

Evan Souza
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(Advisor: Dr. Donald Price)

The little fire and (LFA), Wasmannia auropunctata (Roger) (Hymenoptera: Formicidae) is an invasive fire ant that forms supercolonies when it successfully invades new areas. LFA was first reported in Hawaii in 1999 and has since invaded a variety of sites including nurseries, farms, orchards, pastures, playgrounds, and homes. LFA has also been shown to have detrimental effects on native habitats and invertebrate populations. Amdro (hydromethylnon; in bait stations), Esteem (pyriproxyfen; broadcast bait), and Conserve (spinosad; spray) were tested for their efficacy against LFA in a tropical fruit orchard by making biweekly treatments for 16 weeks. Amdro and Esteem treatments showed a significant reduction in LFA and associated Homoptera on weeks 12 and 16 compared with untreated control treatments. None of the treatments eliminated LFA. Many LFA were found nesting in protected sites in the orchard trees, which may compromise ground-based control methods. Absolute density estimates from thatch samples taken from the orchard suggested the supercolony exceeded 95 million ants per acre. The results of this study are important due to the economic importance of small agriculture in Hawaii, the quarantine status of this ant and the their threat to native ecosystems. The results of this study may also help in the management of problematic non-stinging ants, which also tend Homopterans and can cause exportation delays during product shipment. Reducing populations of LFA in agricultural areas in Hawaii may reduce spread of these highly invasive ants into native ecosystems and therefore decrease their negative effect on native habitats and invertebrate populations.

Effect of Rising Salinity on Egg and Larval Survival of the Orange-black Hawaiian Damselfly

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Decreased ground-water flux associated with urbanization and climate change have the potential to increase the salinity of anchialine pools at Hawaii's Kaloko-Honokohau National Historical Park (KAHO). These unique tidal coastal aquatic features posses an endemic invertebrate fauna including candidate endangered crustaceans and aquatic insects. To assess some of the effects of rising salinities on anchialine pool biota, we are quantifying the survival of egg and early-instar larvae of the Orange-black Hawaiian damselfly (Megalagrion xanthomelas). Preliminary results reveal and inverse relationship between egg and early-instar survival and salinity, with this species possibly exhibiting a threshold response to rising salinity at 20 ppt (approximately 60% seawater). Given the limited information on the effects of increased salinities on anchialine pool biota, this study will provide management implications and raise awareness on the importance of these rare environments.

Development of A Passive Diffusive Sampling Deviece For Time-integrated Assessment of Atrazine in Hawaiian Streams

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(Advidor: JP Michaud)

Atrazine, a globally used herbicide, can enter into and persist in groundwater and aquatic environments. Atrazine was used extensively on sugarcane along the Hamakua coast and may still be in use in Hawaii sporadically as an off-label herbicide for diversified agriculture or weed management on golf courses and athletic fields. Health concerns exist due atrazine's known ability to act as an endocrine disruptor and to accumulate in adipose tissues of organisms. Due to atrazine's potential for biomagnification, effects at higher trophic levels including humans is possible. To help address these concerns, methods of tracking atrazine in the environment are needed. This study describes the development and characterization of a diffusive sampling device designed specifically for the time-integrated measurement of atrazine in streams that experience fluctuations in water quality, such as those present in Hawaii. For the optimization of the diffusive sampler, various membranes were evaluated, biofouling of membranes was assessed, and the effects of pre-wetting, presence of organic solvent or surfactant in C18 coated interstitial spaces of the solid phase sorbent were examined. The effect of change in diffusive boundary path length and the comparability of integration with variation of concentration were also examined. Laboratory and field data gathered demonstrated the applicability of this sampling device for the environmental monitoring of atrazine in time-variant streams and land-use patterns in Hawaii. This device was also designed to be rugged and useful for monitoring atrazine globally in a diverse array of environments.

Hilo Core Genetics Facility at the University of Hawaii at Hilo

Anne Veillet
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(Advisor: Dr. Donald Price)

The Hilo Core Genetics Facility (HCGF) at the University of Hawaii at Hilo was established in 2003 to improve the science and technology infrastructure of Hawaii. The facility gives researchers, educators, and students the opportunity to gain access to technological services and technical training that may not be otherwise available. The bridges built between faculty and students at U.H. Hilo campus create an atmosphere of collaboration throughout the University system, improving the scientific recognition of the University, and ultimately the diversification of the economy. The HCGF provides access to a wide variety of equipment and service available for use by UH faculty, staff, and students, as well as other institutions in the area. The facility provides DNA sequencing, and genotyping services such as AFLP, SNP, tRFLP, STR, and Real-Time PCR. The HCGF functions as a support lab, containing instruments for various molecular biological uses, including CEQ Genetic Analysis Systems, Microarray, PCR instruments, centrifuges, electrophoresis systems, a NanoDrop spectrophotometer, and Revco freezers for storage. Reference materials and analytical software are also available. The facility offers workshops and training sessions to introduce current molecular and biotechnological techniques to students, faculty, and researchers. Current workshops include DNA isolation, PCR Basics, Gel Electrophoresis, Microsatellites Basics, DNA Sequencing, Real-Time PCR Basics, and Molecular Cloning. Future workshops will include tRFLP and Microarray. The facility is currently collaborating with include the University of Hawaii at Hilo, University of Hawaii at Manoa, Hawaii Community College, Bishop Museum, Simthsonian Institution, University of California Berkeley, Humboldt State University, Auburn University, Standford University, Department of Land and Natural Resources, National Parks Service, United States Department of Agriculture, and Unites States Geological Service.

Ciguatoxin Characterization in Hawaiian Archipelago Fishes: Toxicity Identified by N2A Bioassay

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Ciguatera is the most common form of seafood poisoning worldwide. One difficulty in preventing ciguatera is lack of adequate screening methods, due in part to the presence of multiple ciguatoxin (CTX) congeners in fish tissue. CTX congeners are commonly understood to differ by region. As the Hawaiian Archipelago is the most remote island chain in the world, it is reasonable to expect that CTX in Hawai'i's fishes may be unique as well. The objectives of this project were: 1) identify ciguatoxic fishes using a sodium-channel specific neuroblastoma (N2a) bioassay; 2) determine how ciguatoxicity varies among fish species throughout the archipelago; and 3) determine molecular masses of putative ciguatoxin congeners using HPLC/MS. Fishes were opportunistically collected from 14 islands, atolls, and reefs within the archipelago. Muscle tissues were extracted in dichloromethane and analyzed for toxicity using the bioassay. Results for 294 samples indicate 20% of fish exhibited toxicity. Chi square results indicated no significant differences in spatial distributions, or between herbivorous and carnivorous fishes. HPLC/MS analysis of extracts resulted in identification of several candidate peaks that may represent CTX congeners.

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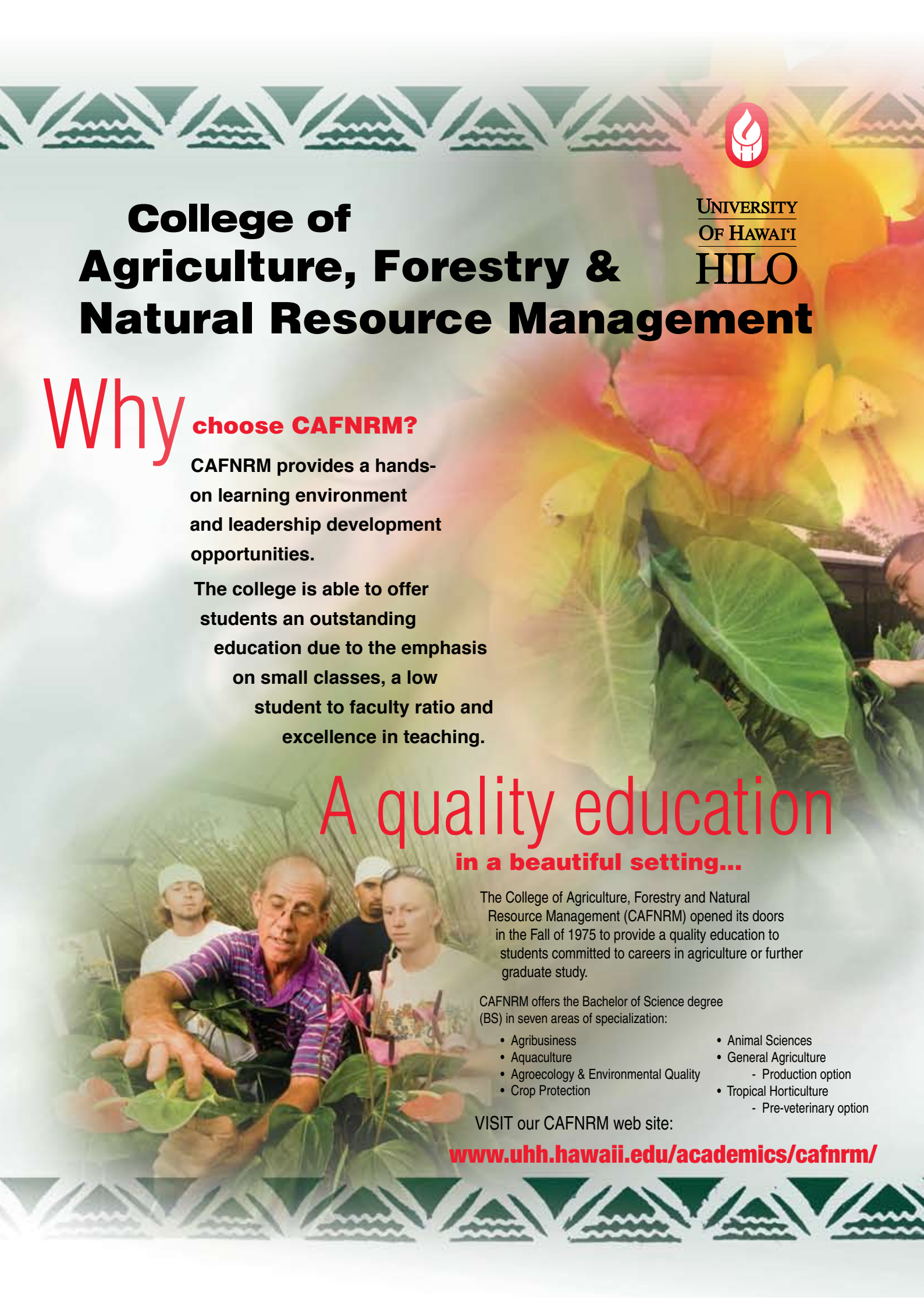

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