72nd ANNUAL MEETING

Subtropical Agriculture and Environments Society

(*Formerly*: Subtropical Plant Science Society & Rio Grande Valley Horticultural Society)

CONFERENCE PROCEEDINGS

February 9, 2018

Rio Farms Inc.

25601 North FM 88, Monte Alto, TX

72nd Annual Meeting

Subtropical Agriculture and Environments Society

Program

Time	Titles & Speakers
07:45-08:20	**Registration**
08:20-08:30	Introductions: Debbie Villalon, President
08:30-09:00	Barry Goldsmith, NOAA, Meteorological update for LRGV agriculture
09:00- 9:30	Shad Nelson, Advancing Student Learning Opportunities in the Agricultural and Biological Sciences through Hispanic Serving Institutional Collaborations
9:30-9:50	Hugh Conway, APHIS, Mexican Fruit Fly –Aerial release of Mexican fruit fly by Drone (UAV)
9:50-10:45	**Poster Session & Break**
10:45-11:05	John Goolsby, Development of methods to treat cattle fever tick infested nilgai antelope with entomopathogenic nematodes
11:05-11:25	Randy Stanko, Utilization of Hair Sheep as an alternative small ruminant in South Texas
11:25-11:45	Larry Smith, Update on Boll Weevil Eradication in the LRGV and Tamualipas
11:45-12:05	Sonja Swiger, Extension, CDC center for excellence in vector borne disease
12:05-12:25	Ninfa Ramos, Development of Some Strategies for the Control of *Spodoptera* sp.
12:25-1:00	Lunch
1:00 – 1:30	SAES: Election of new officers & Potts Award
2:00 -3:00	Student presentations
3:00-4:00	Tour of Rio Farms

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Spring/Summer 2018 Rio Grande Valley Weather Outlook: Confidence Is High for Hot and Dry, but Wild Cards Lurk

Barry S. Goldsmith

NOAA/National Weather Service Forecast Office Brownsville/Rio Grande Valley, Brownsville, TX

Abstract

Winter 2017/2018, meteorologically defined as December-February, has temporarily slowed the persistent warm weather trends that led to near record heat for a majority of calendar years this decade. Three polar-sourced outbreaks of much below average temperatures produced minor freezes over cropland across the Rio Grande Valley, along with three separate instances of winter precipitation – very rare to unprecedented based on available records. With the exception of a stripe of heavy rainfall that impacted agriculturally rich lands along and near U.S. Highway 77 between Brownsville through the southern King Ranch, seasonal precipitation has been one-quarter to one-half of the seasonal average. Combining the low rainfall with "freeze-dried" crop land, the potential exists for a difficult 2018 growing season without sufficient irrigation.

A brief review of the current water year so far (October through January) will be described, followed by a discussion of the 2018 spring and early summer growing season temperature and rainfall outlook, including a range of scenarios based on forecast methods and forecast confidence.. Potential impacts and suggested actions will be offered to help the audience understand what's needed to prepare and mitigate for most likely and reasonable worse case outcomes.

Aerial release of Mexican fruit fly by Drone (UAS)

Hugh Conway[1], Nathan Moses-Gonzales[2], Dustin Krompetz[2], Michael Milam[2], Daryl Hill[3], Ignacio Baez[4]

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[2] *M3 Consulting Group, LLC., Dayton, OH*

[3] *USDA APHIS PPQ, Aircraft and Equipment Operations, Edinburg, TX*

[4] *USDA APHIS PPQ, Science and Technology, Raleigh, NC*

Abstract

During the summer of 2017, a small Vertical Takeoff and Landing Unmanned Aircraft System (UAS) was tested to assess the efficacy of UAS as a platform for the release of sterile Mexican fruit fly (MFF), *Anastrepha ludens* (Loew). The UAS releases were conducted at an altitude of 120m over two days across six replications using two flagged fields. Cessna 206 releases were conducted at altitudes of approximately 120m, 304m and 457m over three weeks using one flagged field. Each field contained a total of 174 trap locations. Each trap was serviced three times per release test or 522 servicing per test with a total of 1,044 traps serviced for UAS aerial release and 1, 566 for Cessna 206 aerial release. Of the approximately 210,000 sterile flies released via UAS, we recaptured ~ 0.96% of total flies released and observed an average swath width of 520m, across the six replications. Of the approximately 105,000 sterile flies released via Cessna 206, we recaptured ~ 0.93% of total flies released and observed an average swath width of 440m, across the three replications. The UAS, when compared to a Cessna 206 achieved a wider swath width, however, the release was also more susceptible to drift than the Cessna 206.

Development of methods to treat cattle fever tick infested nilgai antelope with entomopathogenic nematodes

J. A. Goolsby[3,*], N. K. Singh[3,4], D. I. Shapiro-Ilan[5], R. J. Miller[3], and A. A. Perez de Leon[6]

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Abstract

The southern cattle fever tick, *Rhipicephalus microplus* and bovine babesiosis transmitted by it, caused an annual loss to the U.S. livestock industry of $3 billion in the currency of today before they were eradicated from the U.S. The tick eradication program in the permanent quarantine zone along the Texas-Mexico border needs novel strategies due to growing evidence of resistance to acaricides; invasion of pathogenic landscape-forming weed species such as carrizo cane, *Arundo donax*, and Guineagrass, *Megathyrsus maximus* that enhance survival of cattle fever ticks; and the emerging role of white-tailed deer, *Odocoileus virginianus*, and exotic nilgai antelope, *Boselaphus tragocamelus*, as tick hosts. Nilgai antelope are competent hosts of *R. microplus* and have large home ranges, moving frequently between public lands set aside for wildlife conservation and private lands managed for cattle and/or wildlife. Because they are exotic animals, nilgai do not have a hunting season and are commonly harvested year round in South Texas. Therefore pesticides that have with drawl periods before the meat is consumed from a hunter-harvested animal are not suitable for treatment of cattle fever tick-infested nilgai. Entomopathogenic nematodes have been commercialized as biopesticides may be suitable for treatment of tick-infested nilgai. Remotely activated sprayers have been developed to treat nilgai with the entomopathogenic nematodes. Sprayers can be set up on nilgai common latrines or fence crossings. The nematode species, *Steinernema riobrave* has performed well in laboratory trials, commercially available, and is native to the Rio Grande Valley. The nematode and treatment methods are being field tested in Willacy Co., with the goal of incorporating this technology into the Cattle Fever Tick Eradication Program.

Utilization of Hair Sheep as an alternative small ruminant in South Texas

R.L Stanko[1,2,*], V.V. Flores[1], J. A. Martinez[1], and E.C. Taylor[1]

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[2]*Animal Reproduction Laboratory, Texas A&M University AgriLife, Beeville, TX. 78102*

Abstract

The U.S. sheep industry has changed over the last 60 years. Decreasing profitability of wool production has shifted focus to meat production. High summer temperatures add an additional challenge to wool sheep in Texas. Hair sheep are gaining popularity across the Gulf-Coast states and the Dorper breed is the most popular in Texas due to superior carcass qualities. An accelerated lambing system can produce three lamb crops in two years and profit potential approaching that of beef cattle production. Accelerated lambing requires ewes to breed early postpartum and at all seasons of the year. We hypothesize that Dorper sheep have reproductive characteristics suitable for enhanced lamb production in south Texas. We determined and evaluated Dorper ewe reproductive characteristics. Cumulative percentage of pubertal spring-born ewe lambs (n=15) increased during September (20%), October (67%), November (87%), and December (100%; 39.0 ± 0.7 kg) of the same year. All spring-born ewe lambs became anestrous prior to the following March. Fall-born ewe lambs were pre-treated with intravaginal progesterone implants (n=9; 39.6 ± 1.2 kg, 230.5 ± 2.1 d) or sham (n=9; 39.6 ± 1.2 kg, 230 ± 2.3 d), and were exposed to fertile rams during June of the following year. Only 1 of 18 ewe lambs conceived; however, 94% (16/17; 315 ± 6.4 d) conceived in September. During early summer anestrous, 90-d post-partum ewes (n=13) received progesterone implants or sham, and exposure to fertile rams (30-d). Conception rate (50% vs. 80%), days to conception (4 ± 1.3 d vs. 2.0 ± 1.1 d), and number of lambs born per ewe (2.0 ± 0.3 vs. 1.4 ± 0.3) were similar between treatment groups. Ewes (n=30) in various post-partum stages (100 to >200 d) were exposed to fertile rams (30-d) in May and, nuli- and primiparous ewes (n=24) with varying degrees of fleece were exposed to fertile rams (22-d) in August. A majority of the ewes in both studies conceived (83%). We conclude that Dorper sheep have suitable reproductive characteristics for accelerated lamb production in south Texas.

CDC center of excellence in vector-borne disease

Sonja L. Swiger, PhD, Christopher Vitek, PhD, and Scott Weaver, PhD

Western Gulf Center of Excellence for Vector-Borne Disease

Texas A&M AgriLife Extension, University of Texas Rio Grande Valley, University of Texas Medical Branch - Galveston

Abstract

The Western Gulf Center of Excellence for Vector-Borne Disease was established in 2016 through funding from the CDC. This center is headquartered at the University of Texas Medical Branch, with multiple academic partners including the University of Texas Rio Grande Valley and Texas A&M University, as well as state and local public health partners. The goals of this center are to advance research in the areas of vector-borne disease, including disease surveillance, insecticide resistance, vector biology and ecology, and control strategies, and to educate current and future public health scientists trough diverse educational and outreach programs. Multiple applied research projects are being led by individuals within the center, and will be conducted alongside outreach, education, and training programs for both vector-control personnel and students interested in vector-borne disease. In addition to these efforts, we are also engaged in active disease and vector surveillance efforts in the lower Rio Grande Valley, the site of recent circulation of Zika, chikungunya, dengue and West Nile viruses as well as Chagas disease parasites. The lower Rio Grande Valley (RGV) region of Texas remains a high-risk region for introduction of new vector borne diseases due to extensive land immigration from Latin America. Multiple surveillance and monitoring efforts are currently underway in the RGV.

Development of some strategies for the control of *Spodoptera* sp.

N. M. Rosas-García, M. Mireles-Martínez, J. M. Villegas-Mendoza, and Germán A. Rodríguez-Morales

Laboratorio de Biotecnología Ambiental, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa, Tamp., México.

Abstract

Spodoptera sp. Hübner (Lepidoptera: Noctuidae) are economically important pests that attack a wide range crops worldwide. In Mexico, these pests are distributed in many producing regions attacking maize, sorghum and soy mainly. *Bacillus thuringiensis* is an important control agent that has been used against a variety of lepidopterans. This bacterium has been used to control this pest but results have been inconsistent so far. We developed three formulations containing the *B. thuringiensis* spore-crystal complex with ingredients such as feeding stimulants, adherents, vegetable oil and/or in combination with baculovirus. Each formulation was tested against neonate larvae using artificial diet or plants. Preliminary tests with essential oils were also conducted against larvae. Results indicated that the formulation composed by spore-crystal complex of HD-125 strain, modified corn starch, gelatin and corncob caused 90% and 71% mortality in laboratory and greenhouse tests respectively. The formulation containing spore-crystal complex of HD-133 strain, occlusion bodies of SeMNPV/SfMNPV, carrier suspension with soy oil and powdered soy leaves caused 70% mortality in *S. exigua* larvae, and nearly 100% mortality in *S. frugiperda* larvae. The formulation containing spore-crystal complex of HD-133 strain and garlic vegetable oil caused 100% mortality in neonate larvae showing synergistic interaction. Individual agents caused 36% mortality for *B. thuringiensis* strain and 80% mortality for garlic oil. Preliminary results with fifteen essential oils indicated that eleven of them are 100% toxic to larvae by vapor phase bioassay at medium concentration, while in feeding bioassay they caused mortality below 50% and only oregano essential oil caused 65% mortality in contact bioassays. These results show alternatives that can be used alone or in combination to control *Spodoptera* sp.

Poster Abstracts

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Subtropical Agriculture and Environments Society

Animal Science

1
Ticks in Texas

M. Barreiro-Arevalo[1,3], L. Esteve-Gassent[2,3], T. Oraby[1,3], T.Feria-Arroyo[1,3], B. Doss[2], T. Reeve[2]
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[2]*Texas A&M University College of Veterinary Medicine*
[3]*Talent in Agriculture for Climate Change and Food Security Adaptation*

Ticks have been known to be vectors of several deadly diseases including Lyme disease, which has often gone misdiagnosed. Yet, little is known about whether the abundance of ticks is related with the presence of its host and which climatic variables might limit their distribution. The main goal of this research project is to examine the ecological and climatic factors related to ticks found in several counties of Texas. Passive surveillance, in which samples are not actively being looked for, was conducted between the years 2011 - 2016; during which time, ticks that were found on humans, on animals, or in the environment were voluntarily submitted for pathogen detection. Each specimen was identified using dichotomous keys and identifying body structures (e.g. mouthparts, legs, palps) in conjunction with dissection microscopy for morphological identification of the ticks' species, life-stage, and sex. A total of 1081 tick samples were used in this study. Monthly precipitation and temperature was recorded for each location that a tick was found in for 12 months prior to discovery. Figures were reported from nearest weather tower within county lines according to longitudinal/latitudinal position of tick when discovered. Preliminary results show that the most common genus was found to be *Rhipicephalus spp*., accounting for 469 of the ticks collected. The most collected tick species in our state were *Rhipicephalus sanguineus,* also known as the Brown Dog Tick. The majority of the ticks reported were from the Texas Gulf Coast region, and from this region Brazos county reported the most ticks with 87 samples identified. Further observation of tick variables is needed, and will be examined using cluster analysis and principle component analysis.

2
Spatial Distribution of Nilgai Antelope Latrines: Implications for Control of Cattle Fever Ticks

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Nilgai antelope (*Boselaphus tragocamelus*), are an exotic species of ungulate in Texas. Native to India, Nepal, and Pakistan, nilgai have expanded into northeast Mexico and much of coastal southern Texas since their introduction in 1924–1949. The presence of nilgai in Mexico and south Texas has complicated the eradication of cattle fever ticks (CFT; *Rhipicephalus annulatus* and *R. microplus*). Cattle fever ticks can transmit bovine babesiosis to cattle, a serious economic threat to the U.S. cattle industry. With CFT quarantine zones established in southern Texas, ranches with infested cattle are burdened with expensive and time-consuming eradication requirements. Wildlife can hinder eradication efforts because white-tailed deer (*Odocoileus virginianus*) and nilgai are alternative hosts for CFT. Control methods, such as treated baits, are available for deer. Nilgai do not respond to bait, which is a major challenge for controlling the spread of CFT. One unique aspect of nilgai ecology is their use of communal latrines, or repeated defecation at a localized site. The existence of communal areas which nilgai re-visit presents an opportunity for CFT treatment through an application of acaricides via remotely activated sprayers. We are characterizing the distribution and usage of latrines on ranches in south Texas. We will analyze the density, size, and location of latrines in relation to roads, habitat types, and abundance of nilgai. The results of this study will have important implications for the development of treatment methods for eradication of CFT in the U.S.

3

Movement Patterns of Nilgai Antelope in South Texas: Implications for Cattle Fever Tick Management

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Nilgai antelope (*Boselaphus tragocamelus*) are a free-ranging, introduced ungulate in southern Texas known to carry cattle fever ticks (CFT). CFT are the vector for the etiological agent of bovine babesiosis, a lethal disease causing high mortality in susceptible *Bos taurus* populations and severely affecting the beef cattle industry. Efforts to eradicate CFT from the United States have been successful; however, a quarantine area is maintained between Texas and Mexico to check its entry from the infected areas of neighboring Mexico states as wildlife and stray cattle that carry CFT freely cross the border. In recent years, there has been an increase in CFT infestations outside of the quarantine area in Texas. Nilgai are of interest in understanding how CFT may be spread through the landscape. Thirty nilgai were captured and fitted with satellite radio collars in South Texas to gain information about movement patterns, response to disturbances, and movement barriers. Median annual home range sizes (± SD) were highly variable in females (1,698 ± 9,813 ha) and males (4,814 ± 6,828 ha). Female movement patterns were seasonal with peaks during June-August. These peaks appeared to be a function of break-ups in social groups rather than environmental conditions. Nilgai, which reportedly are sensitive to disturbance, were more likely to relocate into new areas immediately after being captured versus 4 other types of helicopter activities. Nilgai did not cross 1.25 m high cattle fences parallel to paved highways but did cross other fence types. Results indicate that females have a higher chance of spreading CFT though the landscape than males, but spread of CFT may be mitigated via maintenance of fences running parallel with paved highways.

4

Comparing DNA Extraction Methods to Extract Quality *Rhipicephalus microplus* DNA

Jose Guerra[1], Jason Tidwell[2], Guilherme Klafke[3], Ariel Delgado[4], Kennith Prunnet[5], Adalberto Pérez de León[6], Donald Thomas[7]

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The southern cattle tick, *Rhipicephalus (Boophilus) microplus* is a worldwide economic pest costing the agriculture industry billions. This research focuses on a comparison of three different DNA extraction methods: phenol-chloroform extraction (method 1), a modified version of the Aljanabi and Martinez protocol (method 2), and PureLink DNA Mini Kit protocol (method 3); all used to purify nucleic acids and eliminate contaminants for producing good quality DNA from *R. microplus*. Efficacy is based on outcome factors such as purification quality, time efficiency, and average yield of quality DNA collected from each sample. With method 1, 85% of the samples produced quality DNA. Method 2, 78% of the samples produced quality DNA. Lastly method 3, 75% of the samples produced quality DNA. Method 1 was able to extract a higher yield of DNA whereas method 2 and 3 had no significant difference between them. All methods had high quality DNA quality, yet method 2 and 3 are more time efficient than method 1. This research is important because with quality DNA better results from downstream analysis can be done namely on acaricide mutations, RT-PCR, population genetics, and next-gen sequencing.

5

Detection of Mutations in the Sodium Channel of *Rhipicephalus microplus* Using High Resolution Melting Analysis

Ariel Delgado
University of Texas, Rio Grande Valley

The Cattle Fever Tick (CFT), *Rhipicephalus microplus*, is a single-host tick. CFT has the largest economic impact on livestock husbandry costing the agriculture industry billions of dollars each year. The tick distribution occurs in tropical and subtropical areas, for this reason, outbreaks are still present in the southern regions of Texas and its boundary with North East Mexico, which has been established as a permanent quarantine zone for CFT. Pyrethroids are an acaricide class used to control CFT infestations. The unbounded use of pyrethroids in Mexico has led to resistance in CFT populations. The US imports over 1 million head of cattle from Mexico every year and having a rapid detection for pyrethroid resistant ticks is critical for a CFT control program. Tick samples were taken from livestock at the ports of entry into the US from Mexico. Forty-eight samples were tested using high resolution melt analysis for pyrethroid resistant genotypes. This r eal-time PCR technique is fast and versatile where new mutations can also be detected. Primers for the voltage-sensitive sodium channel were used to amplify regions of the gene that contain target site mutations. We used resistant and susceptible references from the colonies at the Cattle Fever Tick Research Laboratory as controls. Acaricide resistant genotypes were identified in the comparative analysis of melting curves of amplicons. This method provides a new path to identify other mutations that provide acaricide resistance. The research of this procedure can serve as a basis to plan outbreak control strategies.

Environmental Science

6

IRIS Tubes to Quantify Soil Anoxia: Results of experiments from natural ecosystems and an experimental proposal

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Flood-irrigation on clayey soils may temporarily create anoxic conditions detrimental to root functioning. Indicator of Reduction in Soil (IRIS) tubes, PVC tubes coated with an iron oxide paint, can be used to observe iron-reducing conditions in soils. Previous applications of IRIS tube will be discussed, and an experiment utilizing IRIS tubes to gauge the possibility of anoxic conditions developing in flood-irrigated citrus soils will be proposed.

7

Effect of Climate Change on The Potential Distribution of Wild Lima Bean (*Phaseolus lunatus L.*, Fabacea)

Cerda-Hurtado Ivon Montserrat[1*], Hernández-Delgado Sanjuana[1], Reyes-Valdés M. Humberto[2], Mayek-Pérez Netzahualcoyotl[3] and González-Prieto Juan Manuel[1]
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The distribution of wild bean *Phaseolus lunatus* in Mexico is broad and adapted to several climatic conditions. Recent studies reveal a low conservation status of wild beans in most populations due anthropogenic disturbance of their habitats. Thus the need to implement strategies of conservation *in situ* or *ex situ* arises. The aim of this study was to evaluate the impact of climate change in the years 2020 and 2080 on the potential distribution model (DM) of wild *P. lunatus* in Mexico. Distributions were modeled by using Maxent (Philips *et al*., 2006); accessions with geographical information (126) were obtained from several herbarium databases and germplasm banks and used as occurrences data. Bioclimatic variables (19) were calculated for Mexico based on climate date of the periods 1961-2009 (reference climatology) and compared with 2020s (2015-2039) and 2080s (2075-2099), under two representative concentration pathways of greenhouse gases (RCP 4.5 y 8.5). Wild lima bean was distributed across 17 states of Mexico, with Chiapas and Campeche being represented by 15.9 and 13.5%, respectively. The current distribution potential of surface of HEA (High Environmental Aptitude) was 416,748.3 km^2, whereas surface of MEA (Medium Environmental aptitude) was 43,421.3 km^2. The projected changes in the distribution of *P. lunatus* are concentrated in Yucatan Peninsula areas for the scenario RCP 4.5 in both periods, as well as in the Trans-Mexican Volcanic Belt present in the Jalisco and Michoacán states. Climate change will decrease the surface with medium and high environmental fitness for distribution of *P. lunatus*. It is necessary to design conservation strategies in the near future, to protect and prevent the loss of these genetic resources.

8

Characterizing Microbial Communities in Lower Rio Grande Freshwater Reservoirs: Donna Lake and Irrigation System versus Delta Lake and Edinburg Non-Sterile Deionized Water

Alisha M. Janiga
University of the Rio Grande Valley

The Donna Reservoir and Irrigation System (DRIS) in Donna, Texas plays an important role in providing farmers and municipalities with freshwater as well as serving as a diverse ecosystem. However, the area has a critical polychlorinated biphenyl (PCB) pollution problem and is on the National Priorities List of the Superfund Program. EA Engineering, Science, and Technology, Inc., PBC prepared a review of the ecological impacts of the pollution on local avia, reptiles, fish, and mammals, but did not include an analysis of any impact of PCB on microbial communities. This investigation compares the growth rate and carbon metabolism capability among microbial communities from the DRIS and from non-PCB polluted waters. When exposed to a solution of 0.0001 g/L of biphenyl in a methanol solution, there was no difference in growth within the samples compare to the control of just methanol. However, there was a significant difference in the growth rates of samples that potentially interacted with plants and those that didn't. This significant difference was seen again when comparing preferred carbons sources among the samples. The results from both test suggest that interaction with plant life has more impact than exposure to PCB pollution on growth and metabolism in microbial communities. Further research on this project include community catalogue with next generation sequencing, comparing presence of microbial eukaryotes, and quantifying the presence of a gene that promotes PCB degradation in bacteria.

9

Drainage system water: Does Water Quality Change During Transport?

William Quidilla and Hudson DeYoe,
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The Lower Rio Grande Valley is a region with relatively flat topography. As a result, the LRGV drainage system was developed over the years to transport agricultural and urban runoff and treated sewage plant effluent to the coast. After the point of discharge, there are no state standards on drainage water quality. Drainage water can be detrimental as high nitrate, ammonium, and phosphate levels directly or indirectly can impact freshwater and marine ecosystems. The main purpose of this study was to see if water quality changed as it moved along a drain. We focused our study on one drain, the Donna drain which receives agricultural runoff and treated effluent from surrounding areas and empties into the Lower Laguna Madre. Water samples were collected four times from February to June 2017 at 10 points along the Donna drain. Water travel time between the collection sites was calculated. Field measurements were recorded for temperature, pH, turbidity, dissolved oxygen, conductivity and water velocity. Water samples collected at each site were analyzed for nitrate-nitrite nitrogen, ammonium-nitrogen and dissolved phosphate levels.

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Identification and Analysis of Genes Related to The Stress in *Azospirillum brasilense* CBG-497.

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The gender Azospirillum is a Plant Growth Promoting Rhizobacteria (PGPR), which belongs to the alpha-proteobacteria subclass. *Azospirillum* shows a wide geographical distribution that goes from tropical regions up to moderate, cold and desert.

Previous studies have informed that exists a plasticity in the *Azospirillum brasilense* genome, awarding to this bacterium a major adaptation to different ecological niches. The pH of the soil plays a significant role in the presence of the species of this gender, mostly they meet in soils values of neutral ph.
A clear example is the strain CBG-497 which was isolated of the rhizosphere of corn cultivated in soils of Tamaulipas with pH alkaline. In the above-mentioned study model there have been identified functional subsystems of genes related to the stress. Nevertheless; there does not exist scientific evidence that sustains the importance and functionality of the above mentioned subsystems, reason for which; the objective of this project is to identify and analyze the genes that are involved in the above mentioned subsystems by bioinformatics tools, in addition to know the biological mechanisms of answer and adaptability of the gene group that allow him this species to resist the unfavorable abiotic factors to be able to survive the different ambiences to which it could be exposed.

11
Impact of Pesticides on Bacterial Communities in Mexican Soils

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The application of chemical fertilizers and pesticides have been used increasingly in the environment soil. Some studies have found that the microbial diversity may have been changed following pesticide use despite unaltered metabolism, and such changes may affect soil fertility. Despite the great help of these products in the economy of agriculture, indiscriminate use has caused damage to human health and the environment, but especially important accumulations of nitrates, nitrites, pesticides and other combinations ecologically harmful to the soil in general. Unfortunately, the widespread use of pesticides in modern agriculture is of increasing concern. The effects of pesticides in the environment have been studied by functional parameters such as microbial activities in soil. However, there are few studies assessments and incorporate a measure of microbial diversity by using the metagenomic approach. The aims of this study was to determine the possible ecological implications of altered bacterial diversity for soil fertility on soils derived from four Mexican agricultural regions by using Next Generation Sequencing (NGS) based analysis of the V3 16s RNA gene region.

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Impacts of Winter Cover Crops on Soil Respiration in the Rio Grande Valley

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Collaboration: Hilltop Gardens, Lyford TX and NCAT

Cover crops are often cited as an appropriate and effective method to restore health of soils. Soil respiration, the carbon dioxide (CO_2) released by microorganisms, insects, plant roots, decomposition, and other soil creatures, is one of the NRCS-recognized biological indicators of soil health. Cover crop trials currently underway in subtropical Texas examine the impact on soil health of four different winter season cover crop treatments. In November 2017, three common winter legumes - crimson clover, hairy vetch, and forage peas - as well as a grass/legume mix of forage peas and triticale were planted in a completely randomized block design on a 15-acre plot to proceed a planned organic sorghum planting in mid-February 2018. Using a LiCor 6400XT portable gas exchange system, we have taken respiration measurements taken over the course of the cover crop season to date. Comparatively, the largest increases in soil respiration have occurred in the forage pea and the forage pea/triticale mix blocks, likely due to their greater biomass compared to the other cover crop treatments

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Metagenomic Analysis of Sludge from Gas Wells in the Burgos Basin Tamaulipas, México.

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The soil is a complex system that harbors a large number and diversity of microorganisms. Until recently, only a small percentage of the microbiota inhabiting this ecosystem could be accessed. Currently, metagenomic analysis has allowed us to identify and study the present genomic material in more detail. On the other hand, biological processes, such as bioremediation, play an important role in the elimination of contaminants and biotechnology takes advantage of the versatility of microorganisms to degrade and convert these compounds. The Burgos basin is the most important natural gas reserve in Mexico, so the metagenomic study of well mud soils in this region will help us determine if there is a possible environmental impact, generated by contamination with organic compounds. The objective of the present work was to identify the main microorganisms present in soils of the Burgos Basin, by extracting metagenomic DNA from soil from wells of gas extraction. The results of the metagenomic analysis show 303 different species grouped in 276 genera (OTUS ID> 97%). Of the identified species they stand out: *Sphingomonas, Fabaceae, Pseudomonas, Paracoccus, Pseudoalteromonas, Stenotrophomonas, Acinetobacter* and *Rhodococcus*. The objective of this work is to make a first identification of the microbiota present in soils of the Burgos basin, which in the future will allow the generation of biotechnological opportunities for the improvement of the environment.

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Metagenomics Characterization of Rhizobacterial Community Structure Over Two Period of Cultivation of Non Modified and Transgenic Cotton

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The main objective was to determine relative abundance, bacterial diversity, and distribution of rhizobacterial communities in each type of genotype plant by using next-generation sequencing (NGS) at the V3 region of 16S ribosomal RNA gene amplicons over a two year cultivation period. We identified the structure of rhizobacterial communities by using QIIME (Quantitative Insights into Microbial Ecology) platform. Soil before sowing and rhizosphere soil derived from non-modified, transgenic 1 (herbicide tolerance) and transgenic 2 (herbicide tolerance and insect resistant) cotton plants were collected at vegetative stage across two years of cultivation period (2015-2016). The taxonomic analysis indicated that the prominent phyla in soils before sowing were Proteobacteria, Acidobacteria, Actinobacteria, and Firmicutes. However, phyla with major abundance at rhizosphere level were Proteobacteria, Acidobacteria, and Actinobacteria during vegetative stage in 2015 and 2016. However, in rhizosphere soil, we found that the abundance of Cyanobacteria phylum was increased at rhizosphere derived from transgenic 2. The alpha diversity was higher in the soil before sowing than rhizosphere soil. Statistical results showed that transgenic cotton (1 and 2) harbored the number of bacterial biomarkers than non-modified plants. Finally, Non-metric multidimensional scaling (NMDS) results showed three main groups: 1) soil samples before sowing, 2) rhizosphere derived from non-modified and transgenic cotton collection in the 2015 year and 3) rhizosphere derived from non-modified and transgenic cotton

collection in the 2016 year. We concluded that plant did a specific selection in some bacterial communities depending periods of cultivation more than genetic modification of cotton plant.

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Characterization of *Microbacterium petrolearium* Strain Isolated from Hydrocarbon-contaminated Soils from Cuenca de Burgos

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In the last years there has been a population increase that has brought with it the need to optimize the obtaining of energy sources, increasing the exploitation of natural resources, such as petroleum and petroleum-based products. In the process to supply this need, leaks and accidental spills occurs during the exploration, refining and transport of petroleum, generating an alarming amount of waste that pollutes the air, soil and seas. Currently the remediation techniques for the treatment of hydrocarbon-contaminated environments are expensive and inefficient, so the presence of microorganisms capable of degrading hydrocarbon components becomes a sustainable alternative for the recovery of contaminated sites. As a result of this situation and in search of a solution, interest has grown in studying those microorganisms capable of growing in contaminated environmental, that is, in contaminated soils and waters, in order to study their mechanisms and their biotechnological potential offering alternatives to reduce the damage in these areas.The main objective of this study was to characterize *Microbacterium petrolearium* and evaluate its tolerance to hydrocarbons, this strain was isolated from hydrocarbon-contaminated soil from Cuenca de Burgos, Tamaulipas.

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Progress and Potential of Two Biological Control Agents of the Invasive Giant Reed (*Arundo donax* L.)

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Biological control, or the use of natural enemies for pest management, may the best long-term option for managing the invasive *Arundo donax,* a noxious weed dominating riparian habitats globally and along the Rio Grande River. This work reports on the progress and potential of two arundo biological control agents permitted for release in Texas and Mexico. The arundo wasp, T*etramesa romana*, released in 2009, is having significant impacts as reported from various field locations around the world, described here using standard exit hole counts. The highest density levels were found in Texas (introduced range) compared to relatively low populations in the native range (ave. exit holes = 79.98, p = 0.001 and 4.81, p = 0.001 respectively). *Lasioptera donacis*, the arundo leaf miner, is currently permitted for release in North America. Field research was conducted in the native range of *L. donacis* (Greece) to evaluate the biotic and abiotic factors that influence population density. *Lasioptera donacis* feeding damage was documented on 40.4 and 67.8 % of dead and decaying leaf sheaths respectively across all sites. *Lasioptera donacis* was active in all locations including highly disturbed sites, but showed a slight

preference for sites near running freshwater sources (R = -0.514, p = 0.000) and lower densities adjacent to salt water sources (R = 0.463, p = 0.000). The environmental preferences of *L. donacis* in Europe signal strong potential for impact in the U.S. where *A. donax* is invasive.

17

Topping *Arundo donax* Increases Diversity and Abundance of Native Plants in the Rio Grande Riparian Zone.

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Arundo donax, also known as giant reed or carrizo cane, is native to Mediterranean Europe. Genetic studies of *A. donax* indicate it was introduced into the Rio Grande Basin of Texas and Northern Mexico from Spain and has become invasive in the riparian habitats of the southwestern United States and northern Mexico. In the Rio Grande Basin, *Arundo donax* has historically dominated these habitats where it competes for scarce water resources, lowers riparian biodiversity, reduces access and visibility for law enforcement, and facilitates the invasion and survival of cattle fever ticks, *Rhipicephalus microplus* and *Rhipicephalus annulatus* from Mexico. Topping of *A. donax* at approximately 4 feet is a method to improve visibility for law enforcement and accelerates the decline of this invasive weed. Topping increases the production of side shoots that are preferred oviposition sites for the arundo wasp, *Tetramesa romana*. Gall formation by the wasp weakens and stunts the growth of *A. donax*. In a yearlong study from January 2017 to February 2018 at the U.S. Fish and Wildlife Service, Lower Rio Grande Valley National Wildlife Refuge, Pate Bend Tract in Hidalgo, TX, we found that topping increased solar penetration and significantly increased the diversity and abundance of native plant species as compared to untopped controls. Topping of *A. donax* produces long-term benefits for the environment and meets national security needs.

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Update on Biological Control of Carrizo Cane in the Rio Grande Basin of Texas and Mexico

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Arundo donax, also known as arundo, giant reed or carrizo cane, is native to Mediterranean Europe. Genetic studies of *A. donax* indicate it was introduced into the Rio Grande Basin of Texas and Northern Mexico from Spain and has become invasive in the riparian habitats of the southwestern United States and northern Mexico. In the Rio Grande Basin, *Arundo donax* has historically dominated these habitats where it competes for scarce water resources, lowers riparian biodiversity, reduces access and visibility for law enforcement, and facilitates the invasion of cattle fever ticks, *Rhipicephalus microplus* and *Rhipicephalus annulatus* from Mexico.

Biological control of *A. donax* with insects may be the best long-term option for managing this highly invasive weed. Two agents, the stem-galling wasp, *Tetramesa romana* and the rhizome-feeding armored scale, *Rhizaspidiotus donacis* are widely established and are having significant impacts in Texas and Mexico. A third agent, *Lasioptera donacis*, a leaf mining defoliator, has been permitted and releases are in progress.

Thus far, we have documented consistent declines in above ground biomass of the invasive weed and return of desirable native vegetation, such as black willow and sugar hackberry trees along the Rio Grande. Economically, the reduction in carrizo cane biomass is estimated to save 6,000 acre-feet of irrigation water per year (which is equal to the yearly needs of McAllen, Texas, a city of 147,000 people), and worth $4.4 million. The biological control technology also is being transferred to the end users along the Rio Grande, including: the U.S. Border Patrol, Big Bend National Park, Comisión Limites y Aguas, Instituto Mexicano, Tecnología del Aguas, in California, and internationally in South Africa and New Zealand.

19

Johnston's Frankenia *(Frankenia Johnstonii)*

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The recent post-delisting monitoring (PDM) plan published for Johnston's frankenia (*Frankenia johnstonii*) (U.S. Fish and Wildlife Service, 2016) outlined the need for monitoring the population status of this species. In this project, we conducted on-site assessments in order to evaluate the potential residual impacts that landscape modifications might have on the populations (e.g., population declines). Fieldwork was conducted from July thru December 2017. Population size was estimated by following a 10 m^2 plot method, except for one of the visited sites where we counted all individuals in the population. The average number of plants per 100 square meters was then calculated and stored in excel files for population estimation purposes. On-site monitoring also took the form of population perimeter mapping in at least portions of three populations. General trends (e.g., qualitative observations) in phenology and the presence/absence of anthropogenic disturbances were also note per each visit site. Photo point updates were conducted at four different sites. First-time photo points were conducted in two populations. Population size was estimated to vary from hundreds to thousands of individuals in the different populations. The immediate future should be stable mainly because of the conservation efforts of land owners in some of the monitored populations (e.g., Dodier Ranch). In some of the monitored populations, the immediate future of the species is predicted to be stable mainly because of the conservation efforts of land owners. However, the long-term persistence of some populations located by adjacent roadways could potentially be jeopardized at some future point.

Poster Abstracts

Entomology

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Texas Psyllid Survey – Zebra Chip Monitoring Program: Comparison of the last two consecutive and contrasting years

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Zebra chip (ZC), an economically important disease of potato (*Solanum tuberosum*), has been recorded to occur in commercial potato fields in the United States, Mexico, Central America, and New Zealand. In the United States, ZC disease was first recorded in the Lower Rio Grande Valley during the early 2000's. Since 2004, Texas potato growers have suffered serious economic losses due to the high incidence of ZC causing tubers to be rejected by processors. The putative causal agent, "*Candidatus* Liberibacter solanacearum" has been later found to be transmitted from plant-to-plant by the potato psyllid, B*actericera cockerelli*. Currently, both vector and pathogen control relies broadly on the use of insecticides and monitoring for potato psyllids is an essential part of a successful integrated pest management program. Thus, a Texas Psyllid Survey – Zebra Chip Monitoring Program was initiated in 2009 to better understand insect population trends and mobility as well as to make more informed and timely pest management decisions. The purpose of this Program is to provide fast and reliable information about insect activity to both farmers and pest managers within their crops and fields. The Texas Psyllid Survey – Zebra Chip Monitoring Program provides this information for the entire potato growing season and throughout the state of Texas at a weekly basis so that cost effective and environmentally sound pest management decisions can be made. Adult psyllids are monitored using yellow sticky cards while eggs and nymphs are monitored by visual inspection of potato leaves. Here we are presenting and comparing the population densities and reproduction level of potato psyllids for the consecutive, contrasting years of 2016 and 2017. This Texas 2018 Program is currently ongoing.

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Assessing Possible Boll Weevil (*Antonomas grandis* Boheman 1843) Resistance to Malathion

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In the late 1890's, *Anthonomus grandis* (boll weevil) migrated from Mexico into the United States and spread throughout the Cotton Belt. In 1958, the National Cotton Council officially recognized the economic havoc the boll weevil was causing on U.S. cotton production. This helped initiate the Boll Weevil Eradication Program sponsored by the USDA, which sought to eradicate the boll weevil in the cotton growing areas of the U. S. This program enabled cotton farmers to greatly reduce overall pesticide use and increase cotton yields. Boll weevil eradication has been successful in all U. S. cotton regions with the exception of lower regions of South Texas, along the Mexico border. Potential reasons for the difficulties in eradicating boll weevil in this area include feral cotton that is present along the Rio Grande and irrigations cannels, regular migrations of weevils north from Mexico, and the potential for the development of Malathion resistance by the boll weevil population. To address the latter issue, we conducted a study on field collected boll weevil adults using technical grade Malathion at different concentrations to determine the presence of resistance to Malathion. Based on our results, this study indicated there is currently no resistance to Malathion in boll weevil populations in south Texas.

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Tissue-specific Rranscriptional Responses of the Potato Psyllid Related to the Horizontal and Vertical Transmission of the Bacterial Pathogen Causing Zebra Chip Disease of Potato

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The potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), is the insect vector of the fastidious alpha-proteobacterium "*Candidatus* Liberibacter solanacearum" (Lso). This bacterial pathogen causes diseases in several solanaceous crops, including zebra chip, an economically important disease of potato in United States, Mexico, Central America, and New Zealand. Lso is transmitted in a persistent propagative manner by *B. cockerelli* where it infects and multiplies in the digestive track, reproductive organs, and salivary glands of its insect vector. Lso infection of the reproductive organs of *B. cockerelli* is hypothesized to be a pre-requisite for transovarial transmission of the pathogen to the insect offspring. It has been previously shown that Lso has a detrimental effect on the fecundity and nymphal survival rate of *B. cockerelli*. To better understand the molecular bases of these biological consequences in the insect vector due to the bacterial infection, we have conducted a tissue-specific transcriptome analysis of *B. cockerelli* organs involved in the horizontal and vertical transmission of Lso. Total RNA was extracted from pools of dissected salivary glands and ovaries from non-infected (Lso-) and infected (Lso+) insects using three biological replicates. Libraries were prepared and sequenced using poly-A enriched RNA coupled with Illumina Hi-Seq technology. Bioinformatics analyses are being conducted to identify the transcriptional changes in these insect tissues in response to the bacterial infection. Identification of responsive candidate genes from *B. cockerelli* is expected to increase our understanding of a vector-bacteria interaction that results in some detrimental effects to the insect host and might ultimately aid in the development of novel control strategies to mitigate losses caused by this economically important pathosystem.

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Developing Methods to Determine Relative Infectivity/Virulence (= efficacy) of Selected Insect Pathogenic Fungi for ACP Using Spray Exposure Bioassays

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As a part of an integrated pest management program and in an attempt to manage populations of *D. citri*, a screening method has been developed to determine the efficacy of commercial entomopathogenic fungi in the Lower Rio Grande Valley. Previous programs, such as the control of sweet potato whitefly, have shown the value of mycoinsecticides in IPM programs, and many of the current commercially available mycoinsecticides are products of these programs. Nevertheless, few have been tested for efficacy against *D. citri*, therefore, this project aims to produce several candidates for field trials under conditions that are highly transferrable to field application. Protocols have been established to screen primary and secondary acquisition of adult *D. citri*, and primary acquisition by *D. citri* nymphs, in an effort to determine the impact of each acquisition method in the field. Likewise, the design and manufacturing of a Potter's spray tower and calibration of the apparatus to administer a spore deposition rate reflective of observed field application rates further increases the transferability of the collected data to field trials. Thus, in combination with climate parameters adherent to observed climate data for the Lower Rio Grande Valley, this study aims to elucidate the expected outcome of the selected mycoinsecticides in future field trials for the management of *D. citri* populations.

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Developing Methods to Collect, Process, and Screen Indigenous Fungal Strains that Naturally Attack the ACP in the Lower Rio Grande Valley

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"Candidatus Liberibacter spp.*"*, the causative agents of Citrus Greening Disease or Huanglongbing (HLB), are vectored by the Asian Citrus Psyllid (*Diaphorina citri)*. Managing population of psyllids in the Lower Rio Grande Valley is imperative given the high mortality rate of HLB-positive citrus trees. A facet of integrated pest management currently being developed is the use of strains of entomopathogenic fungi for the biological control of *D. citri*. By spraying host plants with a concentration of fungal spores, psyllids come into contact with deposited spores via primary and secondary acquisition. The result of this infection is metabolic stress, organ failure, and eventual death of the insect. Psyllid samples collected from local RV and residential areas are surface-sterilized and plated. Post-mortem fungal samples are isolated and cryostored for later identification. In order to avoid much of the regulatory obstacles that come with introducing a foreign species of fungus to the area, this project aims to identify endemic strains of pathogenic fungi that grow favorably under Lower Rio Grande Valley environmental conditions and naturally infect ACP. Such strains already have an established niche in the local ecosystem and are prime candidates for use in psyllid biocontrol. The development of these methods is currently ongoing and much screening is still to come but discovery of local strains has shown promise.

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Cold Temperature Study on Adult Mexican Fruit Fly (*Anastrepha ludens*) Mortality

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A study was conducted to determine the temperature necessary to cause 100% mortality in an adult Mexican fruit fly rearing cage over the period of 2 hours and 15 minutes. A new Mexican Fruit Fly Mass Rearing Facility is currently in the planning stages and this experiment was preformed to help expedite the design for an adult cage kill room to ensure that at least 8 to 9 adult colony cages are killed during one 8 hour shift. The new facility will have a room built large enough to effectively freeze three adult colony cages at a time. The dead flies will then be vacuumed up with a shop vacuum prior to cage cleaning and sanitation procedures. The current protocol for killing an adult colony cage requires rinsing down the cage with water, collecting the flies and waste into trash bags and freezing them for 48 hours to ensure 100% fly mortality. This protocol uses copious amounts of water and results in bags that often get frozen to the bottom of the freezer. These bags often tear resulting in messy spillage during the removal process. The five repetitions of this test utilized freezers that ranged in temperature from -24.4°C (-12°F) to -13°C (8°F) and ranged in times from 30 minutes to 2 hours and 15 minutes. Cages were removed at 15 or 30 minute intervals to determine mortality. Onset HOBO® data loggers (Bourne, MA 02532) were used to measure fluctuations in temperature for each of the freezers during the duration of this test. Results indicated that the most effective temperature was -24°C (11.2°F) resulting in complete mortality of the flies after 2 hours of exposure.

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Strengthening the Mexican fruit fly, *Anastrepha ludens*, Eradication Program with the Use of Attract-and-Kill Devices

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The Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae), is an invasive pest that infests citrus groves in Central and South America and is a recurring pest in South Texas. *A.ludens* is pest of regulatory concern, thus making economic thresholds, including the detection of five wild flies within a three mile radius circle, a wild mated female, or larvae/egg found in fruit calls for a quarantine requiring growers to follow costly strict treatment protocols. The sterile insect technique (SIT) has been used for over 30 years in an area wide preventive release and eradication program. Although largely successful, frequent detections of wild flies are made in the South Texas landscape in which unmanaged residential or abandoned citrus are intermingled with managed commercial groves. The objective of this study is to test the effectiveness of the attract-and-kill (AK) strategy for strengthening *A.ludens* eradication efforts. This strategy relies on the use of AK devices that are comprised of *A.ludens* lures and plasticized PVC infused with beta-cyfluthrin, a pyrethroid insecticide as the killing agent. AK devices will be deployed in unmanaged residential settings and abandoned groves for control of wild *A.ludens* populations. Studies were conducted to evaluate the efficacy of AK fresh and weathered devices as to determine replacement frequencies in the field. Fresh and one week AK devices were equally effective at killing all flies on contact (100% mortality). The efficacy of aged devices gradually declined with the duration of weathering from 4 to 12 weeks. Weathered devices left in the field for 8 weeks resulted in 80% adult *A.ludens* mortality, hence this cut-off date is recommended as the replacement frequency of AK devices in the field.

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Mexican Fruit Fly (*Anastrepha ludens)* 2-Component Cones Validation Test in 2017

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The Mexican Fruit Fly, *Anastrepha ludens*, is an invasive insect species indigenous to Mexico and much of Central America. The species infests fruit orchards including citrus groves in Central and South America, and has become a recurring pest in the lower Rio Grande Valley region of Texas. To combat the problem of this invasive species, the USDA APHIS PPQ along with the Texas Department of Agriculture conducts a Preventative Release Program using the Sterile Insect Technique (SIT), a suppression and control program involving mass rearing, sterilizing, and aerial releasing millions of sterile Mexican fruit flies per week. In order to verify the integrity of the control program, a surveillance and trapping program is in place which implements the most effective lure/trap combination. Capture results between two-component cones (ammonium acetate and putrescene), torula yeast borax tablets, and 10% propylene glycol capture solution (control) were compared using bioassays. For each test period, 10 Multi-lure^{R} traps of each treatment types were distributed amongst the orchard using a Latin Square trap placement with a minimum trap spacing of 30 m between traps. Bags containing approximately 5,000 dyed and sterilized Mexican fruit flies were taken to the test field and evenly released throughout the orchard, providing roughly 1,200 flies per acre release rate. Capture results demonstrated that two-component cones captured more sterile Mexican fruit flies than the torula yeast, and both the cones and torula yeast

captured significantly more sterile Mexican fruit flies than the 10% propylene glycol solution across the eight week testing period. Further comparison indicated that the two-component cones captured more flies than torula yeast at 24 hours and 1, 2, and 8 weeks of servicing.

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The Bioinsecticide *Bacillus thuringiensis*: Interaction of Dosages and Larval Sizes on Mortality of the Caterpillar Pest *Palpita quadristigmalis*

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Palpita quadristigmalis (Lepidoptera: Crambidae), the four-spotted Palpita moth, is a widespread species in North America; its larvae feed on privet, *Ligustrum* spp., olives (*Olea europaea*) and other plants in the Oleaceae. In 2017 larvae of this species caused extensive, severe defoliation of privet at Saltillo, Mexico. This damage caused widespread dieback of branches. Slight damage on olive trees was observed also. Biological insecticides like *Bacillus thuringiensis* are an interesting control alternative because they are effective while causing no mortality of beneficial insects. *Palpita quadristigmalis* larvae were collected from privet at Saltillo and separated among three age categories (sizes) according to cephalic capsule size (small 0.53 mm, medium 0.87 mm and large 1.2 mm). Larvae were exposed to four concentrations [0.0, 1.0, 2.5 and 5.0 gr/L of *B. thuringiensis* (Dipel®)] sprayed on privet leaves used as food. Each treatment (combinations of larval size/bioinsecticide concentration) consisted of eight replicates (five larvae/replicate). Compared to the control, *B. thuringiensis* concentrations caused significant mortality in treated larvae that increased with increasing concentrations of the bioinsecticide. The highest mortality (across larval sizes) was caused by the highest concentration (5 gr/L): 50-57% at 24 h, and 92.5-97.5% at 48 h. The lowest mortality occurred in large larvae exposed to the lowest concentration (1.0 gr/L): 31.6% at 24 h, and 47.1% at 48 h. We observed the normal emergence of beneficial insects (endoparasitoids) from treated larvae: wasps (Ichneumonidae and Braconidae) and flies (Tachinidae). Therefore, *Bacillus thuringiensis* caused high mortality of larvae and did not seem to affect or kill beneficial insects. It can be an alternative to control *Palpita* larvae on privet and possibly other plants like olive.

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Morphological Identification of the Four-spotted Moth *Palpita quadristigmalis* (Lepidoptera: Crambidae) Attacking Ornamental Privet, *Ligustrum japonicum* in Mexico

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The larvae of the four-spotted moth, *Palpita quadristigmalis* Guenée (Lepidoptera: Crambidae) and other *Palpita* species are tree and shrub defoliators. *Palpita quadristigmalis* has been recorded in North America from Quebec and Ontario, south to Florida and west to Arizona. Some *Palpita* species cause economic damage to olive trees and ornamentals like privet, *Ligustrium* spp. (Oleaceae). Privet it is widely planted in Mexico as an ornamental tree. In the spring of 2017, we observed severe defoliation caused by larvae of an unidentified species of Crambidae (apparently *Palpita*) on privet, *Ligustrum japonicum* at Saltillo, Coahuila, Mexico. This defoliation, along with severe drought killed many large branches; more than half of the above-ground parts of hedges were killed in some plants. Eventually many infested plants were uprooted and removed by gardeners. There are not reports on taxonomic identification of *Palpita* spp. in Mexico. In this study, we identified morphologically these crambid larvae causing damage to privet at Saltillo. Adults were reared and identified using external morphological

characteristics, and the internal genitalia were also observed. Adults were cleared from pigmentation and genitalia were examined microscopically. We also examined the larval stage. The lamella postvaginalis of *P. quadristigmalis* adults is circular, flat, rounded and symmetrical, whereas in the male genitalia, the apex of each part of the valva has three short, symmetrical crescent-shaped processes (claspers) that are closer to the base of the valve and are not parallel to the distal margin. These and other characters compared to closely related and sympatric *Palpita* species like *Palpita persimilis* and *Palpita kimballi* are presented.

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Phoretic Dispersal of *Brevipalpus* Mites on *Anastrepha ludens* (MFF)

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The genus *Brevipalpus* includes more than 300 species, is distributed worldwide, and have been reported to infest more than 1,000 different plant species. In addition to causing feeding damage, *Brevipalpus* mites have been associated with the transmission of numerous viral plant diseases. The most important viruses spread by these mites cause Citrus leprosis, a potentially fatal disease of citrus plants. While there is no record of Citrus leprosis viruses in the US, they have steadily spread from South America to Central Mexico. As these viruses cannot move without their vectoring mites, it is paramount to understand methods of dispersal for *Brevipalpus* mites. Mites generally disperse passively through wind driven movement or actively through phoresy, where they attach themselves to another organism for a period of time. Here we demonstrate, for the first time, that phoretic dispersal is possible for *Brevipalpus* mites and evaluate potential drivers of dispersal. Specifically, we established an experiment to test the impact of host quality and mite density on the rate of phoretic dispersal. By varying the density of mites and age of donor fruit, we assessed the rate of movement on *A. ludens* flies to healthy, mite-free destination fruit. Our results showed no association between host quality or mite density on the rate of phoretic movement to clean destination fruit.

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Comparison of Hinojosa and Speedy with Pyramid Pupae Loaders

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The Mexican fruit fly (*Anastrepha ludens*) is considered an invasive pest in the Lower Rio Grande Valley of Texas. Female flies lay their eggs into citrus fruits and the resulting larvae tunnel within the fruit rendering it unmarketable. Mexican fruit fly infestations negatively affect citrus growers and fruit agribusinesses in the Lower Rio Grande Valley. One major method for mitigating the threat imposed by *A. ludens* involves the use of Sterile Insect Technique (SIT), which includes the weekly aerial release of millions of radiosterilized flies. The SIT process incorporates various equipment for the successful rearing of *A. ludens*. Specifically, one machine is responsible for loading the pupae into emergence trays. The old system, Spee-Dee® volumetric cup filler with pyramid pupae loader, posed problems when damp pupae were introduced to the system. The damp pupae caused clogging and caking resulting in blockage, inhibition, and uneven distribution of the pupae into the trays. A new system was developed, Hinojosa

pupae loader, in 2017 to alleviate the problems that occurred with the old system. Testing was conducted to compare the two systems to determine if the new system was an improvement upon the old system. Results from this study indicate that the Hinojosa pupae loader took less time: to prepare for use, to fill a tower with *A. ludens* pupae, to clean up afterwards, and to construct. Furthermore, it resulted in fewer pupae spilling onto the screen and floor, was less costly to build, and it did not affect the quantity or quality of *A. ludens*. The Hinojosa pupae loader is a major improvement that is currently being assimilated into the rearing process.

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The Ventral Receptacle Compression Technique As an Adjunct to Scoring Mated Status in Fruit Flies of Economic Significance

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The determination of mated status in wild female fruit fly detections provides information to program managers that is useful in two respects. Firstly, the mated (or unmated) status, is a factor in triggering quarantine restrictions at the detection location. Invasive female fruit flies that have mated status immediately trigger a quarantine, as they have likely mated with other wild flies in the area and may have already oviposited into host material, whereas two or more unmated females are required to trigger a quarantine. Secondly, the mated status of female flies can give an indication of the sterile male activity and coverage in the area. If a mature female has not mated then it could indicate a problem with sterile male compatibility, competitiveness, or an insufficient release level in the area.

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Optimization of a Methodology to Identify and Quantify Malathion in Dipterous Larvae of Forensic Importance

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Acute pesticide poisoning is a global public health problem. In developing countries pesticides cause up to one million cases of poisoning and up to 20,000 deaths annually. In Mexico, it has been reported that due to intoxications by xenobiotics, among these pesticides, 1,400 people die each year. In the present work a methodology was developed and optimized to detect and quantify malathion in third instar fly larvae. Pieces of pork (*Sus scrofa domesticus*) of 100 grs were used as a substrate which was injected with a dose (LD) of malathion equivalent to 1375mg/ kg. Subsequently they were exposed to the environment and larvae were collected for several days: They were washed with distilled water and kept at -20^{o}C until processing. We worked with a biomass of 1.5g to which was added 1.2 mL of methanol-water phase (70/30), stirred for 5 min and centrifuged for 10 min at 3000 rpm. The supernatant was sent for HPLC

analysis, using a methanol-water phase (70/30). The retention time of malathion is 4.1 min. For the estimation of the concentration, the calibration curve was performed. The detection limit (LD) was calculated at 0.0301073ppm. The limit of quantification or determination (LC), which serves to obtain the minimum amount of the analyte present in a compound, was performed, resulting in 0.0845 ppm. The technique and procedure used may serve as a tool to identify the presence of toxic (organophosphate) in cases of forensic importance.
Keywords: Entomotoxicology, Malathion, HPLC and Diptera

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Development of Integrated Pest Management for Barnacle Scale in South Texas Citrus

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The prevalence of barnacle scale (*Ceroplastes cirripediformis*) populations and the damage caused to citrus trees and fruit in South Texas citrus orchards have demonstrated the need to make improvements on current integrated pest management (IPM) strategies. Although many citrus growers have implemented successful insecticidal spray programs, frequent outbreaks of barnacle scale, as a result of poor pesticide stewardship, require a constant quest of management strategies adapted to the changing pest profiles in citrus groves. Due to the scarcity of information regarding barnacle scale in South Texas groves, several ecological and IPM components of this pest were studied. Population density was monitored throughout several commercial groves and biological control agents were also collected. Identification of barnacle scale natural enemies will foster the implementation of biological control and ensure the sustainability of its control program. A field trial was conducted to test the efficacy of commercially available insecticides and data collected was used to make improved management recommendations to citrus growers. Weeds commonly found harboring barnacle scale within citrus groves were also identified and management of these alternative host plants was suggested in order to prevent resurgence of barnacle scale.

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Testing DNA Extraction Procedures for the Preservation of Morphological Characters of *Anastrepha ludens* (Loew) Third Instar Larvae

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Studies using Scanning Electron Microscopes (SEM) have reported morphological character differences among Tephritid fruit fly larvae of different species confirming that larval characters are useful for diagnosis. This requires examination of multiple specimens from different populations and species. Unfortunately, the procurement of identified larvae for SEM is not feasible for many exotic species. One possible solution to this problem is to identify these larvae using DNA. Methods of isolating DNA that do not require destruction of a specimen are available. It is not known if these methods damage morphological structures on the larva that are needed for SEM examination and description. This study was developed using *Anastrepha ludens* (Loew) as a model species to identify a method of DNA extraction that is appropriate for both SEM and molecular identification. Two extraction procedures were tested using magnetic particle beads for DNA isolation. For method one, an insect pin was used to pierce the center of the larvae and soaked overnight in lysis buffer. For method two, the midsection of the larvae was macerated and used for extraction, while anterior and posterior regions were saved for identification.

Untreated larvae were included as controls. Larvae remnants were placed in 100% ethanol solution and imaged using a SEM. Results indicate the extraction methods negatively impact morphological characters when observed using SEM. Both methods resulted in larvae not suitable for SEM analysis. Regarding molecular results, the piercing method yields a lower DNA concentration than the excision method. Further tests are being conducted to improve the preservation of larval characters post-DNA extraction.

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Molecular Diagnostics of Boll Weevil *Anthonomus grandis* (Boheman) using COI DNA sequencing and leg ratios collected from New Mexico and Mexico during 2017

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The cotton boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), is a major economic pest of cultivated cotton. It was first reported in 1892 in Texas. By 1922, it had spread throughout most of the southeastern U.S. where it damaged commercial cotton fields. Since 1983, eradication programs have been directed at boll weevil populations in the southern United States including New Mexico and northern Mexico states. With the exception of a few regions of Texas, it has been successfully eradicated from cotton growing regions of the United States. As part of a continuing effort by APHIS PPQ to provide diagnostic support to the U.S. and Mexico, the USDA APHIS Mission lab has performed molecular analyses on 774 trapped Boll Weevils from New Mexico (NM) and 52 trapped in Northwestern Mexico during 2017. Sequencing of the cytochrome oxidase subunit I (COI) gene is the primary method used to determine the identity of the suspected *Anthonomus grandis* weevils as either commercial cotton boll weevil (BW) or the wild cotton thurberia weevil (TW). In addition, analyses of profermora leg ratios were performed for a portion of the captures to help in determining the identity of the specimens. For the NM captures, 689 of weevils were designated thurberia weevil (TW), 85 were inconclusive (INC) and 0 were determined as boll weevil (BW) using both methods. Sequencing results showed a substantial quantity of haplotypes, 47 total in New Mexico for TW, suggesting a diverse population. Among the captures from Mexico, 30 were TW, 5 were inconclusive, and 7 were BW. The identification of these captures in the U.S. and Mexico provided cotton programs with timely information to help maintain pest-free areas.

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Evaluation on the Attractiveness of a New Synthetic Pheromone Analog for *Anastrepha ludens*

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An experimental pheromone attractant as a potential lure for the Mexican fruit fly (*Anastrepha ludens)* was evaluated. Native to Mexico and Central America, the Mexfly is considered an invasive pest in the Lower Rio Grande Valley's orchards and crops in Texas. Mexican fruit flies are introduced due to the movement of larval infested fruits or vegetables crossing our southern borders. These members of the Tephritid family cause damage when their larval stages feed and grow in a wide variety of fruit hosts, particularly in citrus flora. Fruit fly quarantines negatively affect local growers and provide obstacles to agricultural development and its business resulting in economic losses and unmarketable fruit. In the

search for new and efficient strategies to support programs aimed at controlling Mexfly infestations, Anastrepha analog DK3-063 (ARS) has been developed as a potential synthetic pheromone attractant. Its attractiveness was measured in field cages by comparing Mexfly capture using four Delta traps; three containing different treatment levels of pheromone and a no treatment control. For the tests, sterile, sexually mature virgin male and female flies were used on each of eight replications; four replications per sex. Results suggest that fly capture increased over time depending upon the amount or increment of pheromone for both male and female except for the one pheromone level of treatment on male flies. The Anastrepha analog DK3-063 (ARS) demonstrated an increased capability of attracting Mexican fruit flies with increasing levels of the pheromone.

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Species Diversity of *Brevipalpus* mites (Tenuipalpidae) in South Texas

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Brevipalpus mites are considered one the most important plant feeding mite genera in the world. In addition to cause direct feeding damage, *Brevipalpus* species have gained special attention because of their ability to transmit several plant viruses, including Citrus Leprosis Virus (CiLV), Coffee Ringspot Virus (CoRSV), and the Passion Fruit Green Spot Virus (PGSV). Understanding the diversity and host range of these mites in South Texas is important because host plants may represent cryptic asymptomatic reservoirs of CiLV and other *Brevipalpus* transmitted viruses. In this study *Brevipalpus* mites were collected from different hosts, including citrus and non-citrus plants in South Texas. Mites were identified morphologically and using a fragment of the Cytochrome Oxidase I (COI) gene. A total of six species were identified among the 258 of individuals gathered in South Texas: *B. yothersi*, *B. californicus*, *B. obovatus*, *B. lewisi* and two unidentified species. The presence of *B. obovatus* and *B. lewisi*, considered species of great economic importance, was confirmed for the first time in Texas using molecular and morphological identification techniques. *B. californicus* was the most common species and was found on six plant species. *B. yothersi* was the most diverse species, with 7 unique haplotypes. In addition, it was recovered from 11 different plant species. "*Brevipalpus* spp. A" was recovered from two different host plants, and "*Brevipalpus* spp. B" from a single host plant species. For the majority of the *Brevipalpus* samples, including the two potential vectors for CiLV; *B. yothersi* and *B. californicus*, we found no host association with mite species.

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Investigation of the *Anastrepha fraterculus* Species Complex Utilizing tRNA Region of Mitochondrial DNA

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Molecular methods used in the identification of agricultural pests have shown great utility. They provide reliable and relatively quick results that allow action agencies and pest programs to make informative decisions and develop more effective management strategies. These methods rely on well-tested diagnostic markers used in determining the identity of pests within the genus *Anastrepha*. The present study examines 99 individuals from 6 species, all belonging within the *fraterculus* taxonomic group. This research ventures in an intergenic region coding for tRNA within the mitochondrial genome. Our results

show this mitochondrial marker successfully distinguishes *A. luden* and *A. suspensa*, and may also be effective for developing source estimation methods for this and other species.

Horticulture

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Comparability of Timed Plant Growth and Development due to Biomass and Inorganic Nutrients

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Extract of ground *Medicago sativa* (alfalfa) was used as plant nutrient to study the growth promoting potential of organic extracts and inorganic fertilizers. *Capsicum annum* (pepper) and *Tagetes erecta* (African marigold) were treated with diluted concentrations of stock solutions prepared with *M. sativa* (3:1:2) and Miracle Gro™ (24:8:16) such that the working solutions had equal concentrations of nitrogen. Two hundred and fifty pots of each species was arranged on greenhouse tables in five randomized complete blocks. Statistical analysis using repeated measure ANOVA was performed on data collected from leaves, buds, and flower initiation; and plant height, and root and vegetative dry weights. Except for height and leaf initiation in *C. annum,* the results indicate that no statistical significant differences exist in all growth parameters studied. The results also indicate that the rate of decomposition is the main reason farmers and natural resource managers depend on inorganic fertilizers because of the quick growth promoting actions of such fertilizers. It was concluded that similar crop yields will be achieved with organic extracts as in inorganic fertilizers if the decomposition process is circumvented.

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Effect of Planting Date and Plastic Mulch on Tomato Production in South Texas

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Due to elevated temperatures in South Texas, tomatoes are produced under plastic mulch to reduce the yield losses. In addition, selecting appropriate planting dates can lead to higher yield and contribute to better vegetable quality. The study objectives were to evaluate the influences of planting date and plastic mulch (black and white) on the tomato yield. The experiments were conducted during the Spring and Fall season of 2016, In the Spring season, nine tomato varieties (Torero, Mykonos, Shourouq, TAM Hot-Ty, Prunus, Rio Grande, Seri, DRP-8551, and SV8579TE) were evaluated under three planting dates (From late-February till late-March) with three treatments (black, white plastic mulch, and bare soil). Whereas, in the fall, seven tomato varieties (Torero, Mykonos, Everglade, Tycoon, TAM-HOT-Ty, Shourouq, SV8579TE, DRP-8551) were evaluated under two planting dates (from early to mid-September) with two treatments (black and white plastic mulch). Higher average tomato yield was observed in the Spring season compared to the fall season. The best varieties during the spring were DRP-8551, SV8597TE, Shourouq, and Seri-first planting date, SV8597TE, TAM-HOT-Ty, Shourouq, Prunus, and Mykonos-second planting date, and prunus-third planting with average yields ranging from 68,630 to 54,061 kg/ha. In the fall, the best varieties were DRP-8551, SV8597TE, and Tycoon in the first and second planting dates with average yields ranging from 47,123 to 60,674 kg/ha. In both seasons, white plastic mulch

resulted in higher yields compared to the black and bare tomato yields. Tomatoes varieties have a great influence on the yield, and the yield is greatly affected by the plastic mulch and planting date.

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Infrared Thermography Pre- and Post-harvest Applications

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Infrared thermography is a non-contact measurement of an objects emittance of long wave radiation that allows us to visualize the temperature of an object. Stomatal conductance is a parameter frequently measured in plant science research; primarily to determine the effects of stressors on stomata and plant water relations. This measurement gives us insight into plant photosynthesis and how stress can affect growth and development of plants. Stomatal conductance can be influenced by a variety of stimuli, usually inducing rapid responses. Porometry is a common measure of stomatal conductance, but it requires contact with the leaf surface, thus disturbing the boundary layer and affecting light interception and temperature at the point of measurement. All of these disturbances can induce stomatal response and therefore influence the measurement being taken. Previous research has found a relationship between thermal imaging data and stomatal conductance, and it offers multiple advantages over porometry. Thermal imaging is well suited for automation, scalable, non-contact and can generate large amounts of data in a short timeframe. Infrared thermography has also been used in post-harvest analysis of fruit to detect bruising and surface drying. The objective of this study is to explore applications of thermal imaging during plant development and post-harvest. This includes correlating thermal images with stomatal conductance and response to drought stress, as well as post-harvest quality analysis.

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Canopy Hedging, Root Pruning, and Fertilization for Maximizing *Murraya paniculata* Branching
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Invasive *Canditatus* Liberibacter *spp.*, induces huanglongbing (HLB), “the citrus greening disease”, reducing saleable fruits worldwide. A biocontrol defense employs the predatory wasp, *Tamarixia radiata*, to parasitize nymphs of HLB’s vector, *Diaphorina citri*, the asian citrus psyllid (ACP). As ACP spawns on new branches, i.e. flush, in the host plant, this study investigates directing vegetative growth in the citrus, *Murraya paniculata* (Orange Jasmine), prior to biocontrol agent production. We hypothesize that canopy hedging induces flushing as the plant prioritizes aboveground biomass replacement, that root pruning compels belowground biomass replacement with younger uptake-efficient roots, and that supplemental fertilizer ensures nutrient availability. Thirty-six, 60 cm tall plants hedged (H) to 15 ± 2 cm in height received root pruning (R), fertilizer (13.0 ± 0.5 g 11 L pot^{-1} 24-12-6 NPK) (F), or both (i.e., HFR). All plants matured under greenhouse conditions in a complete randomized block design, with four additional untreated unhedged control (hfr). New buds were pruned every 2 weeks. Two months post-treatment, the budding leader, hfr, stalled neglecting new growth. HfR weakly headed to flowering then quit budding before resuming weak but balanced vegetative and reproductive priorities. HFr transitioned from heading to strong vegetative growth reaching 6.9 ± 1.1 early flush at 3 months with only 1.7 ± 0.3 buds per pruning. In contrast, HFR achieved 71% fewer early flush, but 76% more buds per pruning after its brief vegetative phase. Thus, hedging with fertilizer induced significantly more flush than unfertilized

treatments. HFr delivered the desired scenario forcing vegetative production rather than balanced growth. Following research compares hedged candidates in a *T. radiata* production cycle against an unhedged fertilized cohort to determine highest *T. radiata* delivery.

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Evaluating Guar Genotypes Under Various Water Regimes in Southwestern New Mexico

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Southwestern New Mexico is an arid irrigated area with water availability becoming a concern due to decrease in water table. Guar (*Cyamopsis Tetragonoloba* L.) is a potential alternative crop that can be grown in the region due to its low water requirements. Guar can be grown as a forage or as a seed crop. Demand of guar gum produced from the guar seeds has been increasing as an additive in oil and gas industry. The objective of this study was to evaluate seed yield, agronomic and physiological parameters of four guar genotypes under five drip-irrigated water regimes including a dryland treatment with or without application of biogenic silica. The dryland treatments were irrigated for the first four weeks and then received no irrigation. A split plot design was used with water regimes as the main-plots and genotypes as sub-plot treatments, and was replicated four times in the current study. The results of the study suggest that the addition of biogenic silica to dryland treatment improved its seed yield, seeds per plant and SPAD values of guar at 100% pod formation; and dryland with biogenic silica yielded similar or higher than other irrigation treatments. Among the genotypes, NMSU 15-G1 had higher 1000 seed weight, compared to all other three varieties and Kinman had higher branches per plant at pod initiation stage.

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Cover Crop Interseeding for Weed Suppression in Organic Broccoli Systems

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Collaboration: PPC Farms, Mission TX and NCAT

Weed management is a major concern for organic farming systems. The integration of cover cover crops into any cropping system may serve to provide and conserve nitrogen for crops, reduce soil erosion, reduce weed pressure, and increase soil organic matter content (Hartwig and Hoffman 1975). Interseeding cover crops can be used to reduce weeds and as an alternative to direct weed removal or herbicides. To ensure no adverse effects to the main cash crop from interseeding, cover crop selection is important. At an organic subtropical farm in the lower Rio Grande Valley, we interseeded transplants of organic broccoli (Brassica oleracea) with three treatments of cool-season cover crops: crimson clover (Trifolium incarnatum), buckwheat (Fagopyrum esculentum), and a mix of buckwheat/crimson clover (50%/50%). Quadruplets of the three treatments and a control were planted in a completely randomized Latin square design on late-December 2018 and left to grow until harvest. Preliminary data will be collected before termination for weed biomass, weed type and total yield to determine the effectiveness of the cover crop in weed suppression.

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Developing Transgenic Sugarcane with Tolerance to Combined Abiotic Stresses

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Sugarcane, a major sugar and biomass producer with a net value of $143 billion per year, is considered as a biofuel feedstock accounting for about 40% of the biofuel production worldwide. The major challenge to producing sugarcane in the U.S. is the limited tolerance of the current commercial varieties that result in considerably low yields and high costs of production under individual or combined abiotic stresses. The major goal of this research was to overexpress stress-responsive transcription factors in drought/salt-sensitive commercial sugarcane varieties in Texas. A total of 10 independent transgenic lines were generated and tested for tolerance to combined drought and salt stresses in the greenhouse. Based on data collected from water usage and measurement of physiological parameters, several transgenic lines showed enhanced stress tolerance to combined drought and salt, with the following characteristics: (a) 95.2% survival with 30.4% decrease in water usage after 10 weeks of sustained drought (50% irrigation), and (b) 92.4% survival with 28.1% decrease in water usage after 10 weeks of 50% watering with high salt (0.4 M sodium chloride). These findings will aid the development of varieties that are better suited to marginal growth environments.

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Sunburn in Citrus: Assessing Physiological Impacts and Mitigation Treatments

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The Lower Rio Grande Valley in Southern Texas is subject to high temperatures and drought which are major environmental stress factors that limit plant growth, metabolism and productivity. Rising ambient temperatures can cause significant stress to trees by impacting transpiration, photosynthesis and yield. Sunburn is a physiological disorder in citrus and other fruit species caused by excess solar radiation where fruit is discolored and exhibit varying degrees of cell death. Previous studies have shown that anti-transpirants can be used to limit water loss by closing stomata in times of drought. Reflectants can help prevent light and heat damage from solar irradiation which may lead to sunburn. This study aims to provide fundamental information on the environmental conditions correlated with sunburn in Texas and its influence on fruit quality. We also evaluated the effects of reflectants and anti-transpirant applications on mitigating sunburn in citrus. Physiological and environmental data were collected for the 2016 and 2017 seasons to determine sunburn incidence, environmental factors and the effects of reflectants and anti-transpirants on tree stress and fruit quality. Sunburn was predominantly found on the west and east side of the anti-transpirant tree canopy compared with the other treatments. Reflectant sprays reduced fruit and leaf temperatures, while the anti-transpirant treatments increased fruit temperature relative the untreated control. While there was no significant difference in sunburn incidence between treatments, anti-transpirant treatments led to conditions conducive to sunburn but these effects could be mitigated by applying reflectants.

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Integration of Bioinformatics and Omics Technologies in Crop Improvement

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Bioinformatics-based technologies play a vital role in agricultural research by providing means to generate improved cultivars with superior traits, which could help meet the rising global demands for food and fuel. In our lab, we are pursuing next-generation sequencing (NGS) and phenotyping technologies to enhance the crop stress tolerance and yields without compromising its beneficial agronomic traits, in response to various abiotic and biotic stresses affecting the productivity of crops. We are implementing and developing a combination of omics-based approaches, including comparative genomics, transcriptomics, phenotyping and gene network analysis in potato, tomato, sugarcane, energycane, and citrus to identify resistance genes, gene interaction, and molecular markers in response to various environmental stresses. These resources will be important for crop improvement using advanced biotechnology and plant breeding techniques. In addition to molecular level discovery, we are developing and implementing new Bioinformatics software and web resources to provide an innovative way to interpret large-scale datasets generated from omics-based research.

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Developing Sugarcane as a Highly Productive Biofactory for Therapeutic Proteins

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Sugarcane is an ideal platform for the production of protein-based therapeutics at commercial levels. It is one of the fastest growing species with a highly efficient carbon fixation pathway, which allows the production of a large biomass and hence a higher yield of recombinant proteins at a cost-effective scale. The specific goal of this project is to develop an efficient expression system in sugarcane to produce large quantities of a recombinant protein with potent antiviral and antitumor activities. Initially, we have recovered protein expression levels up to 0.1% of total soluble protein (TSP) (equivalent to 0.72 mg/kg fresh weight) in sugarcane lines expressing the therapeutic protein under the control of the constitutive *maize ubiquitin 1* (*Ubi1*) promoter. To optimize the expression system, we transformed sugarcane varieties CP72-1210 and CP89-2143 with a combination of constructs carrying the therapeutic protein under the control of multiple stalk-regulated promoters; *dirigent5-1* (*SHDIR5-1*) and *dirigent16* (*SHDIR16*) developed in our laboratory, and constitutive promoters; proline rich protein (*SHPRP*), elongation factor1 (*SHEF1*) and *Sugarcane bacilliform virus* (*SCBV21*). Protein yields of the lines generated with the multiple promoter expression system increased from 0.1% to 1.3% TSP (9.4 mg/kg stalk fresh weight tissue) using the combination of *Ubi1*, *SCBV21*, *SHDIR5-1* and *SHDIR16* promoters, that is over 10x more protein yield than the one obtained with a single promoter.

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Biolistic Transformation of *Carrizo Citrange* with Whole Plasmid and Minimum Cassette using Mcherry Fluorescent Protein as Visual Marker

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A visual selectable marker Mcherry red fluorescent protein was used to establish a protocol to biolistically transform *Carrizo citrange* plants. Whole plasmid containing Mcherry gene (Mcherry3) was coated on gold particles (1.0µm) and bombarded on epicotyl segments. Explants were bombarded at target distances of 6cm and 9cm; 1550psi and 1350psi rupture disk pressures; 0.3cm and 0.6cm bombardment gaps; 25psi of Hg Vacuum pressure. Transgene expression was visualized under fluorescent light with excitation maximum at 587nm and emission at 610nm after 3-4 weeks from bombardment. Number of transient and stably transformed explants were recorded. Effective conditions for shoot regeneration were investigated, with antibiotic (Kanamycin 100µg/ml) selection starting at time zero, and 24h after bombardment. Transformation of Mcherry gene will be verified by PCR after plant regeneration. Preliminary results showed high transgene expression in explants pre-cultured for 5-7 days before transformation. Further, evaluation for high-expression and low copy transgenic plants shall be conducted with whole constructed plasmid and minimum cassette (Fragment of plasmid DNA containing a selectable marker, screenable marker and a gene of interest flanked by restriction sites).

Pathology

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Evaluation of Cinnamon Extract (*Cinnamomum verum*) for the Control of Postharvest Microorganisms in Papaya

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The Papaya, *Carica papaya*, is one of the main fruits of great consumption, nevertheless this presents problems with pathogens like fungi in its postharvest, with a negative impact on the shelf life and the commercial value. Currently, extracts of plants are considered an option for replacing synthetic fungicides. In this study was assessed the fungicidal activity of cinnamon extract (*Cinnamomum verum*) in postharvest against *Colletotrichum gloeosporioides* in a completely randomized design. The results showed that at a concentration of 7% (v / v) of the cinnamon extract applied on surfaces of fruits were minor in comparison to the control fruit. Also influenced in the firmness, where it was significantly different at concentrations of 5 and 7% of the extract of cinnamon. This extract is an alternative for controlling of some microorganisms in postharvest (*C. gloeosporioides*), which cause great losses in these types of fruits.

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Efficacy of Oxiphos, Sanidate 12.0, TerraClean 5.0 and TerraGrow for control of *Phytophthora* root rot of citrus seedlings under greenhouse conditions

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Phytophthora root rot is one of the most widespread and destructive soil borne diseases of citrus in all growing areas of the world. The pathogen infects the plants starting from seedlings which show yellow leaves, die back, steam and root rot and in many cases plant death. The goal of this study was to investigate the efficacy of the chemicals OxiPhos, SaniDate 12.0, TerraClean 5.0 and the microbial inoculant product TerraGrow (BioSafe, East Hartford, CT) as a soil drench for control of *Phytophthora* root rot of citrus seedlings under greenhouse conditions. Ten, one-month old sour orange seedlings were inoculated with a zoospore suspension of *P. nicotianae* (2.5×10^4 spores/ml) before transplanting them to pots. The soil was then treated immediately with the listed products by soil drench. Forty-five days post application, 95, 95 and 90 percent reduction of *Phytophthora* induced dead was observed in SaniDate 12.0, TerraGrow integrated with SaniDate 12.0 + TerraClean 5.0 and OxiPhos, respectively as compared to the untreated inoculated control. Average plant height and root growth parameters of plants treated with the different products were significantly higher *($P<0.05$)* than untreated inoculated control. These findings show that OxiPhos, SaniDate 12.0, TerraClean 5.0 and TerraGrow can be used as fungicide for control of *Phytophthora* root rot and it can improve plant vigor of citrus seedlings.

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Trichoderma spp. Suppress Growth and Sporulation of *Phytophthora nicotianae in vitro*

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Phytophthora foot and root rot of citrus, caused by *Phytophthora nicotianae,* is an important disease affecting citrus production in South Texas. *Trichoderma* spp.*,* a ubiquitous soil-borne fungus, is capable of suppressing several plant pathogenic fungi through its antifungal activity. Our previous studies we have shown that native *Trichoderma* species can actively suppress *P. nicotianae* growth. Five of these species exhibited inhibition by competition and one by mycoparasitism. The aim of the study was to evaluate if antagonistic *Trichoderma* species can suppress *P. nicotianae* sporulation. Nine *Trichoderma* isolates (Three of *T. harzianum,* two of *T. afroharzianum*, two of *T. lixii*, one of *T. inhamatum* and one of *Trichoderma spp*.) collected from five different locations in South Texas were evaluated for their efficacy in suppressing sporangia formation in *P. nicotianae*. Dual cultures of *Trichoderma* and *P. nicotianae* were performed in triplicate and after mycelium encounter, three plugs from *P. nicotianae* were extracted near the point of contact with a cork borer. Mycelium plugs were then stained with acid fuchsin dye, pressed onto microscope slides with a cover glass and quantified under a compound microscope at 40X magnification. All *Trichoderma* isolates showed significant antagonistic activity against *P. nicotianae* by reducing mycelial growth and sporangia counts over control treatment. *T. harzianum* was found highly inhibitory to *P. nicotianae* in dual culture followed by *T. afroharzianum*, *T. lixii* and *T. inhamatum*. *Trichoderma spp.* was the least effective to inhibit the mycelial growth and sporangia counts of *P. nicotianae*. These findings show that *Trichoderma* spp. can suppress phytopathogens by competition and

by inhibition of spore formation. These results support their field evaluations as potential biocontrol agents against *Phytophthora* spp.

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Evaluation of Orondis for the Control of *Phytopthora* Propagules in Citrus orchards

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Citrus foot and root rot in a disease that is widely spread in Texas groves. It is caused by the oomycete *Phytophthora nicotianae* and depending on the rate of infection, it slowly leads to smaller fruit, lower yield, tree decline and tree death. The purpose of this study was to evaluate the product Orondis Gold™ (Syngenta) for the control of *Phytophthora* spp. in grapefruit orchards. In this field experiment, treatments were applied at two different times during the season (spring and fall) in two different plots that were under drip and microjet irrigation. We applied Orondis at 9.6 oz./Ac, Ridomil Gold SL™ (Syngenta) as control at (16 oz/Ac) and compared to an untreated control. *Phytophthora* propagules were quantified four weeks after treatment using standard protocols. After 4 weeks post spring application, all treatments reduced the amount of viable *Phytophthora* propagules in the soil when compared to the untreated control. However, after fall application, all treatments showed less than 10 propagules per cm^3 of soil, which is below the *Phytophthora* disease threshold, suggesting a natural fluctuation of *Phytophthora* propagules in the soil. In addition, fifty fruit were collected randomly from 10 trees per treatment (5 fruit/tree) and assessed size utilizing a caliper. All treatments showed an increase in fruit size compared to untreated control. Moreover, trees that received two treatments (spring and fall) of Orondis or Ridomil Gold SL, had larger fruit than both, untreated control and trees receiving only spring treatments. These results demonstrate that Orondis is effective at controlling *Phytophthora* propagules in the soil and that grapefruit trees respond with increased fruit size after treatment.

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Impact of Selected Amino Acids and Nutrients on HLB Mitigation and Citrus Tree Health

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Huanglongbing (HLB), commonly known as citrus greening is a devastating and presently incurable disease caused by bacteria (*Candidatus Liberibacter* spp.) and transmitted by psyllid vectors. Both the Asian citrus psyllid (*Diaphorina citri*) and the Asian form of HLB have invaded the USA in the late 1990s and early 2000s, now threatening the sustainability of a previously prosperous citrus industry. HLB affected trees gradually lose their vigor; produce less fruit that are lower in quality. Studies have shown that HLB infection results in drastic changes in the amino acid and mineral nutrient profiles of plants. Key elements such as nitrogen (N) and micronutrients such as zinc (Zn), as well as levels of essential amino acids (e.g. proline and glycine) are drastically reduced following an HLB infection. Thus, we hypothesized that foliar sprays of these amino acids and nutrients to citrus will mitigate the negative effects of HLB. In greenhouse studies with potted citrus trees, application of amino acids dramatically increased their respective levels, but also levels of specific nutrients in citrus foliage. Hence, application of proline resulted in increase in N and P level in leaf tissue, while application of glycine led to an increase in Mg level. In ongoing field trials, effects of foliar application of proline, glycine, and their combination on tree health and bacterial titers are being evaluated.

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Interaction Between Copper and Biochar on the Severity of HLB or Citrus Greening Disease in Citrus Groves

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The Asian citrus psyllid (*Diaphorina citri*) is the vector of *Candidatus* Liberibacter asiaticus, a bacterial pathogen that causes Huanglongbing (HLB) disease, which makes citrus fruit lopsided, bitter, discolored and unmarketable. Copper has been used as a fungicide to control various citrus diseases. However, copper accumulation in the soil resulting from heavy use has been linked to toxicity in the root zone and decline in tree health. The combination of HLB and copper toxicity in citrus will lead to rapid tree decline. Biochar, a charcoal obtained from pyrolysis of organic materials, is a possible solution to the problem of overuse of copper. To tests this hypothesis, the interaction between copper and biochar on citrus tree health was evaluated. Leaves were selected and tested for HLB confirmation using a PCR test to quantify Ct Value. Copper was sprayed to the root zone at a rate of 19 grams and biochar applied at 1 pound per tree per month to induce toxicity. Every two months, nutrient analysis was done to track overall tree health. Citrus trees treated with copper showed continual increase in copper in the root zone. Trees that received biochar and copper showed a decrease in root zone copper. Trees only receiving biochar showed the least damage done by copper to the root zone. By adding biochar to infected trees we have shown a decrease in copper and other heavy metals detrimental to the health of the tree and we are seeing increased nutrient uptake of the tree.

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Citrus Fibrous Roots: An Alternative Source Material for Huanglongbing (HLB) Diagnosis atPre-Symptomatic Stage

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Huanglongbing (HLB) is a destructive citrus disease that has been associated with three species of phloem-limited Gram-negative Alpha-proteobacteria belonging to the genus '*Candidatus* Liberibacter'. Among these species, *Ca*. L. asiaticus (CLas) is the most predominant species which is transmitted by Asian citrus psyllids (*Diaphorina citri* Kuwayama) and through propagation of infected material. The disease control strategies include (1) control of psyllid vector population, (2) removal of symptomatic trees and (3) planting disease-free trees. Since there are no known resistant commercial citrus cultivars available, early CLas detection is critical for an efficient disease control. Previously, we have shown that the root system of a HLB-positive tree has more even distribution of CLas than in the tree canopy. Based on this observation, we developed a new HLB diagnostic real-time PCR method optimized for citrus root tissue. The potential of the newly developed method for pre-symptomatic HLB detection was evaluated by testing leaf and fibrous root tissue collected from 289 non-symptomatic mature citrus trees (15-20 years old) growing in three different adjacent blocks of a commercial orchard in the Rio Grande Valley, Texas. The test results showed that 114 root samples were CLas-positive while there were only nine positive leaf samples. This study indicated that root samples serve as better diagnostic samples for CLas detection when there are no obvious HLB symptoms in the tree canopy.

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In vitro Evaluation of *Capsicum annuum* L. Varieties for Their Usefulness in the Investigation of Chili-Pathogen Interactions

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Phytophthora capsici is an important pathogen of chili peppers and other vegetable crops, causing important losses in production. Although pathogen biology, host range and dissemination is known, limited information is available about the recognition process between *P. capsici* and its hosts. Our overall goal is to study the secreted proteins during *Phytophthora capsici- Capsicum annuum* interaction. In order to reach our goal it is important to first evaluate the factors that could affect the appearance and responses of host plants to the growth conditions before the study begins. In this project, our aim is to conduct a phenotypic characterization of three different chili pepper varieties growing *in vitro*. Serrano, Pimiento and Poblano varieties were evaluated considering germination percentage, stem length, and root length. Seeds were washed, imbibed water and treated twice with 10% sodium hypochlorite to be surface sterilized, then germinated on MS medium in the dark. After 17 days the shoots were individually transferred to liquid MS medium, and incubated at 25 ± 2°C under a 16/8 h light/dark photoperiod for two months. Poblano displayed 98% germination, followed by Serrano with 82% and Pimiento 70%. Average root length was significantly higher in Poblano (21.18 cm) compared to Serrano (16.85 cm) and Pimiento (9.89cm). As expressed by the coefficient of variation, plant size homogeneity was best in Poblano 2.14%, in contrast with the other two varieties that were up to 23.24%. These results show that the Poblano variety is the best candidate to be selected for the in vitro study of chili-pathogen interactions.

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The Inhibition of the Histone Deacetylases and its Effect on the Morphology and Virulence of the Fungus *Macrophomina phaseolina* (Tassi) Goid.

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Fungal phytopathogens require different skills to infect the plants and complete its lifecycle. The strategies to infect the host consist mainly of adaptation to stress conditions, penetration of physical barriers and overcoming the plant defense system. Essentials proteins for pathogenesis are responsible of these skills and its production is regulated by epigenetic mechanism through chromatin-remodeling. Furthermore, there is evidence that these mechanisms participate in the gene-regulation involved in the development of the fungal pathogenic process, because their alteration can reduce the phytopathogens infective ability and virulence. *Macrophomina phaseolina* is an important phytopathogen that leads considerable losses of different crops, especially during drought conditions. For the above, its features of morphology and virulence have been widely studied. In the present work, we studied the role of some

histone deacetylases by means of chemical inhibition. The *M. phaseolina* morphology and virulence were analyzed under the effect of histone deacetylases (HDACs) inhibitors, valproic acid (VPA) and sodium butyrate (SBT). The main effects in *M. phaseolina* strains with the application of HDACs inhibitors (HDACi) VPA and SBT were a reduction in aerial mycelia production, vegetative growth, change in the cells pigmentation, diameter and number of microsclerotia per mm^2. Also, the HDACi VPA and SBT affected significantly ($P < 0.05$) the virulence of *M. phaseolina* in BAT-477 bean variety.