

### Western Pacific Tropical Research Center College of Natural and Applied Sciences University of Guam

### Buenas yan Hafa Adai,

Once again the diversity of the Western Pacific Tropical Research Center is exemplified in our 2011 Impact Report. Our front cover is a classic example of how ancient and modern meet with a traditional fishing method (throw net or Talaya) being used to catch juvenile rabbitfish with of one of our electrical producing power plants in the background. As a small tropical research institute in the Western Pacific, our scientists are known regionally, nationally and internationally.

The 2011 impact stories include updates on a new invasive species of fire ants to a tuna-roe-based diet for shrimp. We highlight a unique international research project by one of our leading research scientists who spends a great deal of his time in the Philippines. In addition, we offer a list of WPTRC 2011 publications.

In 2012, the University of Guam marks it 60th Anniversary as an institute of higher education and in June 2012, we mark 40 years as a designated Land Grant Institution. During the month of June, we invite all our community stakeholders to participate in the numerous activities we have planned to showcase the impact of our college.

Lastly, I personally want to thank all the gifted individuals that have contributed to this report, and I hope you will find this year's impact report a true example of the excellence we strive for at WPTRC.

### Dear Readers,

Once again interesting research activities have been highlighted in this year's impact report.

We are especially pleased to showcase projects having regional scope and those that were conducted in collaboration with scientists from other countries and universities. Even if this is just a small sampling of many activities conducted by WPTRC, it reflects the diversity of our research and demonstrates the impact our work has on tropical agriculture and our natural environment.

In spite of tough economic times across the nation, our hard working faculty and staff remained competitive in securing funding. Every year, the number and diversity of funded grants goes up. In 2011 we conducted more research, collaborated with more off-island scientists, provided more local employment and published more papers than ever.

I would like to extend a warm Si Yu'us ma'ase to our media specialist Olympia Terral for developing this quality publication and to all faculty and staff who contributed to this report. WPTRC serves you, so feel free to contact my office if you have questions or need additional information.

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# Credifs

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Steve Lindfield Marine Lab student Dave Benevente throws a talaya net from Guam's west coast

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1 Guam is on Fire with Ants

3 Marianas Mañahak are Homies

5 Home on the Range: Tracking Unicornfish in Piti Preserve

7 Weeding out Invasive Species with Classical Biological Control

9 Roe-based Diet for Shrimp

11 A Volcano Starts Over

13 The Unique Pineapple

15 The Give and Take of Wind

17 Yes, We Have Some Bananas Today

19 Biological Control of the Coconut Rhinoceros Beetle

21 Protecting Fruit Crops with Pheromones

23 2011 Publications

## Table Of Confenss



### Guam is on Fire with Ants



### Funded by WPTRC

As of the first week in November 2011 Guam's newest invasive species, the little fire ant has been found on island. The little fire ant (LFA), Wasmannia auropunctata, was most likely accidentally introduced to Guam because of human activity and it will take the collaboration of the general public, local scientists and government officials to make the difference in whether this ant is here to stay or just on holiday.

"At this time we are not sure exactly how the little fire ant got to Guam, but it is being spread around the island by people moving plants, soil, dumping green waste, and possibly cutting tangantangan or other plants to feed their livestock," says WPTRC entomologist Ross Miller.

LFA is on the list of the top 100 nastiest invasive species worldwide and is considered to be the greatest invasive ant threat to the Pacific region. Dr. Miller has been on the look out for this tramp ant for several years, as it was found in the Hawaiian Islands in 1999 and with all the traffic between Guam and Hawaii it was only a matter of time for these ants to find their way to Guam.

Dr. Miller's Entomology Laboratory received the first LFA sample from the

staff of the Rhinoceros Beetle Eradication Project and positively identified the ant as *Wasmannia auropunctata*. His lab is currently the ant identification center for the region.

LFA has been found in Yigo, Nimitz Hill, Piti, Santa Rita, Umatac, and Merizo. "Thanks to the rapid response of the public in bringing in ant samples to my lab as well as to the Government of Guam territorial entomologist, Russ Campbell, we have been able to get a good idea where little fire ant populations are located. The next step is to get a plan in place to contain it," says Dr. Miller.

At this time there is no evidence of little fire ants in the CNMI. Everyone is asked to do their part to see that this ant is not spread to other Micronesian islands. Follow all Customs and Quarantine rules and regulations. Do

not bring in or take out plants or soil as they may contain unwanted hitchhikers like the three invasive species that are currently wreaking havoc on Guam: the little fire ant, coconut rhinoceros beetle, and Asian cycad scale.

"Any invasive species we find on Guam poses a threat to our neighboring islands due to our frequent air and sea connections," says Dr. Miller, "If people follow the rules and get a permit and certificate to bring in plants this will ensure that no unwanted pests will be accidentally introduced and could save the government millions of dollars trying to eradicate an invasive species."



Ross Miller (671) 735-2141 rmiller@uguam.uog.edu Funded by US Fish & Wildlife Service Sport Fish Restoration Program

The annual juvenile rabbitfish (Siganus spinus) run is a much-anticipated event for fisher folk on Guam as these little fish are considered a delicacy on the island. The local name is mañahak and in good years the harvest can make up the bulk of the coral-reef inshore fishery catch. Marine lab scientists were curious to find out where Guam's mañahak come from. Are larvae coming to Guam's reefs from other parts of Micronesia or the Philippines? This information is important in order to determine how well connected or isolated rabbitfish populations are in the region.

Using genetic sampling and DNA profiling of rabbitfish from Guam, Saipan, Philippines and Micronesia, Mark Priest, an MSc student of Dr. Jennifer McIlwain found that mañahak from Guam and Saipan were genetically similar but were very different from fish collected throughout the rest of Micronesia and the Philippines. Fish were sampled from four separate *mañahak* runs on Guam to work out the origin of Guam's rabbitfish. The results showed that all of the mañahak had genetic profiles similar to the Guam and Saipan adults and did not come from any other source. This suggests that the rabbitfish from the Mariana Islands are isolated from the rest of the Pacific and have very

limited, if any, connections with other areas. "It is important that local adult rabbitfish populations are effectively managed, as these local adults must be the source of the island's *mañahak* runs. Sufficient protection of adult spawning stocks would protect the population from human disturbances and overfishing, helping to ensure the future sustainability of Guam's mañahak fishery," says McIlwain.

Understanding and quantifying patterns of connectivity are essential for the effective management and conservation of marine ecosystems. The degree to which individuals disperse, and populations are subsequently connected, has a major influence on the population dynamics of a species and determines the most appropriate management strategy. If a population is isolated and does not receive a supply of larvae from anywhere else, it

is important the local adult population is well protected as any decrease in large adult fish (they produce the vast majority of eggs) would result in a large decrease in the number of larvae returning to the reefs to replenish the population. Populations that are well connected may rely on larvae arriving from elsewhere for replenishment; in this case it is important that the source population(s) that is supplying larvae has adequate protection to ensure the long-term delivery of new larvae. "By understanding population connectivity and determining which populations are sources and sinks of larvae we can scale fisheries management accordingly. However, what works for one species may not be suitable for another as different species have different reproductive and larval characteristics," cautions Dr. McIlwain.



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## Home on the Range: Tracking Unicornfish in Piti Preserve



Funded by US Fish & Wildlife Service Sport Fish Restoration Program

**G**uam's Marine Preserves have been in existence since 1997 when the law to create them was established. As there are no specific regulations pertaining to the capture of reef fish (e.g. bag limits and minimum size limits), the preserves act as the primary management tool. It was only in 2001 that the law began to be enforced and since then several research projects have

shown there is a significant buildup of fish biomass inside these areas closed to fishing. However, nothing was known about the distances fish roam within the marine preserves and whether the reserves are large enough to protect the popular, targeted species.

Marine Lab researcher Dr. Jenny McIlwain and her graduate student Alyssa Marshell used passive acoustic telemetry to investigate the movement patterns of two heavily exploited unicornfish Naso unicornis (Tåtaga') and Naso lituratus (Hangon). Fifteen fish were implanted with internal acoustic tags and tracked continuously for over four months using a remote acoustic receiver array in the Piti Bomb Holes Preserve. Twelve receivers were set up at the western end of the preserve in reef flat and reef slope habitats where they recorded the presence and timing of all tagged fish. Individual receivers were also positioned outside of the eastern and western preserve boundaries to detect potential spillover. All receivers were retrieved and data downloaded monthly.

This study was the first to quantify longterm home range area and movement patterns of *N. unicornis* and *N. lituratus*  within a marine preserve in the central Pacific. The results indicated that the home range area increases with an increase in fish size. The peak times of recorded activity coincided with sunrise and sunset with both species primarily utilizing sheltered, shallow coral habitats. Overall, the tagged fish demonstrated strong site fidelity and a preference for habitat that provided protection from predators. Only one fish was recorded outside the preserve boundaries, and although the results suggest that unicornfish are highly site-attached, extensive movements outside the daily home range were also recorded.

"Assuming the fish tracked in this study are representative of these species, then the Piti preserve seems effective in protecting local unicornfish populations," says Dr. McIlwain. "Knowledge of fish home range size and movement patterns provides local managers with guidelines for designing effective marine reserves, which greatly improve the management of our coral reef fisheries."



"the Piti preserve seems effective in protecting local unicornfish"

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## Weeding out Invasive Species with Classical Biological Control



### Funded by USDA-APHIS

**T**he mile-a-minute vine, *Mikania* micranthra is an invasive weed on Guam and other islands in the Pacific. Mikania's native range is tropical and subtropical areas of North, Central, and South America. This prolific weed is on the top ten list of important weeds of Pacific island countries and territories (PICTs) due to its aggressive climbing abilities, which allow it to strangle young establishing plants. This can negatively impact agriculture and agroforestry plants greatly affecting plant biodiversity in the areas it colonizes. Estimates have the infestation of mikania on Guam around 2,581 acres.

Dr. Gadi V. P. Reddy and his team at the WPTRC Chemical Ecology and Entomology Laboratory have been investigating a proven biological agent to control the spread of the mile-aminute vine on the island and in the CNMI. Although Puccinia spegazzinii sounds like an appetizing menu item, it is a rust fungus that has been used in various places around the world to contain the spread of mikania. Working in collaboration with Secretariat of the Pacific Community (SPC), Fiji, Dr. Reddy's team has received a permit to release the strain of P. spegazzinii that was released in Fiji and Papua New Guinea for the same purpose. "The rust is highly damaging to the leaves, petioles and stems of M. micranthra causing cankers and often resulting in death of the plant. Tests have shown that *Puccinia spegazzinii* is completely host specific and will not cause damage to any other plants or humans," says Dr. Reddy.

One of the greatest advantages of using classical biological control is that it eliminates the need to use toxic chemicals such as herbicides that may contaminate ground water, rivers or the ocean through runoff. Aside from the environmental damage herbicides may cause, they have proven ineffective in containing the spread of mikania.

Dr. Reddy will be traveling to Papua New Guinea to collect live spores of *P. spegazzinii* to cultivate on Guam for release into the field. He fully expects the fungus to thrive in the hot humid conditions on the island and reduce the severity of the mile-a-minute infestation. The rust fungus will also be released in the Northern Marianas Islands.

Protecting Guam's natural environment from the devastating effects of invasive species is an important part of the work carried out by Dr. Reddy and his team.



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### **Roe-based Diet for Shrimp**

Funded by USDA Center for Tropical and Subtropical Agriculture

There is a need for the development of a nutritionally complete, cost-effective and disease-free maturation diet for shrimp broodstock utilizing locally available ingredients. Dr. Hui Gong and researchers at the Guam Aquaculture Development and Training Center (GADTC) are studying the possibilities of using tuna roe as the main component for a shrimp maturation diet.

Migratory tuna species are the most important fish resource in the Pacific region, with a harvest worth about \$3 billion US dollars annually. Guam is the largest tuna transshipment center in the Western Pacific, and has several operations that process the fish before the loins are flown to Japan. Roe comprises 16% of the total scraps from the loining operation. Roe is high in HUFAs, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and are not known to carry any shrimp pathogens. Preliminary trails with young shrimp and tilapia showed that the tuna roe was well accepted. However, it is unknown how effectively

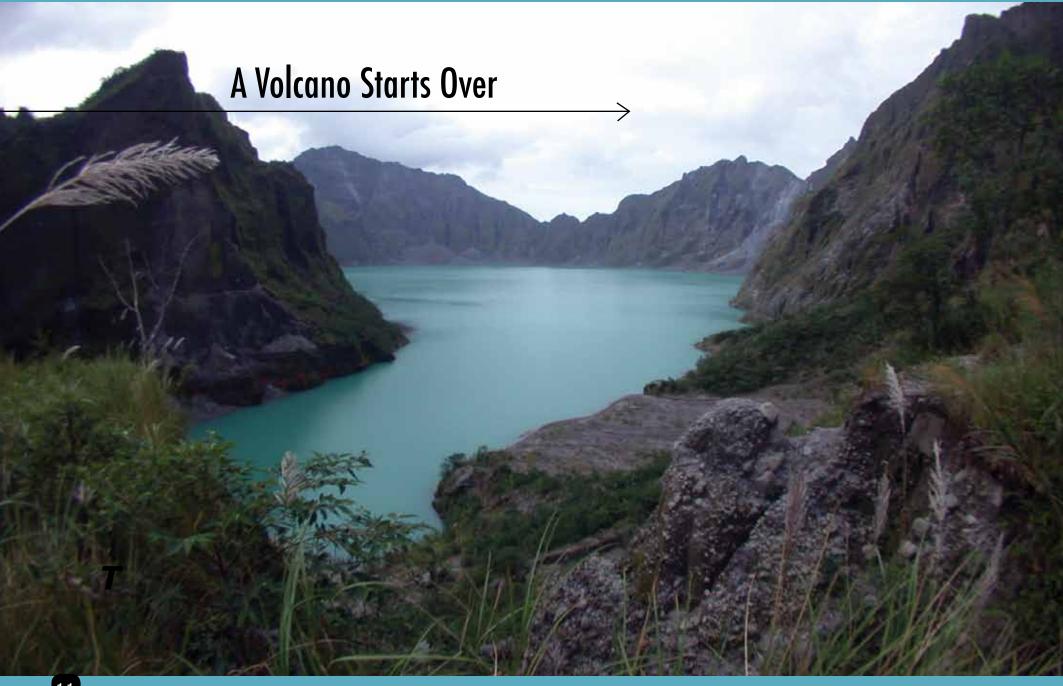
tuna roe can boost the maturation process as compared to conventional fresh-frozen maturation feeding regimes from the point of view of both health and nutrition.

This study was designed to assess the nutritional and health status of fish roe based on analytical results and to develop practical ways to utilize the roe in maturation diets. A freeze-drying protocol was established, and then the tuna roe was randomly sampled and submitted for analyses of fatty acid and mineral profiles and proximate analytical values. In addition, six pooled raw tuna roe samples were collected for DNA and RNA extraction, followed by PCR and RT-PCR diagnosis for detecting shrimp pathogens such as WSSV, TSV,

YHV, IHHNV, IMNV, etc. Results showed that the tuna roe samples were free of the previously listed pathogens. Semi-moist maturation diets, which incorporated the tuna fish roe as the main ingredient, were developed to achieve suitable density, stability and palatability for broodstock feed. The reproductive performance of female shrimp fed the tuna-roe-based diet, including gonad development, fertilization rate and hatching rate was also evaluated in comparison with the normal maturation feeding regime at the hatchery. The findings provide researchers with some baseline information for further evaluation studies.



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Funded by WPTRC

Mount Pinatubo's 1991 eruption caused immeasurable damage to the local environment in western Luzon, Philippines. The vegetation in the highest elevations of the mountain was cooked or blown sky-high. Many of the forest plants that survived the event were later uprooted or buried during the subsequent years of lahars that plagued the descending slopes.

Following a natural disaster like a volcanic eruption or landslide that kills most or



all of the plants in a habitat, studying the initial vegetation resurgence is called primary succession. This form of ecology research is important for understanding how ecosystems recover and how human actions may help or hinder that recovery. As a result, WPTRC scientist Thomas Marler has been traveling to Mount Pinatubo since 2006 to document which plants have begun colonizing the volcano's previously barren soil surfaces and determine how these initial plant immigrants will steer future ecosystem developments.

The years of research resulted in three publications in 2011. The exhaustive list of plant species and the manner in which they assembled on the various soil surfaces revealed that the large grass Saccharum spontaneum and the small woody tree *Parasponia rugosa* dominated the mountain's vegetation.

According to Marler, the manner in which these two native species influence continued forest development will be highly contrasting and will likely result in a patchy mosaic of plant assemblies during sustained forest development.

Several highly invasive plant species including *Chromolaena odorata* and *Pennisetum setaceum* were also widespread in the Pinatubo surveys. "These species have been known to degrade forest health in other tropical island habitats, and their influence on forest recovery of this famous volcano should be monitored in the coming years," said Marler.

Further reading: Pacific Science 65:157-173 (2011); Communicative & Integrative Biology 4:788-790 (2011); Plant Signaling & Behavior 6:1380-1382 (2011).



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### The Unique Pineapple



Funded by WPTRC

The flavor and aroma of tropical fruits provide Guam residents with endless pleasures. As a freshly harvested fruit is enjoyed, we rarely consider the miracles that occurred in order to produce the fruit. The building blocks for the sugars and phytochemicals that enter the manufacturing of a tropical fruit originate in the process of photosynthesis. As the sun's energy is captured by

green plant tissues, this energy is used to drive the process.

Pineapple is easy to propagate, requires very little care, and withstands Guam's devastating typhoons. It is an ideal choice for the island home garden. This unusual plant recently became the subject of WPTRC physiology research because of the unique characteristics of how it approaches photosynthesis. The Guam research resulted in two publications in 2011.

Most of Earth's plants immediately use the air's carbon dioxide to make sugars such that the entire process occurs during daytime. The process causes a lot of water to be lost from the plant during this prevalent form of photosynthesis. Pineapple plants add a step that captures the air's carbon dioxide at night by storing it in a temporary acid, only to release it during the following day when light energy is available to enable the production of sugars. This unusual feature leads to highly efficient use of available water during photosynthesis.

A second unique characteristic of pineapple is how it constructs various leaf forms. When a pineapple plant produces a fruit, it adds leaves on top of the fruit known as "crowns" and immediately below the fruit known as "slips". This unusual approach of adding new forms of leaves means that

photosynthesis to support fruit growth can occur in the original plant's leaves, in the crown's leaves, and in the slip's leaves.

WPTRC scientist Thomas Marler has determined that the highest level of photosynthesis occurs in the crown, and the lowest level of photosynthesis occurs in the original plant's leaves. Photosynthesis in the leaves of pineapple slips is intermediate. "One common phenomenon among plants is that the production of a fruit tends to increase photosynthesis in the original leaves that are close to the fruit," said Marler. "This did not occur in our pineapple research, probably because the newly added slips and crowns supply the extra food demands that are required by the plant during the production of a fruit."

Further reading: Acta Horticulturae 902:239-243 (2011); Acta Horticulturae 902:253-256 (2011).





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Funded by WPTRC

**G**uam's trade winds can decrease plant growth and cause wind-scar on fruits and vegetables. For these reasons, farmers are encouraged to use trees to create windbreaks. Past research at the WPTRC has shown that papaya is one crop that increases in growth and productivity when protected from Guam's trade winds.

"Unfortunately, Guam's farmers must also plan for damage caused by the passage of a typhoon," said WPTRC scientist Thomas Marler. "And this presents a challenge because we know that placing a load on tissues of plants and animals can increase strength." Indeed, exposure to the swaying motion caused by mild winds has been shown to strengthen trees in many climates.

A recent WPTRC publication discussed some of the leaf, stem, and root responses of papaya plants to mild wind exposure. Results showed that these three plant organs responded differently to wind exposure, with expansion rates declining for leaves and stems but not for roots. A trait that was highly responsive to wind exposure was the branching of roots, as plants receiving wind greatly increased their number of root tips. Research on storm

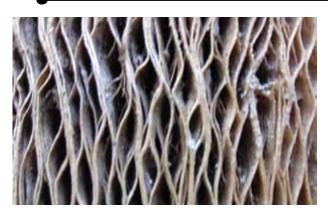
damage in many climates has shown that increased root tip density improves the ability of a plant to resist toppling during catastrophic winds.

Like most of life's challenges, wind exposure generates positives and negatives for plants in a typhoon-prone climate. Appropriate farm management requires an ability to balance all of the information while formulating plant care decisions.

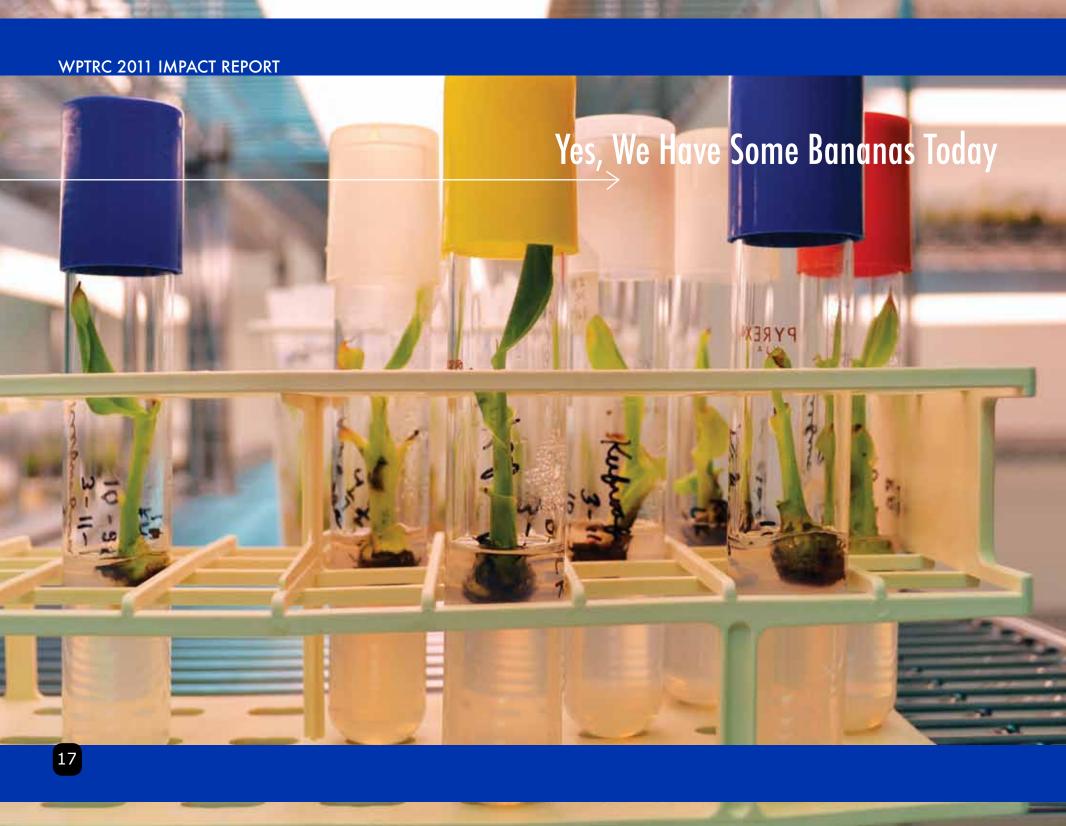
Further reading: HortScience 46:1105-1109 (2011)

"we know that placing a load on tissues of plants and animals can increase strength"





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Funded by USDA

**G**uam Department of Agriculture and the University of Guam are partnering to make clean planting stock available to farmers for local banana production.

Growers and farmers have been calling Dr. George C. Wall, UOG plant pathologist, to find out how to obtain disease-free banana plants. To satisfy this need, GDA and UOG are collaborating on a three-year project to make disease-free planting material available to local growers.

In 2009, six varieties of tissue-cultured bananas from the SPC (South Pacific Commission) laboratory in Suva, Fiji were brought to the WPTRC Plant Pathology Laboratory for studies. These included sweet dessert bananas and the less sweet cooking bananas. Another popular dessert variety, Silk (known on Guam as Manila) was imported from a laboratory in Belgium. All varieties were planted in the WPTRC nursery and then grown in the field under controlled conditions. In early 2011, Dr. Wall and his staff collected healthy suckers from each variety and propagated seedlings in the tissue-culture lab they set up at the Department of Agriculture. They are providing tissue culture training to GDA personnel to clone the diseasefree material ensuring purity and plant health.

The advantages to planting in vitro propagated plants are many:

- Since they are disease and insect free they will grow faster and more vigorously than suckers taken from the field
- All tissue-cultured plants are guaranteed to be of the same variety
- They can be ordered in advance and available in large numbers at one time

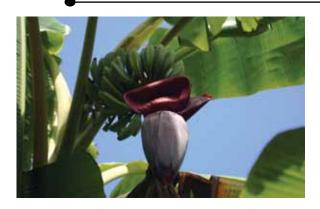
"I highly encourage all banana lovers and farmers to take advantage of this fantastic opportunity to buy these disease-free banana plants at the Department of Agriculture," says Dr. Wall. "There is nothing like tree-ripened locally-grown fruit in terms of nutrition, taste and value. Bananas are the fourth most important food in the world because they provide a good source of fiber, potassium and vitamin C. They also contain essential amino acids that

our body is unable to produce on its own."

Within the three-year time period of this contract with UOG, GDA plans to propagate via tissue culture thousands of banana plants per year to be distributed to growers. These disease-free plants are already available at the Guam Department of Agriculture.

The University of Guam and Guam Department of Agriculture are working together for the benefit of Guam's farmers and consumers.





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Funded by: USDA-APHIS, US Forest Service, Government of Guam

When insect pests first arrive on Guam and other tropical islands, there is often a population explosion, resulting in much higher damage than is ever seen in the pest's homeland. A major cause of pest population explosions is what entomologists refer to as "escape from natural enemies". Populations of pests in their native range are controlled by "natural enemies" which include predators, parasites and diseases. Newly arrived pests on Guam may have no natural enemies to control population growth. That is why entomologists often introduced biological control agents in an attempt to establish a natural balance. Biological control agents for insect pests are usually predators or parasites, but sometimes they are pathogens, which cause insect disease.

UOG entomologist, Dr. Aubrey Moore, is trying to use insect diseases to prevent an outbreak of the coconut rhinoceros beetle (CRB). This major pest of palm trees was first discovered on Guam in 2007. Adult beetles kill palms when they bore into the crowns to feed on sap. If beetle populations are not controlled, they may kill 50% of coconut palms on Guam. This is what happened a few years after the CRB arrived in Palau during the 1940's.

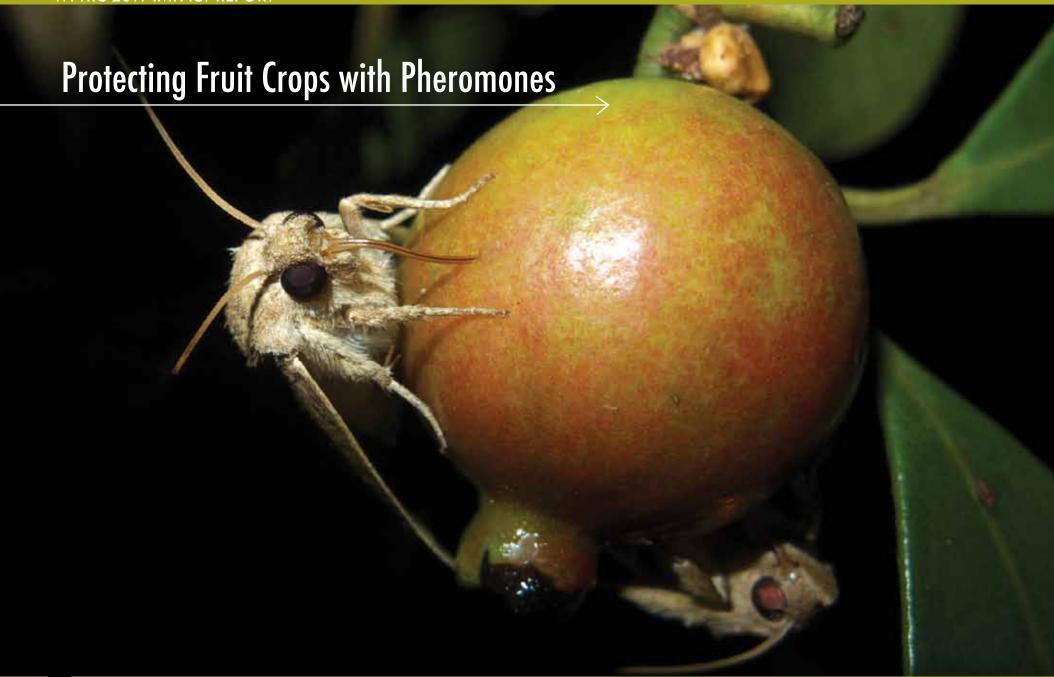
Moore's first attempt at controlling CRB with a virus was an unexpected failure. This virus, which only infects rhinoceros beetles, has been used successfully on many Pacific Islands. Unfortunately, the virus had no affect on Guam's beetles. Eight strains of the virus produced by a cell culture lab in New Zealand were tested. Either Guam's beetles are resistant to the virus, or the virus strains have lost their potency. Moore plans to find the reason for the failure.

A second attempt to establish biological control for the CRB uses a strain of green muscardine fungus (GMF). This work is being done in collaboration with Dr. Ambrosio Alfiler at the Philippines Coconut Authority. As with the virus, the strain of GMF being used is known to kill only rhino beetles and no other kinds of insects. Dr. Alfiler produces GMF spores in his lab by growing the fungus on sterilized corn. These spores, in the form of a powder, are distributed to Filipino coconut farmers who incorporate the powder into CRB breeding sites. CRB grubs and adults that come in contact with the spores become sick and die within a couple of weeks. By using GMF, Filipino coconut growers are able to minimize CRB crop damage without using chemical insecticides.

Use of GMF started on Guam in September, 2011 and results are promising. The Guam CRB Eradication

Project crew have been incorporating GMF wherever they find CRB breeding sites. When the crew revisits these sites after a few weeks, they find that many of the beetles are dead or dying from fungal infection. Project staff are also using a technique called "autodissemination" to get the fungus delivered to undiscovered breeding sites. When adult males are caught in the 1,000 pheromone traps currently deployed island-wide, these beetles are dusted with spores and allowed to fly off. The idea is to get these beetles to deliver spores to undiscovered breeding sites before they themselves succumb to fungal disease. Females are not released because they may lay viable eggs, thus increasing the beetle population. CRB population levels and damage caused by them are still relatively low on Guam. If Moore's biological control implementation is successful, the GMF will keep it that way.

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### Funded by USDA-ARS

The adult fruit-piercing moth is an important pest of citrus and other fruit crops. With more than 100 plant species in its diet *Eudocima phalonia* (Clerck) (Lepidoptera: Noctuidae) enjoys munching on some commercially important crops like apple, pear, star fruit, melon, tomato, banana, mango, papaya, and others.

Its common name, fruit-piercing moth, accurately describes how this insect feeds. Using its tough proboscis it pierces fruit macerating the pulp and sucking up the juices, which leaves a blemish on the fruit that becomes a site for pathogens to take up residence. Damaged fruits are unmarketable and pose a contamination threat if packed with other fruit.

Dr. Gadi V. P. Reddy has been using fruit puree bait laced with a small amount of pesticide to control the moth and is currently investigating integrated biological control methods of using semiochemicals to assist farmers in getting their fruit to market with the least amount of loss from the fruit-piercing moth. His expertise is in analysis, isolation and identification of natural products from insects and plants and his research focuses on the identification of insect pheromones, understanding the behavior of invasive insect species and developing more

environmentally friendly methods of insect pest control. As there is no pheromone particular to the fruit-piercing moth currently available, Dr. Reddy is collaborating with scientists at USDA-ARS, Hilo to extract the pheromone to use in his research.

His team has been collecting larvae in the field from the host plant, *Erythrina variegata* and rearing them in the lab. *E. variegata* is a showy, spreading tree with brilliant red blossoms commonly known as the Indian coral tree in Asia or tropical coral tree in the Pacific. Interestingly, the fruit piercing moth larvae feed only on the coral tree while adults suck the sap from all available fruits and lay their eggs on the coral tree.

Once the larvae pupate, Dr. Reddy's team extracts the pheromone-producing gland from the females by gently squeezing their abdomen

causing the gland to extrude. Using fine scissors they snip off the gland and put it in the solvent hexane for a few hours at room temperature. This solvent, which contains the pheromones, is shipped to Hawaii. In Hilo, researchers will use Gas Chromatography (GC) and GC-Mass Spectrometry (GC-MS) machines to isolate the pheromone. "These machines can be used to identify and isolate semiochemicals such as pheromones in insects. Then these chemicals can be employed in pest control programs," states Dr. Reddy. "Although we do have these machines in our lab we have had difficulty getting the chemicals necessary for the procedures shipped to Guam."

Dr. Reddy's research continues to benefit the environment as well as farmers in the region.



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# 2011 Publications

Cibrian-Jaramillo, A. and T.E. Marler. 2011. Novel tools for an old lineage. Communicative & Integrative Biology 4:466-468.

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