Evaluation of *Metarhizium anisopliae* strains to control the grass spittlebug *Prosapia simulans*

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Abstract

The grass spittlebug is a very important pest that attacks several crops such as grasses and sugarcane. One of the most important genera is *Prosapia* which has been reported in nine Mexican states including Tamaulipas. Particularly in Tamaulipas, this pest has caused important damages on grasses so a good alternative to control it is the application of the entomopathogenic fungus *Metarhizium anisopliae.* The *M. anisopliae* strains provided by the ARSEF, were tested in laboratory bioassays using Buffel grass plants artificially infested with adults of *P. simulans*. Results indicated that 3 strains (798, 3019 and 6347) were highly efficient to kill adults of *P. simulans* which caused 100% mortality. However we consider that strains 798 and 3019 are the most suitable strains to control *P. simulans* due to the mycelia formation and sporulation, which could also serve as inoculum for infection propagation. It is also important to mention that spore concentration used was not so high (10^9 conidia/ml) indicating the high virulence of these fungi. In addition the presence of protease genes in these strains may indicate the ability of these fungi to degrade the insect cuticle. Further studies should be conducted in order to determine the application procedures to control this pest.

Gypsum application to two soil management systems improves broccoli floret tissue sulfur

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Abstract

Soils in semi-arid areas can be deficient in sulfur (S). Field-grown broccoli, *Brassica oleracea* Italica group (L.), cultivars Waltham 29 and Gypsy, were direct seeded on 15 Oct. 10 into raised beds and into stripped tilled sudex residue at a site near Weslaco, TX (Lat. 26° 08'). Gypsum (23% Ca and 16.5% S) at rates of 0, 500, 1000, and 2000 kg/ha were applied and incorporated on 10 Oct. 10 in order to determine the effects of added soil S on soil properties and broccoli head floret mineral nutrients. Results indicate that the open-pollinated 'Waltham' is more nutrient dense that the 'Gypsy' hybrid; that tillage methods do not effect broccoli floret nutrients or yield but do influence stand and floret head size; and that increased gypsum rates increase floret S concentrations (linearly) but do not affect plant stand or yield. Soil that was bedded tested higher in soil organic matter, K, Mn, B, and Zn compared to strip-tilled soil. Gypsum application increased the soil cation exchange capacity, soil Ca and S, but decreased soil Mn, B and pH (all linearly) and

organic matter (quadratically). Initial soil penetration force was greater in strip-tilled vs. bedded plantings but these differences declined after 80 days. At the end of the study there was both a linear and quadratic reduction in penetration force to 15 cm depth resulting from increased gypsum application.

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Abiotic factors affecting development of the arundo armored scale *Rhizaspidiotus donacis* in mass rearing systems

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Arundo donax (giant reed) is a nonnative invasive grass. Its invasion in Texas and Mexico has increased the risk of cattle fever tick invasion and reduced water resources. The arundo armored scale insect (*Rhizaspidiotus donacis*) has been released in Texas for biological control. *R. donacis* stunts shoot growth and reduces rhizome mass. This project aims to investigate how abiotic factors such as soil type, temperature, and humidity affect scale survival rate. Our objectives are to determine the effect of soil type on *A. donax* growth, moisture content and scale survival, and to compare temperature and humidity in different mass rearing locations. Soil was collected from three sites: Moore Airbase (loam), and Los Indios (silt) and Laredo (sand) along the Rio Grande. Pea gravel, used for mass-rearing, was used as a standard for comparison. To compare temperature and humidity in mass-rearing, soil moisture probes were placed on rhizomes in six different locations: a Moore Airbase greenhouse near to and far from the evaporative cooler; Moore Airbase pond; outdoor field trench; greenhouse trench; and laboratory. Within the Moore Airbase greenhouse, humidity was highest at a position close to the cooler and was higher under a rhizome then on top of the rhizome. Among the four soil types, arundo shoot growth rate was higher in Moore Airbase loam and Los Indios silt then in gravel and Laredo sand.

Moisture conditions in the four soil types will be evaluated, and rhizomes and shoots raised in these soil types and in the six mass-rearing locations will be dissected after infestation by the arundo armored scale to determine survival rate. This research will lead to more productive mass rearing techniques.

Behavioral observations of the arundo leafminer (*Lasioptera donacis*) to develop rearing and host-range testing procedures for biological control

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The arundo leafminer (*Lasioptera donacis*) from Mediterranean Europe is a candidate for biological control of giant reed (*Arundo donax*), a non-native invasive grass that consumes water and damages riparian ecosystems in Texas and Mexico. Adult female leafminers deposit eggs into wounds in leaf sheaths wrapped around the stem of the arundo plant. A fungus colonizes the wounds and larvae of the leafminer feed on both plant material and fungus. The importance of plant condition, fungal source, and sheath location on the stem in determining egg-laying preferences for the leafminer is unknown, and this information is critical for host-range testing and mass rearing. Our objectives are: 1) to determine preference of the leafminer female for arundo stems either wounded with the tips of forceps or unwounded; 2) to determine preferences for fungal material of European versus Texas origin; and 3) to determine positional preferences of females within the arundo stem. Leafminers were confined in cages and observed every five to ten minutes to determine percentage adults flying, feeding on sugar substrate, alighting on the cage, stem, or fungal material, or depositing eggs. In a large cage (1 m. tall), 12.8% of the leafminers landed on a wounded stem versus 6.4% on an unwounded stem. In small cylindrical cages (50 cm tall), 5 to 20% of leafminers landed on a stem provisioned with European material, while virtually none landed on a stem with Texas material. Tests are ongoing to determine stem positional preferences. Additional studies are determining the importance of light and humidity levels. This research will facilitate determination of the Texas fungus, and efficient colony production and evaluation of the arundo leafminer for biological control.

The role of predators in limiting the establishment of the arundo armored scale *Rhizaspidiotus donacis*

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The sap-feeding armored arundo scale insect (*Rhizaspidiotus donacis*) has been released for biological control of giant reed (*Arundo donax* L.), a non-native, highly invasive giant grass that infests waterways and damages riparian ecosystems in Texas, the southwestern US and Mexico. Mass-rearing and optimal field release protocols are in development. Generalist predators such as ants, spiders, ground dwelling beetles and other arthropods may limit the successful establishment and rearing of the scale. The objectives of this project were: (1) to determine if a cycad scale (*Aulacaspis yasumatsui)* could be used as a surrogate for the arundo scale in predation tests with ants; (2) to determine the rate of predation on the arundo scale by ants; and (3) to characterize the arthropod predator fauna at field sites by collecting specimens using pitfall traps. In tests done on six ant trails near an arundo stand, arundo scales were preferentially taken by ants over cycad scales within a two hour observation period, indicating that the cycad scale is not a suitable surrogate for predation tests. Almost all of the arundo scales (starting with 30 adult females) were taken in two hours, demonstrating the role of ants as predators when scales are exposed directly to ants. Winter pitfall trap collections at three sites, including one along the Rio Grande, yielded few specimens, ants being most abundant. These results will be used as a baseline to compare the establishment level of the arundo scale in the field in the presence and absence of predators. Strategies to reduce pressures by generalist predators may help increase arundo armored scale survival and reproduction for initial field establishment, and also inform mass rearing techniques.

Effects of Microclimate Changes in Different Vegetation Types on Cattle Fever Tick Larval Survival

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Cattle Fever Ticks (CFT), *Rhipicephalus annulatus and R. microplus*, serve as vectors for *Babesia* spp., which is the cause of cattle fever. Past research on CFT has shown that combinations of temperature and relative humidity are key factors influencing tick larval survival. While macroclimatic conditions are similar for cover types, local microclimates can vary considerably by location.

Our objectives were to record temperature and relative humidity data in three different vegetation types in Zapata, TX and determine if vegetative cover affects CFT

larval survival through changing seasons. HOBO Data Loggers™ were placed in the field using satellite imagery to identify areas of varying vegetative cover categorized as grass, brush, and dense vegetation. HOBO Data Loggers were programmed to collect temperature and relative humidity within microclimates every 15 minutes. In addition, data are offloaded monthly and field sites are sampled for the presence of CFT larvae by walking transects around the data loggers using flannel panels pinned to jeans.

These data will be used to describe daily and monthly microclimate for each vegetation type. Transect sampling will provide data on CFT larvae presence in the study area during seasonal changes in temperature and humidity levels. These data will be used to provide a better understanding of the role microclimate differences play in the survival of CFT larvae in southern Texas.

Rearing technique development: rearing of the armored arundo scale (*Rhizaspidiotus donacis*) using *Arundo donax* micro plants supplemented with micro-nutrients

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The armored arundo scale insect (*Rhizaspidiotus donacis*) has been permitted for use as a biological control agent for giant reed (*Arundo donax* L.), a non-native, invasive woody grass that consumes water and damages riparian ecosystems in Texas and throughout the southwestern US and Mexico. Mass rearing methods for the arundo scale are under development. Earlier studies found that the arundo scale contains high concentrations of essential plant micronutrients (copper, zinc, iron, magnesium, manganese, calcium) that may enhance scale survival. To test this hypothesis, we developed a micro-plant that could be manipulated with micronutrients. Our objectives were to: (1) determine if the soil type (sand vs. peat moss based potting soil) has an effect on giant reed micro-plant survival and production of new side shoots; (2) determine if the micronutrients have an effect on plant growth; (3) determine if survival and population development of the arundo scale on micro-plants can be improved with micronutrients. Cuttings were collected from one year-old arundo shoots containing a bud or small side shoot, and were placed under 90-100% humidity until rooted. Measures of growth, including diameter of the main stem, number of side shoots above and below the soil line, and cutting survival were recorded. Micro-plants treated with rooting hormone survived better in sand than in soil, but soil was logistically more practical. Micronutrient treatments resulted in longer and more abundant side shoots, with some negative effects at the highest concentration. The next step is to evaluate the effect of micronutrients on the arundo scale. The results of this research may improve the efficiency of mass rearing by identifying key micronutrients needed by the arundo scale and/or giant reed.

Behavior of *Rhizaspidiotus donacis* in the presence of diverse *Arundo donax* stimuli

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The sap-feeding arundo armored scale insect (*Rhizaspidiotus donacis*) is biological control agent targeting giant reed (*Arundo donax*), a non-native, invasive grass that consumes water and damages riparian ecosystems in Texas and throughout the southwestern US and Mexico. Efficient mass rearing and release of the scale depends on a thorough understanding of the behavior of the 'crawlers' produced by females, which must rapidly disperse and settle on rhizome or stem tissues to survive. The objectives for this project are: (1) to determine the key olfactory (smell) or gustatory (taste) stimuli that cause crawlers to move; and (2) to determine the importance of phototropism (attraction to light) and gravitropism (response to downward gravitational force) in crawler movement. Crawlers were collected from females in gelatin capsules and released into an olfactometer, which picked up airborne stimuli from two vessels containing rhizomes or other arundo tissues, and then fed the stimuli into opposite ends of a tube. Crawlers were released at the center. The device could be oriented horizontally or vertically. In both horizontal and vertical tests, two to three times more crawlers moved towards rhizome stimuli than towards either leaf stimuli or blank air, consistent with the superior suitability of rhizome tissues for scale development. In vertical tests with rhizome stimuli at both ends, 1.5 times more crawlers moved downward than upwards, suggesting a positive gravitropic response. Future studies will determine the effect of crawler storage on behavioral responsiveness, and the importance of rhizome condition on establishment of the crawlers. The results of this project will lead to the development of crawler release strategies that maximize their survival, improving the efficiency of mass rearing and field releases.

Differential Growth Rates of Native South Texas Species in Invasive Grass Communities

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In South Texas, a suite of African grasses represents the single-most immediate threat to natural plant communities. Guinea grass (*Megathyrsus maximus*), buffelgrass (*Pennistum ciliare*) and Kleberg bluestem (*Dichanthium annulatum*) outcompete native species, such as *Ebenopsis ebano*, *Guaiacum angustifolium*, *Mimosa malacophylla*, *Phaulothamnus spinescens*, and *Pithecellobium pallens*. Because African invasive plants rarely establish themselves in undisturbed native scrub of South Texas, and few native plants occur in well established invasive grass stands, we designed experimental plots to quantify the growth rates of native plants and invasive grasses under disturbed (invaded) and undisturbed scenarios. We transplanted 20 stolons of all three invasive grasses under

mature canopies of the aforementioned native tree species and compared their growth rates to control populations planted in open terrain. We also transplanted seedlings of the woody species within dense stands of Guinea grass and buffel grass to measure their growth rates in relation to control seedlings in the absence of grasses. Preliminary results indicate high mortality rates for Kleberg bluestem and significantly slower growth rates of Guinea grass and buffel grass under mature native shrubs. However, native seedlings of all species except mesquite (*Prosopis glandulosa*) grew significantly faster in association with invasive grasses than in our control groups. This unexpected result might be due to the extreme drought of 2011, which seems to have favored seedlings that were shaded by grasses. We will continue observations in the upcoming year.

Over Expression of a Calcium Signal Modifier Gene (CSM-1) in Sweet Orange Cultivars and Molecular Characterization of Transgenic Plants

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Texas is ranked the third highest producer of citrus in the United States, following Florida and California respectively. The Texas citrus industry has a total annual value of more than $200 million and provides significant employment opportunities. While this crop has been constantly challenged by many biotic and abiotic threats, it has so far survived. The introduction of the bacterium *Ca*. Liberibacter asiaticus (Las), causal agent of Huanglongbing (HLB), or citrus greening and its vector, the Asian citrus psyllid (ACP), have completely changed this scenario and the industry is faced with its worse nightmare. Currently other major diseases attacking citrus and causing detrimental economic loss are Citrus Canker, Citrus Tristeza Virus (CTV), as well as disease caused by fungal pathogens. Disease control can be costly and may only minimize damages while the production of disease resistant cultivars through breeding techniques, such as genetic transformation, will benefit the industry tremendously and could lead to a lasting solution. Genetically modified 'Hamlin' and 'Valencia' sweet orange cultivars were produced through *Agrobacterium*-genetic transformation containing a calcium signal modifier gene (CSM-1), which amplifies calcium signaling in an attempt to create broad-spectrum disease resistance. Real-time polymerase chain reaction (qPCR) confirmed the over expression of the CSM-1 gene and pathogenicity tests to evaluate the transgenic plants to the soil borne Oomycete *Phytophthora nicotianae* confirmed strong resistance characteristics. Preliminary results of transgenic grapefruit containing the CSM-1 gene have shown resistance to one bacterium, one oomycete and one fungus.

EFFECTS OF OSMOTIC AND HEAT STRESSES IN *Macrophomina phaseolina* (Tassi) Goid.

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The fungus *Macrophomina phaseolina* (Mp) causes charcoal rot in sorghum, maize and beans at northern Tamaulipas, Mexico under both water and heat stresses. The knowledge about mechanisms of Mp to tolerate environmental stress could help us to design proper integrated management strategies. The effects of variable osmotic potentials and temperatures on *in vitro* growth, pathogenicity and osmolyte production were measured in Mp. In the first experiment, isolates from maize (HMP4), bean (HMP5), sorghum (HMP46) and soybean (HMP49) were grown in PDA added with 0, 120, and 240 mM of NaCl; in the second, the same isolates were grown under 30 and 37°C and finally, isolates from tropical (HMP5, 23, 33, 35) and arid (HMP1, 14, 15, 47) regions were grown under osmotic (0, 500 mM NaCl) and heat (30, 40°C) stresses. Pathogenicity was assessed in seeds of cultivars BAT 477 and Pinto UI-114 and osmolytes were identified and quantified by HPLC. NaCl and heat stresses reduced growth, size and production of microsclerotia of Mp isolates as well pathogenicity, although increased osmolyte concentrations (glycerol, arabitol, and galactitol under osmotic stress; erythritol and mannitol under heat stress). Maize strain was more tolerant to variable stress based on *in vitro* growth, pathogenicity and osmolyte synthesis. Osmotic and heat stresses affected negatively Mp growth, although isolates from tropical regions were more affected and then produced the highest polyol concentrations. Production of osmolytes as glycerol was higher when osmotic and heat stresses were combined. Data indicated *de novo* synthesis of active osmolytes by *M. phaseolina* under osmotic and/or heat stress and they could favor the survival under adverse environmental conditions without affecting fungus parasitic ability.

Abscisic Acid Increases Cold Tolerance in Citrus

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The Rio Grande Valley is a target of freezes that cause millions of dollars in damage to the Citrus Industry. In leaves freezes cause water inside the plant cells to form ice crystals that draw out the water inside the cells and make water unavailable to plant tissues as well as cause damage by disrupting cell membranes. Damage to the fruits is observed as juice vesicles rupture due to the formation of crystals leading to water loss and desiccation. Abscisic acid (ABA) is a hormone that is involved in the cold tolerance response of many plants. Drought stress causes an accumulation of ABA inside the plant resulting in hormonal signaling of stomata closure. Our hypothesis is that the interaction between drought stress and foliar application of ABA to trees will result in an increase of cold tolerance. In this experiment, four treatments were applied to 3-year-old Rio Red grapefruit trees grown under greenhouse conditions. The treatments consisted in the combination of two irrigation regimes (drought stress, where trees were exposed to three weeks of continuous drought, and normal irrigation), and two concentrations of foliar ABA application (0 and 1mM ABA). Significant differences in stem water potential were

recorded between well- watered and drought-stressed trees, confirming differences in tree water status. Lethal freezing temperature was determined by exposing leaf samples to a range of temperatures ranging between 0 and-12 °C, at intervals of -2°C/h. Results showed that foliar application of ABA increased cold tolerance in grapefruit trees. Nevertheless, there was an interaction with tree water status since ABA application on drought-stressed trees increased cold tolerance more than on well-watered trees (2.3°C *vs*. 0.5°C).

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Effect of sowing date in *Fusarium* incidences and mycotoxin production in maize germplasm

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Cereal crops can be attacked by different fungi as *Fusarium, Aspergillus* and *Penicillum* species under field and/or stored conditions and they can provoke the synthesis of mycotoxins with negative effects in humans and cattle. In this work we measured the effects of nine sowing dates (from December 2007 to February 2008) of yellow and white maize hybrids on *Fusarium* incidences as well as the major mycotoxins produced by the genus infecting maize in Río Bravo, Tamaulipas in order to identify maize germplasm with tolerance to *Fusarium* infection of seeds. Each fungal genus and species was identified based on microscopic morphology after the growth in two culture media and sequencing of ITS ribosomal sequences and their comparison with gene databases. Finally, we identified and quantified the mycotoxins by HPLC. Genera *Penicillium, Aspergillus* and *Fusarium* infected maize grains with incidences up 70%, from 24 to 46% and lower than 6%, respectively. The highest fungal incidences were found at late sowings which coincided with high temperatures during maize flowering. HPLC analysis of maize grains indicated the presence of fumonisin B_1 in concentrations lower than 50 ppb at date of sowing nine. In three hybrids concentrations were lower 50 ppb were found and only P-31G98 showed up 270 ppb. Based on ITS region of 48 *Fusarium* isolates we identified 47 as *F. verticillioides* and the other as *F. chlamydosporum*. In 36 of 48 cases (70%) results were similar between culture media and sequencing and in 37 of 48 between the both media. The hybrid H-440 released by INIFAP showed low incidences of fungi, and drought and disease tolerance.

Agronomic Factors of Forage Sorghum Production in South Texas

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Grain sorghum production is usually grown in areas where rainfall is insufficient and or the temperatures are extreme. Forage sorghums have multiple uses which include silage for livestock, hay, or as green chop. The objectives of this research was to compare brown midrib (BMR), photoperiod sensitive (PS), and conventional forage sorghum and sorghum/sudangrass (SS) cultivars under dryland and limited irrigated production in South Texas. Forage sorghum hybrids were planted in Kingsville, Texas. Each variety was planted in a randomized block design replicated four times. Data was collected on lodging, height, moisture, and forage yield. Each cultivar was harvested when grain reached the soft dough stage (approximately 65% whole plant moisture). Preliminary data indicates that the highest yields were observed in forage sorghums. Because of the extreme drought conditions during 2011, dryland yields were extremely low and difficult to compare yields.

Use Biotyping to Establish Temporal and Spatial Relationship between Potato Psyllid, *Bactericera cockerelli*, Populations Colonizing Potatoes in Texas

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Zebra chip, which causes an internal brown discoloration and dark brown stripes in tubers, first appeared in 1994 in southern Mexico and later spread to Texas in 2000. This disease is associated to the potato psyllid, *Bactericera cockerelli* which transmits the bacterium, *Candidatus Liberbacter*. Different sampling methods and techniques are being used to understand this pathogen and determine if resistance and tolerance can be developed in the potato plants. If in fact these psyllids are migration from one location to another prevention methods can be established to help reduce the incidence of ZC. This project will use bitotyping to establish a relationship between potato psyllid populations colonizing in Texas. *Candidatus Liberbacter* testing will be done on psyllids collected from three different locations in Texas, including Weslaco, Pearsall and Kingsville. For each location several different planting times were established to have an early, optimum, and late planting date. Adults, nymphs and eggs were identified, separated and collected in ependorff tubes. Ten percent of each weekly sampled collected had DNA extracted from individual psyllids using the Qiagen DNEasy kit. This DNA was used for *Candidatus Liberbacter* testing, Melt curve analysis and PCR/ ISSR reactions. Once testing has been conducted and positive insects were identified, speciation between locations and relationships of the psyllids at the different locations were determined using Melt Curve, Standard PCR and ISSR methods. Once melt curve analysis was done, insects were then grouped according to melt curve and then PCR and ISSR methods were conducted.

Molecular Analysis of Citrus Rust *(Phyllocoptruta oleivora)* Populations in Texas

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Phyllocoptruta olevoria (Acari:Eriophyidae) or the citrus rust mite (CRM) is one of the most economically important mite pests of citrus worldwide. CRM originated from Asia, however it is found in most citrus producing areas, such as, Brazil, Morocco and the United States. Though CRM is a major pest and studied worldwide, there are currently no molecular studies available. Molecular studies are important because they can provide a baseline of knowledge that can be applied to management programs, such as, proper taxonomic identification and point of origin. In this study we discuss DNA extraction techniques, optimization of mitochondrial gene, cytochrome oxidase subunit I (COI) and a nuclear ribosomal gene, internal transcribed spacer region I (ITSI), for molecular analysis of CRM. We also implemented Inter-Simple Sequence Repeat Polymerase Chain Reactions (ISSR-PCR), which has been proven to be a valuable tool for population genetic studies because they can assist in determining genetic structure and resolving genetic relationships.

Recent Advances in Indoor Remote Sensing of Giant Reed, *Arundo donax*

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Recent studies have demonstrated the feasibility of using indoor remote sensing in measuring the physiological stress of *Arundo donax* and similar giant reeds. *Arundo donax* is an invasive specie that has caused extensive damage to the Rio Grande Valley and is currently a target of major control efforts. During past several years, many techniques have been developed to accurately measure spectral reflectance of *A. donax* using close up remote sensing. The results of the studies have demonstrated the viability of using abscised leaves of *A. donax* and similar giant reeds to measure spectral reflectance under physiological stress. The results have also shown the practicality of using Glad-Bags® and equivalent plastic bags as an appropriate medium for storage of abscised foliage for transportation from field to laboratory conditions; with only slight changes in the Visible and Near Infrared Wavelength.

GENETIC DIVERSITY ANALYSIS OF *Phaseolus coccineus* L. GERMPLASM FROM VERACRUZ, MÉXICO

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Ayocote beans (*Phaseolus coccineus* L.) is the second major species in the genus *Phaseolus*. In this work we analyzed the genetic variability by using eleven SSR markers of 107 ayocote landraces from the state of Veracruz and then compared with 30 landraces

from Puebla, and 15 wild populations from different regions of México. As external accessions we used seven common bean (*P. vulgaris*) landraces from Veracruz; three common bean cultivars (BAT 477, Pinto UI-114, P. Villa) as well as one accession from the species *P. glabellus* and *P. lunatus*. AMOVA detected significant differences among and within accessions with 46 and 45% of variation, respectively. We found from 14 to 48 alleles per SSR where GATs91 showed the highest numbers of amplified alleles. Cluster analysis differentiated *Phaseolus* accessions from *P. coccineus* accessions and constructed one cluster which included 30% of ayocote beans from Puebla inside accessions from Veracruz. Results indicated that during domestication of ayocote beans could have happened reproductive isolation. Ayocote beans analyzed included germplasm with high levels of genetic variability which must be conserved, analyzed and properly used in bean breeding.

Effects of Micro and Macro Nutrients on major citrus pests in Texas

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One of the major diseases affecting citrus production in Florida is Citrus greening disease (*Citrus huanglongbing*), whose major vector is the Asian Citrus Psyllid (*Diaphorina citri*). The destructive disease has affected citrus crops in Florida and Brazil, but the insect vector has spread from Florida to Texas and Mexico. Being that there is no cure for this bacterial disease, many approaches have been made in order to repel and control ACP, from intensive chemical control programs to mechanical control; but another approach was tried in Florida, nutritional sprays containing different combinations of micro and macro nutrients. Our study focuses on manipulating different micro and macro nutrients associated with the Maury Boyd cocktail (Potassium, Calcium, etc.), and their effects on major insect populations, associated with citrus production such as ACP, Citrus Rust mite (*Phyllocoptruta oleivora) and Citrus Leaf Miner (Phyllocnistis citrella*). We also take into account the effect of the nutrients on tree physiology, fruit production and yield. Preliminary results have showed that Calcium alone and combined with micro-nutrients, has an impact on ACP populations as well as fruit quality.

Recovery of the microbial endophytic fraction of *Citrus limon* var. Eureka

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The study of endophytic genomes is a major challenge because the plant genome represents the largest percentage of existing DNA and microbial genomes are just a minor fraction. In this work we developed a process for endophytic DNA isolation, based on an enrichment protocol from extracts of lemon leaves and then the recovery of the fraction of microbial

DNA by an affinity method and cold and heat shocks to release the bacterial DNA and a parallel method using urea to release fungal DNA. As a preliminary step to the extraction, an enrichment of the microbial fraction was obtained by preparative double centrifugation, where we got two pellets and two supernatant phases (pellets 1 and 3, supernatants 2 and 4), and the four phases were visualized by DAPI staining technique. The DNA of pellets and supernatants were individually extracted by the method describe above, and were amplified with 16S and 26S primers for bacteria and fungi, respectively. The amplification products were subjected to the SSCP technique (Strand Conformation Polymorphism Simple) on acrylamide gels to obtain the microbial profiles of the phases. As conclusion, we developed a reproducible method to isolate the endophytic DNA fraction present in lemon tissue. A positive amplification of bacteria was obtained in the four phases. The use of DAPI staining corroborated the presence of different kinds of bacterial and fungal cells within plant tissues.

Effects of Potato Planting Timing in Texas on Zebra Chip Incidence and Liberibacter Infection Rate in Potato Psyllids

Jennifer Trevino, Greta Schuster, Shad Nelson, Blake Bextine, Joe Munyaneza

Zebra Chip (ZC) is a destructive disease affecting the potato chipping industry resulting in millions of dollars in losses. ZC is associated with the bacterium, *Candidatus Liberibacter*, which is vectored by the potato psyllid *Bactericera cockerelli*. The objective of this research was to evaluate if time of planting affected ZC incidence. Four different planting dates were used in three regions within south central Texas. Adult insects were collected weekly by Kiss sampler as well as leaf samples. Both adults and leaves were taken to lab for accurate identification of egg, nymph, and adult. Samples collected were used for DNA extraction and PCR was conducted to determine if the insects tested positive for ZC. At harvest, individual tubers were cut at both ends and visually scored using a simple yes or no based scoring system. Tubers that exhibited ZC symptoms were cut into .05mm slices, fried, and examined for severity. The 2010 December planting in Weslaco had higher ZC incidence compared to those planted later in the season. The early January and March plantings in Pearsall showed higher ZC incidence than those planted earlier in the season. In the Weslaco region, data indicates that planting late in the season could reduce ZC incidence. However, in the Pearsall area, the data has been erratic with hot vs. cold environmental conditions. Therefore, there is no solid data to support a late or early planting to reduce incidence.

Recent studies on sweet orange scab (SOS) in Texas

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Sweet orange scab (SOS) of citrus caused by ***Elsinoë australis*** was detected for the first time in the United States on lemon sample collected in Spring, TX in 2010. The sample was taken during a citrus commodity survey conducted by the Texas A & M University-Kingsville, Citrus Center. Polymerase chain reaction (PCR) technique was used to confirm the presence of *E. australis* from scab-like symptoms. Since then, PCR was used to test the presence of *E.australis* on various cultivars of citrus fruit at the Citrus Center, Weslaco. USDA-APHIS-PPQ has confirmed the presence of SOS in all citrus producing states except California. To date, 370 SOS suspected fruit were collected and 185 were confirmed PCR positive from several counties including Hidalgo, Cameron and Willacy. The symptomatology of SOS as observed has been consistent with the PCR results. Isolation of pure culture of *E.australis* and inoculation, re-isolation, followed by PCR and sequence data has fulfilled the Koch's postulates confirming its status as a new citrus pathogen in the US. Study of the pathogenicity was done with different citrus cultivars with detached leaf assay. To infect leaves, sporulation experiments were done. From over 10 studies we have now perfected a system to readily produce conidia for artificial inoculation. As part of an effort for a practical solution for the packers, we studied the effect of heat under light and dark conditions. The results show that with the detached leaf assay, infections occur more readily under dark. Out of five separate studies, temperature regimes above 40°C seem to have an inhibitory effect; however, these need to be verified.

Potential impact of parasitoids on the biological control agent *Tetramesa romana* (Hymenoptera: Eurytomidae)

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The gall-inducing arundo wasp (*Tetramesa romana*) is a biological control agent for giant reed (*Arundo donax L.*), a non-native, highly invasive woody grass that consumes water and damages riparian ecosystems along the Rio Grande and throughout the southwestern US and Mexico. The arundo wasp has been released at numerous sites along the Rio Grande, but little is known about biotic factors influencing establishment and efficacy of this wasp in the field. Potential parasitoids, including other wasps (Hymenoptera) and flies (Diptera) have been collected at field sites. The aim of this project is to determine the impact of these potential natural enemies on arundo wasp populations. To test whether certain factors (time of arundo wasp establishment and collection season) impact parasitoid dynamics, collections of arundo wasps and parasitoids and observations of arundo wasp-induced galls are being made at four field sites, including two at which the wasps have been present for 5+ years, and two at which wasps were released in 2009-2010. Collections are being made in the winter, spring, and summer to determine the season in which arundo

wasp and potential parasitoid populations are highest. In winter collections, Laredo shoots yielded the most wasps, but parasitoids were not collected from any site due to winter conditions. Ongoing studies are determining if gall size or color affects attack rate and wasp parasitoid yield. This research will benefit the arundo biological control project by providing information about the degree of impact parasitoids have on the population size and effectiveness of the arundo wasp in the field.

Evaluation of Forage Sorghum and Sorghum x Sudangrass for Forage Yield and Nutritional Value

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Forage sorghum (FS) and sorghum x sudangrass (SS) varieties can be used as a source of roughage in cattle feeding programs; thus, they may be a viable alternative to traditional grain sorghum production in South Texas, especially during times of drought when traditional forage resources are limited. Identifying cultivars that have a high potential for forage yield while maintaining nutritional value is important for both cattle feeders and farmers. Genetic traits, such as the photoperiod sensitive and brown midrib traits, have the potential to improve forage yield and digestibility, respectively. This study was designed to evaluate a total of 12 FS and SS varieties for forage yield and fiber composition. Preliminary results confirm that, when analyzed by FS or SS, there are differences in forage dry matter yield ($P < 0.03$), neutral detergent fiber ($P < 0.01$), acid detergent fiber ($P < 0.01$), and crude protein ($P < 0.01$) for the different sorghum traits. Therefore, traits, in addition to type (FS or SS), is an important factor to consider when making forage production decisions.

Genes involved in the synthesis of indole acetic acid in *Trichoderma* spp.

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*Trichoderm*a spp has proven to be a symbiont in plants provides a defense system against pathogens and stimulates plant growth, in different varieties of agriculturally important crops, including corn. The aim of this study is to look in *Trichoderma asperellum* (HK703) and *Trichoderma koningiopsis* (HTE808) genes involved in the production of indole acetic acid through *aldh* gene amplification, *amd*, *ipdc* and *nit* by PCR species *Trichoderma* spp

studied the results show the presence of genes coding amd *aldh* and aldehyde dehydrogenase enzymes and amidase, respectively, as those responsible for the synthesis of indole acetic acid, through tryptamine and amidase. We confirmed the presence of the gene *aldh* in *Trichoderma* (HK703) at the level of expression of the transcript, having increased expression when the culture medium was supplemented with tryptophan.

Use of Trichoderma spp. in cotton

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The Experimental Biotechnology Laboratory of the Biotechnology Genomics Center, during the Fall-Winter 2009-2010 cycles, is dedicated to finding a solution to the diseases that cause damage to cotton, making the isolation of native fungi in contaminated lots. To combat the disease have been isolated strains of Trichoderma fungi which possesses to control plant diseases caused by soil pathogens, mainly of the genus Phytophthora, Sclerotium, Pythium and Fusarium and others. Trichoderma species act as competitive hyperparasites produce antifungal metabolites and hydrolytic enzymes to which are attributed to structural changes at the cellular level, such as vacuolization, granulation, and disintegration of the cytoplasm and cell lysis, found in organisms with which it interacts. The mechanisms by which Trichoderma strains displace phytopathogenic are essentially two main types: "direct competition" for space or nutrients, and "direct parasitism" of certain Trichoderma species on phytopathogenic fungi. The results are prosperous in these 3 years because it has the support of farmers and associations, and especially cotton. Studies in cotton in 2011, show that T asperellum increases the gain with respect to control. The biological material is being evaluated in other regions of the country with these and other economically important crops. *T. harzianum* can be recovered from roots of treated plants and *T. koningiopsis* is the most widely used as inoculum, in the region in sorghum and maize.

Prickly Pear on the Texas-Mexican Border: a potential cash crop in Texas.

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Prickly pears (*Opuntia* species) native to the southern extremes of Texas and northern parts of Mexico have the potential to become marketable products in the United States. Mexico produces an annual amount estimated to be 128 million USD in *Opuntia* pads and approximately 71 million USD in tunas, or prickly pear fruits. We can enrich our society by incorporating this plant into our diet, perfect for the new green and organic revolution craze. Among other things, *Opuntia* has a hypoglycemic effect in non-insulin-dependent diabetes mellitus patients. Additionally, *Opuntia* species provide wildlife habitat and are ecosystem structures. Prickly pear species are used as emergency forage for cattle during harsh droughts and in the landscape nursery industry. We mapped the current distribution

of all *Opuntia* species in the United States and Mexico using the geographic information system ArcGIS and by gathering geographic information from different online databases, by request, and through literature research. We overlapped the distribution of *Opuntia* with a map of suitable habitat for one of the main threats to prickly pear species: *Cactoblastis cactorum*. This was in order to determine potential areas in which to monitor the spread of this disease. It is necessary to find alternative cash crops that will grow in Texas, particularly the Lower Rio Grande Valley, because of the drastic global changes that are occurring. Since irrigation is not necessary; Texas should consider *Opuntia ficus-indica* as a potential commercial crop. Further research will be developed to establish potential areas in which to grow this crop in Texas.

Preliminary Evaluation of Organic Black Spanish Grapes in the Rio Grande Valley

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Homalodisca vitripennis otherwise known as the Sharp-shooter bug, the entomological characteristic of this insect relate to the glassy structured wings, similarities to that of the (*Onnometopia Orbona*). Surveys have concluded that this insect is native in the area around Northern Mexico and southeastern regions of the United States (Goolsby, Bextine, Skevington, Coleman 2007). *H.vitripennis* has parasitic contributions if transmitting Pierce's Disease that is bacteria which deteriorates the plants outer characteristics by blocking vital nutrients to the plants system. *Atta texana* known as the leaf cutter ant is a fungivore that is converted from plants to generate food for the colony and queen in order to reproduce grow larger.

***Momoridica charantia* (Bitter Melon) Phytochemistry for Human Health: the Bitter-Sweet Phenomenon- Bitter for Diabetes and Food Pathogens and Sweet for Us**

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Over 60% of the current cancer medications currently in the market today are derived from natural products, additionally several other types of medications also use plants for base molecules. The health benefits of many of these plant products have been attributed to the endogenous levels of secondary metabolites. Several studies have reported bitter melon to poses several health promoting properties such as antioxidant, anti-bacterial and anti-

diabetic activities. In this study, we will identify the phytochemical constituents on bitter melon fruit, seeds, leaf and vines. Furthermore, extracts of these components and purified compounds will be used on human adipose and skeletal muscle cells to investigate bitter melon's anti-diabetic properties. Additionally, extracts and purified compounds will be used to investigate the efficiency of bitter melon against food pathogens such as *E. coli* and *Salmonella.* Lastly, agricultural, pre- and post- harvest methods, such as water stress, nutrient, and drying methods, will be evaluated to determine an optimal production strategy for healthier bitter melons. Here we present methods developed for the identification of polyphenols, polyamines, and amino acids by HPLC, in addition to preliminary results on the influence of water stress and post-harvest drying on phytochemical content.

Momoridica charantia (Bitter Melon) Phytochemistry for Human Health: the Bitter-Sweet Phenomenon- Bitter for Diabetes and Food Pathogens and Sweet for Us

Jose Luis Perez[1,2,3], Nasir Malik[1], Mani Skaria[2], Bhimu Patil[3*]

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Effect of parasitism by *Cotesia flavipes* on growth and metabolic efficiency of *Diatraea saccharalis*

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The alteration of host growth by parasites is seen in wide variety of animal species, including many insects. In some species of insect hosts attacked by gregarious endoparasitoids, heavily parasitized individuals ultimately attain a larger final mass compared to lightly parasitized ones, suggesting host growth may be manipulated by the parasitoids to compensate for competition and thereby optimize nutrient transfer to the wasps. The present series of experiments showed that feeding and growth of the sugarcane borer, *D. saccharalis* were inhibited after parasitization of the fourth-instar larvae by *C. flavipes*. Parasitism of *Diatraea saccharalis* larvae by the endoparasitoid *Cotesia flavipes* resulted in a reduction in the amount of food consumed by parasitized larvae, and showed significant decrease in total hemolymph proteins after 8 days of parasitization. Following parasitization of fifth-instar larvae, more heavily parasitized larvae grew larger compared to those containing fewer parasitoids due to an increase in host dry weight. A dose-dependent enhancement of host dry weight would appear nutritionally beneficial for the parasitoids developing in more crowded hosts. The efficiencies of conversion of ingested and digested food to body mass and approximate digestibility of the diet ingested by host caterpillar decreased significantly in parasitized hosts. The effect of parasitism on the ability of *D. saccharalis* larvae to utilize ingested food was partially reduced by parasitism. Larvae parasitized by the wasp females did not convert as much food to body substance as did non-parasitized larvae. Larval parasitoids developing in the presence of many competitors weighed up to 50 % less than those developing in hosts with fewer endoparasitoids, although the weight of adult female parasitoids did not vary with was clutch size. The ratio of the parasitoid emergence to non-emerging decreased as parasitoid clutch size increased, with fewer or none emerging from very heavily parasitized host containing more than 100 parasitoids.

Keywords: Parasitism, Metabolic effieciency, gregarious parasitoid, *Diatraea saccharalis, Cotesia flavipes*

Phylogeographic Analysis of Harrisia Cactus Mealybug, *Hypogeococcus pungens* (Hemiptera: Pseudoccidae) Populations: Work in Progress

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Harrisia cactus mealybug (HCM) *Hypogeococcus pungens* (Hemiptera: Pseudoccidae) Granara de Willink (1981) is infesting and killing cacti in the southern coast of Puerto Rico, covering an area of about 1,400 km^2. The 13 species of cacti occurring in Puerto Rico are threatened by this new pest, three of which are endemic and two are endangered. HCM may also be threatening Florida and Barbados. A biological control program has been initiated in Puerto Rico to search for natural enemies of HCM native to South America. Beyond its native range HCM has been reported in Australia, the Caribbean, Europe, and North America. HCM were first described on the host family plant Amaranthaceae and its host range includes species from Portulacaceae and Cactacea. In collecting HCM in South America, we noticed biological differences when rearing HCM on the different host plants, and therefore we suspected that HCM may be part of a species complex. We must first clarify this situation in order to import the correct natural enemies. We asked the following questions, 1) Did HCM originate from Argentina, 2) Is HCM part of a species complex, and 3) Are HCM populations plant-host specific. The mitochondrial cytochrome oxidase subunit I gene (COI) and the nuclear D2 Loop of the 28S gene were sequenced. Neighbor-joining and maximum parsimony phylograms of the COI sequence data clustered the various populations into 5-6 clades, most of them with strong bootstrap support values. A haplotype match was found in Florida, but not in Puerto Rico. With the exception of Florida, an association with host plant was also identified. The D2 sequence phylograms confirmed the presence of at least five mealybug species.

Title: The Psyllid Fauna of South Texas
Author: Donald B. Thomas

Abstract.
The Asian Citrus psyllid, *Diaphorina citri*, is the vector of the bacteria that causes Citrus Greening, aka, Huanglongbing. D. *citri* is now the commonest insect on south Texas Citrus perhaps due to an absence of its natural enemies. Yet, it is not the only psyllid found in the citrus groves. Of some concern is that the other psyllid species might play a role in the transmission of the disease. Although the "Jumping Plant Lice" are typically narrowly specific in terms of breeding hosts, they will feed on a wider array of plants. Thus some of the natives, *Leuronota maculata* in particular, though breeding on hackberry, is commonly found in the Citrus groves and likewise, *D. citri* is sometimes found on non-Citrus plants, especially in the winter months when Citrus is dormant. At that time D. citri and other psyllids, can be found on succulent winter annuals, such as sow thistle (Compositae). *D. citri* seems to like fig trees (*Ficus carica* L.) and even breeds adventitiously on this plant (Thomas 2011, Fla. Entomol. 94: 1081-1083). It will also breed on a native shrub called Colima (Rutaceae), but only when the plant is stressed. Cross-feeding among hosts raises the possibility of the bacteria being vectored to plants that even if asymptomatic could serve as reservoirs of the disease. It is known for example that Periwinkles, a favorite of south Texas gardeners, can harbor the agent and we have found D. citri on this plant. Herein is a rogue's gallery, a line-up of potential perps, representing the psyllid guild in south Texas and their preferred breeding host-plants.

Differential Growth Rates of Native South Texas Species in Invasive Grass Communities

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In South Texas, a suite of African grasses represents the single-most immediate threat to natural plant communities. Guinea grass (*Megathyrsus maximus*), buffelgrass (*Pennistum ciliare*) and Kleberg bluestem (*Dichanthium annulatum*) outcompete native species, such as *Ebenopsis ebano*, *Guaiacum angustifolium*, *Mimosa malacophylla*, *Phaulothamnus spinescens*, and *Pithecellobium pallens*. Because African invasive plants rarely establish themselves in undisturbed native scrub of South Texas, and few native plants occur in well established invasive grass stands, we designed experimental plots to quantify the growth rates of native plants and invasive grasses under disturbed (invaded) and undisturbed scenarios. We transplanted 20 stolons of all three invasive grasses under mature canopies of the aforementioned native tree species and compared their growth rates to control populations planted in open terrain. We also transplanted seedlings of the woody species within dense stands of Guinea grass and buffel grass to measure their growth rates in relation to control seedlings in the absence of grasses. Preliminary results indicate high mortality rates for Kleberg bluestem and significantly slower growth rates of Guinea grass and buffel grass under mature native shrubs. However, native seedlings of all species except mesquite (*Prosopis glandulosa*) grew significantly faster in association with invasive grasses than in our control groups. This unexpected result might be due to the extreme drought of 2011, which seems to have favored seedlings that were shaded by grasses. We will continue observations in the upcoming year.

Prickly Pear on the Texas-Mexican Border: a potential cash crop in Texas.

Simon Del Alto[1], Brenda Garcia[1], Samantha Vazquez[1], and Teresa P. Feria[1]
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Prickly pears (Opuntia species) native to the southern extremes of Texas and northern parts of Mexico have the potential to become marketable products in the United States. Mexico produces an annual amount estimated to be 128 million USD in Opuntia pads and approximately 71 million USD in tunas, or prickly pear fruits. We can enrich our society by incorporating this plant into our diet, perfect for the new green and organic revolution craze. Among other things, Opuntia has a hypoglycemic effect in non-insulin-dependent diabetes mellitus patients. Additionally, Opuntia species provide wildlife habitat and are ecosystem structures. Prickly pear species are used as an emergency forage for cattle during harsh droughts and in the landscape nursery industry. We mapped the current distribution of all Opuntia species in the United States and Mexico using the geographic information system ArcGIS and by gathering geographic information from different online databases, by request, and through literature research. We overlapped the distribution of Opuntia with a map of suitable habitat for one of the main threats to prickly pear species: Cactoblastis cactorum. This was in order to determine potential areas in which to monitor the spread of this disease. It is necessary to find alternative cash crops that will grow in Texas,

particularly the Lower Rio Grande Valley, because of the drastic global changes that are occurring. Since irrigation is not necessary; Texas should consider Opuntia ficus-indica as a potential commercial crop. Further research will be developed to establish potential areas in which to grow this crop in Texas.

Using Archived CIR Photographs to Map Vegetation On the Spoil Islands of the Lower Laguna Madre

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Abstract

The spoil islands of the Lower Laguna Madre were created by dredging of the Gulf IntraCoastal Waterway in 1947. During the past six decades, most of these islands have been vegetated by natural processes and now serve as valuable wildlife habitat. However, many of these artificial islands are currently threatened by severe erosion caused by waves. In an effort to reduce shoreline erosion, UTPA is currently conducting research to stabilize shorelines using black mangrove, *Avicennia germinans*. Our study will support these efforts by mapping the distribution of black mangrove and other vegetation on the spoil island chains extending north and south of the Arroyo Colorado, and chains located near the Mansfield Channel. We will use high-resolution color infrared (CIR) photographs from archives of the Texas Natural Resources Information System (TNRIS) to develop computer-generated vegetation maps for each island. These vegetation maps will then be draped over digital elevation models developed from topographic surveys based on Global Positioning System (GPS). These three-dimensional maps will allow us to determine elevation ranges suitable for each plant species and to measure and evaluate the effectiveness of the planting strategies for black mangrove and other plants for erosion control.