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Animal Science

AS1

Epigenetic marks within the swine placenta are differentially expressed due to season

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Previous studies from our lab reported breeding season altered epigenetic activity within swine placental and fetal tissues. External factors influence DNA methylation patterns in extra-embryonic tissues and could be related to fetal growth and herd longevity. The objective of this project was to validate gene expression of known chromatin modification and downstream targets that were influenced by summer or winter breeding season and semen storage. Three genes; ATF2, ASH2L, and SMYD3, were validated. Downstream gene targets, ILIB and NANOG, were investigated. Placental ASH2L had a tendency for increased expression from summer breedings ($P>0.05$). Previously placental expression of ATF2 indicated an interaction ($P<0.05$) between breeding season and semen storage existed, but generally, expression was greatest from placenta derived by summer breedings. In the current study, placental expression of ATF2 was greater from summer breedings ($P<0.05$). A downstream target of ATF2, ILIB, had greater placental expression from summer breedings and semen that was cooled-extended as compared to cryopreserved ($P<0.05$). Validation of placental expression of SMYD3 was greater from cooled-extended semen in contrast to cryopreserved semen ($P<0.05$). These data were unexpected, as previously we determined a difference by breeding season only. However, NANOG, downstream of SMYD3, had greater placental expression from summer breedings ($P<0.05$). The current study validated previous results indicating chromatin modification genes were primarily influenced by breeding season. Furthermore, downstream targets, were also influenced by breeding season and may subsequently influence prenatal development. These data support epigenetic modifications persist even though modern swine systems regulate and control temperature and lighting. Future studies will discern the relationship between prenatal seasonal environment, production, and herd longevity.

AS2

ASSESSING PRONGHORN MOVEMENT AT MULTIPLE SPATIAL AND TEMPORAL SCALES IN AGRICULTURAL LANDSCAPES IN THE TEXAS PANHANDLE

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Pronghorn (*Antilocapra Americana*) are located in 27 counties of the Texas Panhandle. Their behavior and movement is easily influenced by the quantity and quality of forage as well as other resources. However, data to support these influencing factors are lacking in this region and studies are required to obtain further information. The objective of this project is to determine the spatial and temporal movement distribution of these ungulates in

relation to landscape features. For this study we used two locations, one near Pampa, TX and the other near Dalhart, TX. The study collected data from 86 collars and we are currently analyzing data to determine the number of animals and number of months that we can use for further analysis. We are currently partitioning the data by months and we plan to estimate distance travelled per month, number of location per land cover, and home range. This will provide very useful information about the spatial and temporal dynamics of pronghorn movement across the landscape. By examining the habitat preferences at different scales, we can gain more knowledge regarding components of the area as well. It is essential to understand the effects of structures, barriers, habitat fragmentation, and crop production. The results of this project can then assist in the development of future management plans and provide insight on the effectiveness of the applied methods.

AS3

ACCEPTANCE OF MOTION-ACTIVATED SPRAYER SYSTEMS BY WHITE-TAILED DEER AT SUPPLEMENTAL FEEDERS

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Cattle fever ticks (CFT), *Rhipicephalus microplus* and *R. annulatus*, are vectors of the protozoan parasite responsible for bovine babesiosis, an economically important disease in the livestock industry. Along the border of Texas and Mexico, CFT are an issue because bovine babesiosis is prevalent in Mexico. Besides cattle, white-tailed deer (*Odocoileus virginianus*) can act as hosts of CFT. South Texas is home to thousands of free-roaming deer leading to the potential spread of CFT. The USDA-APHIS approved method for treating deer for CFT is using ivermectin treated corn at supplemental feed sites. Ivermectin is an anti-parasitic drug used to control tick vectors including CFT. Due to a 60-day withdrawal period before deer hunting season, medicated corn cannot provide year-round treatment. In this study, we tested the acceptance of motion-activated sprayer systems at 4 supplemental feeder sites frequented by deer. The sprayers were filled with a solution of water and nematodes, which may be used as a non-toxic biological control agent with the potential to kill ticks. The objectives of this research are to (1) quantify the use of feeders by deer when no sprayers are active, (2) compare the use of feeders by deer when sprayers are activated, and (3) determine if nematodes alter the deer's use of feeders. Our initial results did not show a difference in deer usage when sprayers were turned on or when nematodes were added. More research still needs to be done as this is a pilot run for a future larger study.

AS4

Use of water troughs by nilgai and white-tailed deer at ranches in South Texas

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Field studies were conducted near Rio Hondo, TX from Sept. to Nov. 2019 to evaluate the use of water troughs by free-ranging nilgai (*Boselaphus tragocamelus* Pallas) and white-tailed deer *Odocoileus virginianus* (Zimmerman) to support research for the Cattle Fever Tick Eradication Program in South Texas. In this study we are looking at the use of concrete and plastic water troughs by deer and nilgai at private ranches in Cameron Co. Remotely activated

sprayers could potentially be deployed at water troughs to treat deer and nilgai for eradication of southern cattle fever ticks *Rhipicephalus* (=*Boophilus*) *microplus* Canestrini (Acari: Ixodidae). Game cameras are placed at the water troughs to record use by wildlife and livestock. At ranch one concrete troughs were enclosed on 3 sides with wire fencing to force animals to drink at short end of trough. For comparison at ranch 2, troughs are not enclosed. As a positive control, cameras were placed at ponds to record the natural water use by wildlife. The most recent results show that deer (100+) and nilgai (6) have accessed the enclosed water troughs. Nilgai took longer to become accustomed to the troughs than the deer. More than 50+ deer and 5 nilgai have been observed at the unenclosed troughs, with 100+ deer and 51 nilgai at the ranch ponds. Data gathered is also being compared to weather variables such as rain, temperature, etc. Troughs may serve as an alternate treatment location for cattle fever tick infested nilgai.

AS5

Nematode Deployment Utilizing Water Troughs

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Infestation of the cattle fever tick has been an arising problem, but a more difficult task is being able to eradicate it. We have found that the nematode we are using is an effective way to kill the fever tick. Finding a way to apply the nematodes to the white tail deer and nilgai has been a challenge. Utilizing water troughs to get the deer and nilgai to get close enough to get sprayed is the experiment in the works. In this study we are looking at the use of concrete and plastic water troughs by WTD and nilgai at the Russell Ranch in Cameron Co. Concrete troughs were closed on 3 sides with wire fencing to force animals to drink at short end of trough. At this end we placed a sonic sensor facing up to activate the nilgai nematode sprayer. According to the most recent results and data gathered, so far only the WT deer have gone to the troughs and drank from them. It is believed that nilgai may take a bit more time to get used to the troughs and eventually starts using them. Given the fact that previous to the fence around the troughs, nilgai had been spotted drinking from the troughs various times. Data gathered is also being compared to weather variables such as rain, temperature etc. Rain might be the most important weather variable affecting nilgai and white tail behavior towards the troughs.

AS6

Tick-Borne Pathogen Prevalence in Puerto Rican Livestock

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Tick-borne pathogens (TBPs) are a significant source of health and economic burden in the Puerto Rican livestock industry. Previous research suggests that upwards of U.S. $6.7 million is lost annually to TBP related infestations. Unfortunately, despite two attempts at tick eradication programs, several species of ticks capable of transmitting pathogens remain widespread across the island. Additionally, changes in climate have been known to disrupt tick distribution and the prevalence of TBPs. This project seeks to evaluate the prevalence and distribution of various TBPs in ticks sampled from Puerto Rican livestock, post-Hurricane Maria in 2018. To accomplish this goal, our team collaborated with USDA APHIS officials in Puerto Rico. To this end, USDA-APHIS veterinarians collected samples from livestock (cattle and horses) from different premises across Puerto Rico. Samples were submitted to our laboratory and were screened for pathogens using both conventional PCR and real-time quantitative PCR. Currently, in the cattle samples tested we have detected a high prevalence (78.9%) of *Anaplasma marginale*, the causative agent of bovine anaplasmosis as well as the hemoparasites *Babesia bovis* and *Babesia bigemina* (overall

Babesia sp. 23.7%). In addition, sequencing data reveal the presence of *A. platys*-like organisms in 7.9% of the tested cattle samples. Furthermore, piroplasmas as well as rickettsial pathogens were detected in horses with very low prevalence (1.2% and 2.4% respectively). The results of this study will inform stakeholders of the TBP diversity affecting Puerto Rico livestock, as well as the distribution of those pathogens throughout the island. This information will be useful in future programs focused on effective eradication and management of TBP transmitting ticks.

AS7

EVALUATION OF NATIVE AND EXOTIC UNGULATE SPECIES AT SUPPLEMENTAL FEED SITES

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Texas has the most privatized land compared to any other state in the United States. Texas' history has aided in the introduction of various practices not commonly found elsewhere. Two of these practices include the use of supplemental feeders and the intentional introduction of exotic species. Private ranches in Texas bring exotic wildlife species onto their property as a source of income through hunting and wildlife viewing. These ranches also use supplemental feed to provide additional sources of nutrients for the wildlife to use. Understanding species' feeder preferences along with interactions between native and exotic wildlife at feeders can help landowners make decisions regarding best management practices. The objectives for this project are to (1) quantify individual species' use at each feeder, and (2) assess white-tailed deer(*Odocoileus virginianus*) and exotic interactions at supplemental feed sites. We used a 654 acre ranch in south Texas with 6 supplemental feed sites that supplied feed to 11 different ungulate species. Ten of the 11 species are exotic to North America, with the only native species being white-tailed deer. We examined roughly 251,000 photos from the 6 feeders from February to May 2019. We recorded the number of individuals of each species present at each feeder and analyzed them for interactions. The data from this study will provide land managers potential insight on feeder use and interactions of various species.

AS8

CAN NILGAI BE TRAINED TO CONSUME MEDICATED FEED TO TREAT CATTLE FEVER TICK INFESTATIONS?

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Rhipicephalus (Boophilus) microplus and *R. (B.) annulatus*, collectively known as cattle fever ticks (CFT), are vectors of the protozoan parasites causing bovine babesiosis. This disease is prevalent in Mexico, putting U.S. livestock herds at risk. In the borderlands of South Texas, wildlife have free access to Mexico, which increases the risk for disease transmission. Cattle are the preferred host, but white-tailed deer (*Odocoileus virginianus*) and nilgai antelope (*Boselaphus tragocamelus*) are suitable CFT hosts. Nilgai, an exotic species native to India, were first

introduced to South Texas in 1924 and are now widespread throughout South Texas. Nilgai are a major concern as a host species due to their large home range sizes and ability to make long-distance movements. There are currently no methods for treating nilgai for CFT. Delivery of treatment through feed is a common practice for wild and domestic animals. Nilgai typically do not eat from supplemental feed sites, although they can be conditioned to eat from feeders when forage is lacking. Using a 250-acre pasture and 4 feed sites, we monitored the interactions of 6 nilgai naïve to feeder use and 6 nilgai conditioned to eat feed. The objectives of this research are to: 1) determine if naïve nilgai can be influence by conditioned nilgai to use supplemental feed, and 2) assess the interactions of white-tailed deer and nilgai at supplemental feed sites. Preliminary data indicates that naïve nilgai were not influenced by conditioned nilgai to eat supplemental feed and minimally interacted with deer at feeders.

AS9

ANALYSIS OF SPACE USE BY NILGAI ANTELOPE IN SOUTH TEXAS USING A STEP-SELECTION FUNCTION

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Nilgai antelope (*Boselaphus tragocamelus*) are an introduced species that have become common in parts of South Texas. Nilgai are known hosts to the southern cattle fever tick (*Rhipicephalus microplus*) which can carry bovine babesiosis, a disease that affects cattle and thus, is a significant concern to cattle ranchers and the beef industry. The Cattle Fever Tick Eradication Program was established by AHPIS in the early 20th century and a permanent quarantine zone is maintained along the Texas – Mexico border to prevent new establishments of these ticks. Recently, cattle fever ticks have been detected on cattle north of the quarantine zone and it is thought that nilgai may be partially responsible. Little is known about nilgai behavior, and more information is needed to better understand the threat nilgai pose to the spread of cattle fever ticks. Our objectives for this study were to quantify nilgai movement behavior and space use. We deployed GPS satellite collars on 30 nilgai (10 male, 20 female) on 2 study areas in Cameron County, TX. We conducted a Step Selection Function using hourly fixes collected between April and October 2019. This method uses consecutive animal locations, or steps, to determine how covariates affect the selection of these steps over random, available steps from a distribution of step lengths and turning angles. We included covariates, such as woody vegetation, fences, and roads to see how these may influence nilgai space use and movement patterns. Our results will have important implications for the management of cattle fever ticks.

AS10

Ticks infesting and Tick-borne Pathogens infecting White-tailed Deer in south Texas

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White-tailed deer (*Odocoileus virginianus*) were censused for tick infestations at the Laguna Atascosa National Wildlife Refuge, Cameron County, Texas, as part of surveillance for invasive Cattle Fever Ticks. A total of 82 deer were taken in 2018-19 public hunts on the wildlife refuge (Fig. 1) and examined by USDA and TAHC inspectors for ticks at the hunter check-out stations. All ticks (N = 3380) were collected and delivered to the CFT research lab where they were counted and identified to species. The border region of south Texas shares a tropical tick fauna with Mexico that is unlike that found in the rest of the USA. A total of seven tick species, including cattle fever ticks, were encountered of which *Anocentor nitens* was the most common. In preliminary screening, 306 adult (117 ♀; 189 ♂) *A. nitens* from 31 WT deer and 5 nilgai were tested for *Rickettsia* sp. Anaplasmataceae sp., and

Theileria/Babesia DNA. In addition 127 nymphs and 55 larval pools (5-10 ea) were screened. Our results indicate that piroplasms and Rickettsial organisms are circulating in south Texas white-tailed deer populations.

AS11

Field evaluation entomopathogenic nematodes applied via remotely activated sprayer for eradication of southern cattle fever ticks on nilgai

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Nilgai antelope *Boselaphus tragocamelus* (Pallas) are implicated in the long-range dispersal and cycling of southern cattle fever ticks, *Rhipicephalus microplus* Canestrini (Acari: Ixodidae) in the environment, especially in Cameron and Willacy Counties. Treatment methods for nilgai are needed to support the Cattle Fever Tick Eradication Program. Remotely activated sprayers developed for application of entomopathogenic nematodes at fence crossings were tested in the summer of 2019 at private ranches in Cameron Co., TX. Sprayers are activated by ultra-sonic sensors as nilgai transit through fence crossings. Nilgai are treated directly with *Steinernema riobrave* entomopathogenic nematodes (Nemasys-R, BASF) and passively as they contact wetted foliage and soil. Satellite collars and game cameras were used to determine movements of nilgai through established fence crossings and within the treated and untreated ranches. Pre and post treatment counts of *R. microplus* and other tick species on the satellite collared nilgai in the study areas showed a significant treatment effect. These methods show promise for eradication of *R. microplus* on nilgai and the study will be refined and repeated in 2020.

Environmental Science

ENV12

Bioinformatic analysis of the metabolic pathways of indole acetic acid production in *Azospirillum brasilense*

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An alternative mechanism in the recovery of agricultural soils is the use of rhizobacteria that improve the morphological characteristics of plant species and soil structure. It has been observed that inoculation with *Azospirillum* generates benefits such as plant growth and increase in crop yield. The generation of phytohormones has been the mechanism by which it exerts this effect, in particular indole acetic acid (IAA) which is an auxin

capable of controlling various physiological processes such as elongation and cell division, etc. IAA synthesis routes in *Azospirillum* involve 3 dependent Tryptophan (TRP) pathways and the independent TRP route has not been reported. Therefore, the objective is the bioinformatic analysis of the indole acetic acid synthesis pathways in *A. brasilense*. In this sense, the genomes and proteomes of the complete genus available in the NCBI were carried out, which made the analysis of virtual genomic footprint and generation of a phylogenomic tree that showed the homogeneity of the species *A. brasilense*. Subsequently, he carried out the search and obtaining of protein sequences related to the IAA routes, the seed alignments linked to related families, as well as the annotation of the complete genomes of *A. brasilense*. Finally, the construction and search of the hidden Markov models was carried out. With this, the hypothetical map of the synthesis of AIA in general and in particular of the study bacteria for different strains was obtained. Differences in the presence of 4 dependent TRP pathways are shown and the presence of the independent TRP pathway is confirmed in all strains of *A. brasilense.*

ENV13

Changes in Agricultural Land Cover in the Texas Panhandle: 2004-2019

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The Texas Panhandle is known for large portions of irrigated agriculture focused on sorghum, cotton and wheat. The area and number of fields used to grow these crops are traditionally considered rangelands. Currently there is evidence that irrigation is depleting the non-rechargeable Ogallala aquifer, located under the Great Plains of the North America. However, there is little information on how the amount and spatial distribution of these cropland areas affect rangeland connectivity and impacts on wildlife. Our objective is to quantify the spatial distribution of crop fields and the changes that have occurred in the past 15 years in the Texas Panhandle. We used 1-m resolution digital orthoquandrangles provided by Texas Natural Resources Information Systems from the years 2004, 2006, 2010, 2012, 2016 and Sentinel-2 satellite imagery for 2019 over 24 counties within the Texas Panhandle. We digitized all cropland areas within the imagery for each tie period using a scale of 1:24,000. We observed that total agricultural area decreased from 2004 to 2012 and increased after 2012. The number of files followed a similar pattern than total agricultural area, but mean field size increased between 2004 and 2012 and decreased after 2012. We are currently digitizing crop areas from 2019. Once we complete this process, we will quantify the amount and spatial distribution of land cover data by sub ecoregion and county for each period. The findings of this study will provide a detailed picture of the spatial and temporal dynamics of land cover changes in the panhandle in the last 15 years. It will also provide insights into the effects of rangelands fragmentation and its potential impacts for wildlife species across the region.

ENV14

Tamaulipan brushland reforestation: Weed-seedling-arthropod interactions.

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Poster Abstracts

The Tamaulipan brushland forest in the lower Rio Grande Valley is a unique ecosystem home to over 50 endangered plant and animal species. Since the early 1900's, over 95% of this habitat has been cleared for agricultural and recreational purposes. Despite previous reforestation attempts, multiple factors cause high mortality in target seedlings. In addition, arthropod community dynamics and their role in seedling success is an area that is poorly understood. Over the next 12 months, 160 native tree and shrub seedlings will be monitored at La Sal del Rey National Wildlife Refuge in the RGV in search of a relationship between the herbaceous weeds: Cowpen Daisy (*Verbesina encelioides*), Honey Mesquite (*Prosopis glandulosa*) among others, our target trees: *Celtis pallida, Acacia rigidula, Amyris texana, Havardia pallens, Zanthoxylum fagara, Forestiera angustifolia* and *Acacia greggi var. wrghtii*, and the associated arthropod community. We will specifically study insect-plant interactions in the microhabitats offered by the herbaceous weeds, whether the weeds provide protection from potentially harmful insects/herbivores or reduce the number of beneficial insects around the seedlings. To accomplish this, we have conducted a weed exclusion experiment in which ten replicates of each six tree species were subjected to mowing and a control. Pitfall traps were installed and are collected bimonthly to count and identify insect populations. Our preliminary analysis suggest that certain seedling species are more associated with beneficial insects than herbivores, clearly demonstrating weed-seedling-arthropod interactions that are species-specific. We predict that these associations will have a vital role in the growth, development and success of the seedlings, thereby influencing restoration efforts in the Rio Grande Valley and beyond.

ENV15

Deep Convolutional Neural Networks Land Cover Classification For Wildlife Habitat in South Texas

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Recent advancements in remote sensing have paved the way for artificial intelligence algorithms known as machine learning. From these advancements in machine learning, Deep Learning is an approach that relies on multiple layers of nonlinear processing for feature identification and patterns. This approach can potentially save time and effort as compared to current image classification protocols. Our goals for this study is to create deep learning protocols that can be used to classify NAIP imagery in South Texas. Our specific objectives are to 1) Create a functional code model; 2) Create an accurately classified landscape image; and 3) Validate created model using field accuracy assessments. Using a created python coded model, we expect to be able to perform an image classification on an image with over 95% accuracy for each cover class. The implications of performing faster image processing is that we can continually input up to date imagery and receive up to date landscape changes to assess larger temporal areas. A successful Deep Learning, landscape classification model can assist with wildlife habitat studies by allowing researchers to cover more area of the species range in a fraction of the time as compared to the current methodologies. Once the protocols are developed we plan to use these to apply to satellite platform as well as unmanned aerial vehicle imagery.

ENV16

Evaluating Mesquite Canopy Height Using UAVs

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Recent advancements in technology such as Unmanned Aerial Vehicles (UAVs) have opened new opportunities for Natural Resource personnel to better quantify features within the landscape. Encroachment of invasive plant species on rangelands is of critical concern and Honey Mesquite (*Prosopis glandulosa*) is one of the most invasive species in Texas and the southwest. During the summer of 2019, we collected UAV vehicle imagery to assess the feasibility of using 3-D models from UAV imagery to quantify tree height. Different methodologies were used to evaluate mesquite height on a ranch in Hood County, TX. The methods used in this project included: on-site tree height field measurements and UAV outputs from two different altitude levels of 50m and 100m. A DJI Phantom IV paired with Pix4D® software was used to collect the UAV images. Drone2Map for ArcGIS® was used to process the images to create 2D and 3D outputs using Esri ArcPro® 2.3.3 to evaluate and perform analyses for mesquite distribution and tree heights. Results suggest good agreement between on-site field measurement plant height values and UAV plant height methods such as interactively using the vertical measuring tool from the Esri ArcPro® 2.3.3 software and height analysis using the UAV created LAS Point Cloud Layer. These methods showed efficient ways to monitor mesquite within the landscape. This type of information can be used for wildlife studies to quantify woody cover properties not captured by traditional satellite imagery.

ENV17

Integrating Emerging Technologies in Understanding Complex Agroecosystems: Evaluating Cover Crops Using Unmanned Aerial Systems in South Texas

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The traditional above-ground biomass collection technique is burdensome, inaccurate, and time consuming, yet it is one of the most basic agronomic parameters used to monitor crop growth and evaluate the effects of management practices. Remote sensing using unmanned aerial systems (UAS) has the potential to reduce the time, inaccuracy, and burden of collecting biomass especially in cover crops. This project looks at plant-based indicators, spectral properties of leaves, and plant height to estimate biomass of cover crops in the subtropics of South Texas. We use a lightweight UAS and multispectral sensor to capture images of fall cover crops both as stand-alone trials and mixes. Post-processed data products like normalized difference vegetation index (NDVI) and crop height models are used to correlate ground-based biomass measurements and canopy coverage. Our project successfully develops data products using UAS-based imagery to evaluate cover crop trials that researchers, extension agents, and farmers can use as an alternative to traditional techniques.

ENV18

Surveying of Cactus Moth in the Lower Rio Grande Valley

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Opuntia species are important components of the ecosystem because many species depend on them for food and survival, and the Cactus moth (Cactoblastis cactorum; Pyralidae, Lepidoptera) can cause severe damage on multiple Opuntia species.The Cactus moth is an invasive species that has been shown to damage prickle pear species, also called Nopales by Mexican Americans. Prickle pear species belong to the Opuntia genus. Six Opuntia species have been recorded in the Lower Rio Grande Valley (LRGV), Texas. The Cactus moth has been recently spotted in northeast Texas, and it is possible that it can spread into the LRGV. The purpose of this study is to monitor high risk areas like farms, conservation, roadside, landowner, private properly, and the gulf coast/beach, where cactus moths could potentially be found. For this purpose, 100 traps were placed between Hidalgo and Cameron counties. Two different type of traps were placed depending on the environment, hang and base wood traps. A map of distribution of traps was created in ArcGIS. Monitoring started May 15, 2019 and will end on September 30, 2020. The Cactus moth has not been detected. However, constant monitoring efforts need to be implemented with strong bi-national in the border of Texas (USA) and Tamaulipas (Mexico) in order to prevent the expansion of this invasive species.

Poster Abstracts

ENV19

Assessing changes in composition and age structure of restored Tamaulipan thorn forest in Hidalgo county, Texas

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Reforestation of federally managed land in the lower Rio Grande Valley (LRGV) of Texas has been ongoing since the 1980s, but follow-up surveys of reforested areas have not been consistently conducted. Furthermore, while anecdotal evidence suggests that natural recruitment of trees in highly invaded areas is low, no formal surveys of tree recruitment in restored forests have been carried out to date. To address these issues, we have begun follow-up surveys to assess the species diversity, demography, density, and biomass of restored forests at La Sal del Rey in northern Hidalgo county, part of the Lower Rio Grande Valley National Wildlife Refuge. We are using a chronosequence of planting ages - four plots reforested at intervals between 1996 and 2016 - in order to compare current forest diversity, demography, density, and biomass with those at the time of planting, as well as to assess the time scale over which any observed changes in these measures occurs. Preliminary results show that several species increase in density, one species persists without significant mortality or recruitment, and many species decrease in density or disappear over time. The number of mature trees per area decreases as the time since reforestation increases, but those that persist are on average larger than their conspecifics in more recent reforestations. Both species richness and species diversity of mature trees were seen to increase with the time since reforestation. The results of this study will be directly useful to those involved in forest restoration and other types of land management in southern Texas and northeastern Mexico.

ENV20

Potential Suitable Habitat for *Manihot walkerae*, an Endemic Endangered Plant of the Tamaulipan Thornscrub Ecoregion

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Walker's Manihot, *Manihot walkerae*, is an endangered plant that is endemic to the Tamaulipan thornscrub ecoregion. Historically, *M. walkerae's* distribution is known from only a few localities in South Texas and Tamaulipas, Mexico, but the use of Species Distribution Models (SDM) and Geographic Information Systems (GIS) can reveal areas of potentially suitable habitat for this species. The maximum entropy algorithm, better known as MaxEnt, is a modeling technique that uses presence-only data along with a set of relevant environmental variables to produce a model depicting potential species distribution. MaxEnt assigns a value of habitat suitability for each grid cell in a study area, ranging from 0 to 1, where a value of 1 represents the highest similitude with the known habitat, and values close to 0 represent low or no similitude. Using known historical occurrences for *M. walkerae* and relevant environmental variables, 50 models were produced through MaxEnt. These models were visualized in ArcGIS and a consensus model with an ensuing binary model was produced to better visualize the consensus model. To quantitatively evaluate the model, the Area Under the ROC curve (AUC) was used. The AUC value ranges between 0.5 and 1 where 0.5 is equivalent to a random classification and a value of 1 indicates a perfect adjustment. The AUC value for the consensus map was calculated as 0.93098 indicating statistically significant results. Ongoing fieldwork in Texas and Mexico is incorporating the use of this model to find new populations. With concurrent

studies being done on population dynamics and species interactions, the objective is to contribute to the long-term conservation of *M. walkerae.*

ENV21

Investigating the use of Native Plants of South Texas in Agroecosystem through Germination Experiments

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Regenerative ecological applications in agriculture are a growing field of research to develop practices investing in the health of the production system for both economic and environment benefit. An area less explored is the implementation of native plants, which could add biodiversity by creating hubs of refuge and food for wildlife and reconnect the ever-fragmented natural world of America. Native plants, through their adaptations of the subtropical climate of the Rio Grande Valley, could also prove valuable allies in dry agricultural practices utilized in the region. To move forward in this initiative, viability and germination experiments are necessary steps to understand the seeding rate and cost, which are critical considerations for farmers for incorporation. Twelve native species of grasses, forbs, and legumes were tested with 1% TTC to determine viability and set a baseline of potential. The same species underwent five methods of germination – cold stratification, H_2SO_4 acid scarification, sandpaper scarification, bubbling, and a control treatment with just water – to better understand suitability for field application. *Ratibidia columnifera* (Nutt.) Wooton & Standl. was the top contender of all forb species with a viability rate of 78.64% and germination of 70%. *Pappophorum bicolor* outcompeted all other species with an average germination of 76.50%. These species show promise as additional implements to restorative agricultural practices and will be used for further research to measure possible relationships between plant and insect interactions.

ENV22

Management of King Ranch and Kleberg Bluestems in South Texas

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King Ranch (KR) bluestem (*Bothriochloa ischemum* var. Songarica) and Kleberg bluestem (*Dichanthium annulatum* Forssk.) are invasive grass species that are overtaking rangelands and pastures throughout South Texas. Because of their competitive ability, these bluestem species leave pastures unproductive for livestock grazing and hay practices and more importantly pose a threat to native ecosystems. The purpose of this experiment was to determine the best herbicide options to manage KR and Kleberg bluestems. This study was conducted in Nueces County located in the Coastal Prairie Region (27°44'08.1"N 97°49'53.9"W). The soil type at the experiment site is Victoria clay. The

experimental design was a randomized complete block design (RCBD) with 3 by 6 meter plots consisting of three replications. This study consisted of 4 herbicidal treatments as well as an untreated control (UTC). Herbicide treatments consisted of glyphosate (280 g ai/ha), glyphosate tank-mixed with high and low rate of indaziflam (26 g ai/ha and 44 g ai/ha, respectively), and glyphosate tank-mixed with pendimethalin (7 kg ai/ha). A second application of glyphosate was applied after recovery of plant material before KR and Kleberg bluestems could produce a seed head. Data consisted of counting the number of crowns in a 0.3x0.3 m^2 square that was randomly tossed in each plot three times. Preliminary data shows 14 days after treatment (DAT) glyphosate tank-mixed with the high and low rates of indaziflam resulted with 79 and 72 crowns, respectively, compared to the glyphosate only treatment which resulted with 90 crowns (P=0.0005). Our data suggest that these herbicide treatments did suppress plant growth however more research is needed to compare other glyphosate tank-mix options to better manage these invasive KR and Kleberg bluestems.

ENV23

Exploring Social and Environmental Predictors of Heavy Metal Contamination in Rio Grande Valley Agricultural Soils

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The Rio Grande Valley of Texas is the state-wide leader in the production of fruits, vegetables, and grains. This fertile region is heavily irrigated by the Rio Grande itself, in which heavy metal contaminants above legal standards have been found by the International Boundary Water Commission and the United States Geological Survey. This work attempts to observe and document patterns in heavy metal pooling in order to mitigate the increased risk of heavy metal contamination due to urbanization. Heavy metals are of public health concern, as they are known carcinogens. We hypothesize that there exist three relationships between heavy metal content and the following: proximity to roads, household income, and soil type/elevation. We predict a direct relationship between soil proximity to road and heavy metal content and indirect relationships between household income and soil elevation/slope with contamination. This research tests these relationships through soil sampling in the Rio Grande Valley and utilizes atomic absorption spectrometry, inductively coupled plasma-optical emission spectrometry, and x-ray fluorescence spectrometry. Results are provided for each machine for As, Cd, Pb, Fe, Ni, Cu, Zn, Cr, and Se and are compared with standards for heavy metal content from the Texas Commission on Environmental Quality and the Environmental Protection Agency.

ENV24

ASSESSMENT OF COMPOST IN AGRICULTURAL SOILS IN THE NORTH OF TAMAULIPAS AS A SUSTAINABLE STRATEGY FOR DEGRADED SOILS

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The soil is a valuable non-renewable resource, which takes hundreds of years to form and acquire the necessary conditions to maintain terrestrial ecosystems; It is also necessary to produce human and animal food. However, human activities and climate change, coupled with natural phenomena, have caused the amount of this resource to decrease or lose its properties. An alternative to improve the soils, mainly those of agricultural use, is the insertion

of organic matter which occurs naturally, however the recovery time is extremely slow, but, it can be done in a guided way with specific direct materials that change the parameters The use of organic matter processed at the laboratory level applied to degraded soils is presented in this study. Organic waste, such as food waste, from pruning of parks or gardens, is a good source of organic matter to improve the physical, chemical and even microbiological quality of the soil; Also, the use of such waste helps to eliminate contaminants, since in some cases they are burned for disposal. Therefore, the objective of this work was to evaluate the insertion of organic waste (fruit and vegetable peel) in agricultural soils obtained in the municipality of Río Bravo, Tamaulipas; about the physicochemical properties of these. To evaluate the efficacy of these wastes, an experimental design was carried out morphologically and agronomically characterized, white grain sorghum grown in the greenhouse.

Entomology

ENT25

Mutations in the Voltage-gated Sodium Channel Gene of Pyrethroid-Resistant *Amblyomma mixtum* (Acari: Ixodidae) from Mexico

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Amblyomma mixtum is a vector of rickettsiosis to humans and also affects domestic animals. Tick infestations are controlled by using acaricides. The most used are the pyrethroids, however, studies have shown increasing reports of resistance to this pesticide class. The most common mechanism of resistance to pyrethroids is the presence of mutations in the voltage-gated sodium channel gene (Na-channel). This research tested the hypothesis that mutations in the Na-channel gene are associated with resistance to pyrethroids in *A. mixtum*. Adult female ticks were collected in 5 different ranches in the state of Veracruz, Mexico, and incubated at laboratory conditions to obtain larvae used in pyrethroid resistance detection bioassays (larval packet test with permethrin). One population was obtained from a Nilgai antelope captured in Los Fresnos, TX. Ticks were used to obtain the genomic DNA used in real-time PCR followed by high resolution melting (PCR-HRM) analysis of the amplicons to identify different haplotypes at the second domain of the Na-channel gene. There was a total of six populations tested for this study. The studied populations presented varying levels of resistance to permethrin. Population A had a low level of resistance. Populations D and E were moderately resistant. Population F was susceptible. For populations B and C (Nilgai collection) there was no bioassay information. After running PCR-HRM on all these samples, 6 different haplotypes (*H*) were found. *H*1 was found in all the resistant populations, in frequencies between 60 to 81.3%. *H*2 was highly frequent in the susceptible population (62.5%). The other haplotypes appeared in lower frequencies among the populations. The sequences of the haplotypes will be further investigated to search for nucleotide polymorphisms possibly associated with pyrethroid resistance.

ENT26

Influence of Precipitation on the Abundance of the Asian Citrus Psyllid

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The Asian Citrus Psyllid (ACP), *Diaphorina citri*, is the vector of citrus greening, an incurable disease affecting citrus trees worldwide and which ultimately leads to a decline in the marketable fruit production of these plant. The main goal of this research exercise is to become more familiar with the climatic preferences of ACP within the Rio Grande Valley of Texas. Our study's hypothesis is that the abundance of psyllids will be positively correlated with the amount of precipitation. The data was gathered from Texas' commercial citrus industry and consisted of ACP population indices derived from bi-weekly trapping efforts throughout the production zone (Cameron, Hidalgo and Willacy Counties) over a 2-year period, (2017-2019). Indices were compared over time across five geographically distinct trapping routes and against mean precipitation recorded by CDO NOAA. Resulting analysis produced a T-test p-value of less than 0.5, thus supporting our hypothesis. An increase of precipitation correlated with the rapid increase of ACP. Therefore, precipitation does affect the ACP population levels found in commercial groves and this will likely have implications for ACP management during traditional high precipitation windows (e.g., September). We postulate that precipitation acts to alleviate environmental stresses present throughout most of the year in this region, specifically the periods of extreme heat.

ENT27

Viability and longevity of *Steinernema riobrave* entomopathogenic nematodes for treatment of cattle fever tick-infested nilgai under field conditions in South Texas

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Field studies were conducted near Rio Hondo and Edinburg, TX from Oct. to Nov. 2019 to evaluate the longevity of entomopathogenic nematodes, *Steinernema riobrave* (Cabanillas, Poinar, and Raulston) used in remotely activated sprayers deployed for eradication of southern cattle fever ticks, *Rhipicephalus microplus* (Canestrini) infesting free-ranging nilgai (*Boselaphus tragocamelus* Pallas) in South Texas. Samples were collected over a period of 7 days from the tanks of the sprayers to determine the viability and longevity of infective juvenile entomopathogenic nematodes in the oxygenated water solution which ranged from 20 to 30°C. A Beileshi Universal clip-type LED cellphone 60x microscope attached to an iPhone 8 plus was used to capture images of the nematodes in the water solution at each sample date. Images were analyzed to determine the percentage of live and dead nematodes to determine viability. *Steinernema riobrave*, (Nemasys-R, BASF Co.) used in the weekly studies were held in cold storage for 7 months at 3-5º C with a mean viability of 81 ± 2.4 SE % live infective juveniles. Viability at day 3 and 7 in the water tank solution was 58.8 ± 6.1 SE and 42.0 % infective juveniles respectively. Viability at the nozzle head followed a similar trend but had approximately 7.1% less live infective juveniles. These results indicate that the viability and longevity of the nematodes in the sprayers were sufficiently viable to be effective against *R. microplus* for a one-week maintenance replacement schedule.

ENT28

Efficacy of X-ray irradiation to sterilize Mexican fruit fly (*Anastrepha ludens)* in a Sterile Insect Technique program

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Mexican fruit fly (*Anastrepha ludens*, Loew), "Mexfly", is a reoccurring threat in South Texas that can lead to quarantines that negatively affect Texas citrus growers. Since 1986, the US Department of Agriculture Animal Plant Health Inspection Service has carried out an integrated pest management program utilizing the Sterile Insect Technique (SIT), which involves the sterilization and aerial release of millions of irradiated Mexflies throughout the LRGV in an effort to disrupt wild fly mating and eradicate wild populations. This paper examines the efficacy of X-ray as an alternative radiation source for Mexfly sterilization. Fertile and irradiated Mexflies were mated to determine if flies exposed to X-ray irradiation at the recommended dose of 70 Grays maintained a similar level of sterility compared to Gamma irradiated flies using quality control guidelines set by the International Atomic Energy Agency.

ENT29

Anti-Tick Vaccines, "One size does not fit all"

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Cattle fever ticks are the highest pests in the livestock industry globally. Cattle fever ticks impede the development and sustainability of the livestock industry. A vaccine using the Bm86 antigen was developed for multi tick control with primary intent to control *Rhipicephalus microplus and Rhipicephalus annulatus.* The current study seeks to evaluate the efficacy of a novel vaccine (Rm86TX) developed by our collaborators at the USDA-ARS. To this end New Zealand white rabbits were immunized at day 0 with 35µg per animal of Rm86TX, followed by a dose of 25µg/animals for the first boost 3-weeks post-priming, and 7µg/animal for the second booster dose 6-weeks post-priming. Each dose was formulated in Montanide™ ISA 61 VG. Immune response to the vaccine antigen was followed by collecting 3ml of blood prior to immunization and 3, 6, 8, and 12-weeks post-priming. Rabbits were infested 12-weeks post-priming with adult ticks of either *Ixodes scapularis* or *Amblyomma americanum* species. Reproductive fitness of the ticks was measured throughout the study. *Ixodes scapularis* ticks that feed on animals immunized with Rm86TX, feed for longer and produced a larger egg mass than that of the control group. However, *A. americanum* ticks that feed on animals immunized with Rm86TX had no observable effect on feeding time or reproductive fitness. Thus, our study suggests that the use of Rm86TX is only recommended to control infestations with both *R. microplus* and *R. annulatus* as previously documented. Therefore, no cross-protection was evidenced in this study, and we do not recommend the use of this vaccine antigen to control tick infestations in wildlife.

ENT30

Molecular characterization of entomopathogenic fungal field isolates collected from *Diaphorina citri* in the Lower Rio Grande Valley

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Entomopathogenic fungi are ubiquitous microbes that induce disease in arthropods and can also be found as endophytes and in soils. These fungi have been used as a means of biological control for various insect pest species. Our laboratory is examining these entomopathogens as one additional facet in an existing integrated pest management strategy for the control of the citrus pest *Diaphorina citri*, a vector for the fastidious bacterium *"Candidatus* Liberibacter asiaticus", the disease-causing agent of Citrus Greening disease. In order to examine for the prevalence of entomopathogenic fungi within *D. citri* populations, field collections were conducted and fungal isolates were collected. Preliminary morphological analyses identified the isolates belong within the genera *Beauveria*, *Isaria*, and *Lecanicillium*. Molecular analyses were performed to confirm these identifications using previously published molecular markers for each of three morphological types. Phylogenetic results confirmed the identity of the *Beauveria* samples and isolates as *B. bassiana*. The molecular marker used for *Isaria* samples provided insufficient resolution to identify to the genus-level. Sequences from the *Lecanicillium* morphotype did not match *Lecanicillium* database in GenBank. Rather, sequences for samples initially identified as *Lecanicillium* were more closely related to *Fusarium* species, sharing greater than 99% identity matches with the genus. Future work will investigate additional gene loci to hopefully improve the resolution of current tests in order to conclusively determine the identity of these isolates.

ENT31

Developing methods to collect, process, and screen indigenous fungal strains that naturally attack the Asian citrus psyllid in the Lower Rio Grande Valley

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The Asian citrus psyllid (ACP) (*Diaphorina citri*) vectors "*Candidatus* Liberibacter spp.", the causative agents of Citrus Greening Disease or Huanglongbing (HLB). Managing the population of psyllids in the Lower Rio Grande Valley (LRGV) is imperative given the continuous increase in detection of HLB-positive trees. An aspect of integrated pest management (IPM) in development is the use of strains of entomopathogenic fungi for the biological control of *D. citri*. In an attempt to find endemic strains of entomopathogenic fungi that grow favorably under LRGV environmental conditions and naturally infect ACP, psyllid samples were collected from local RV and residential areas, surface sterilized, and plated. Post-mortem fungal samples were isolated and cryostored in glycerol for later identification. Over 8,500 samples collected from 267 sites throughout the LRGV led to the positive identification of two *Beauveria* spp. isolates (ACP01 & ACP02) which were grown in liquid culture and solid substrate fermentation (SSF). Additionally, four new potential isolates including one *Lecanicillium*, one *Isaria*, and two *Beauveria* spp. were discovered and are pending genetic identification. Current efforts include the implementation of isolates into applicable bioassays and characterization of the fungus via radial growth plates, UV tolerance, among other techniques. ACP01 and ACP02 have shown relative success in spray exposure bioassays and growth kinetics, and may prove to be good candidates for the control of ACP populations in the LRGV.

ENT32

Analysis of *Wolbachia* co-infections on ACP worldwide populations using Next Generation Sequencing

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Diaphorina citri is the most effective vector of the bacteria, *Candidatus* Liberibacter asiaticus (CLas) that causes citrus greening disease. Like many other insects, *D. citri* can be infected with the bacterium *Wolbachia*. *Wolbachia* is an endosymbiont that can influence the survival, reproduction, and defenses of infected insects. For this reason, *Wolbachia* has the potential to be used for insect biocontrol. Previous studies have found a break in population structure for *D. citri*, concordant with *Wolbachia* strain distribution, suggesting possible cytoplasmic incompability. However, co-infections with multiple *Wolbachia* lineages was also observed in some specific populations. The distribution of co-infection patterns may inhibit or enhance cytoplasmic incompability. Unfortunately, the extent of co-infection and the identity of co-infection members is still unknown. In this study we analyzed the genetic diversity of *Wolbachia* among *D. citri* populations from 24 different countries. DNA was extracted from 384 individuals. Six *Wolbachia* genes *wsp, coxA, fbpA, ftsZ, gatB,* and *hcpA,* and general bacterial 16S were amplified by PCR, and a library was constructed using Illumina indexing primers for Next Generation Sequencing (NGS) analyses. The results from this study will provide the rate of co-infection on these *D. citri* populations, and will provide more information about possible cytoplasmic incompability. This information could be used in future biological control programs for *D. citri*.

ENT33

Investigating alternative radiation sources in sterile insect technique programs: X-ray radiation effects on *Anastrepha ludens* flight ability and emergence

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The Mexican Fruit Fly (Mexfly), *Anastrepha ludens* Loew, is an invasive pest of citrus in the Lower Rio Grande Valley (LRGV). Citrus growers in the LRGV rely on a multi-faceted integrated pest management approach to control Mexfly that includes pesticide applications and an intensive trapping and Sterile Insect Technique (SIT) program led by the USDA-APHIS. Successful SIT programs require an efficient and reliable source of radiation to deliver a low dose (70 Grays) of radiation to developing pupae. Gamma radiation has been the primary source for global SIT programs in the last 60 years, but efforts are underway to move to other sources of radiation for security purposes. Quality control tests were conducted on Mexfly pupae irradiated in a Rad Source 2400V X-ray irradiator at 70 Grays. Results indicate that X-ray irradiators adequately sterilize Mexfly adults while limiting cell damage to non-target tissues when flies were chilled prior to irradiating. These tests and results are consistent with previous studies investigating the use of X-ray on other Tephritid pests.

ENT34

Wild Strain Development of the Mexican Fruit Fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae)

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The Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), is a pest of numerous crops and fruits in its native range of Mexico and Central America. The detection of either five wild adult flies within a three-mile radius circle, a wild mated female, or larva/egg found in fruit causes a quarantine requiring growers to follow costly and strict treatment protocols before fruits can be harvested or transport out of the quarantine area. To ensure that *A. ludens* does not permanently establish itself, a Preventative Release Program (PRP) comprised of mass-producing and releasing irradiated adult flies has been ongoing for over 30 years. The goal of releasing irradiated adult flies is to over-flood the wild population with sterile flies, thus reducing the chance of successful mating among wild flies. To ensure the best quality of an *A. ludens* strain for use in the PRP program, it is necessary to have: (1) a back-up *A. ludens* strain capable of replacing the current 'Willacy' strain or (2) an additional wild strain to infuse desirable traits into the current mass-produced strain. The protocol to develop a wild strain consists of a fruit sampling and

surveying for *A. ludens* larvae when wild flies are detected in a grove. Possible larvae infested fruit are transported to the Arthropod Quarantine facility on Moore Air Base and placed on larvae collection trays. If larvae are found, a new strain is reared and numbers increased on grapefruit. The resulting pupae and adult flies are subjected to quality control measures that must meet or exceed the IAEA standards before the prospective strain is considered for use in the Mass Rearing Facility.

ENT35

Biological control of Asian citrus psyllid in Mexico using *Tamarixia radiata*

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Huanglongbing (HLB) is a devastating disease affecting millions of citrus trees worldwide. It is spread by *Diaphorina citri*, a ubiquitous pest that acts as a vector for the bacterium "Candidatus Liberibacter asiaticus", the cause of HLB. *Tamarixia radiata*, an ectoparasitic wasp that attacks the nymph stages of *D. citri*, was introduced as a biological control agent to suppress populations. *T. radiata* is mass produced at the USDA S&T Mission Lab and then distributed to Mexico, specifically Tamaulipas and Tijuana. Tamaulipas and Tijuana began releases in 2013 and 2014 respectively. As of the end of FY2019, the S&T Mission Lab has provided 1,938,315 parasitoids for release in Tamaulipas and 2,605,191 have been released in Tijuana. From 2013 to 2015 *D. citri* nymphs decreased, however, from 2016 to 2019 that number has shown a 73.38% increase. In contrast, Tijuana *D. citri* nymph populations decreased 75.75% from 2014 to 2019.

ENT36

Development of a Loop-mediated isothermal amplification (LAMP) assay for identification of *Rhagoletis cerasi*

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The species of tephritid fruit fly known as European cherry fruit fly (ECFF), *Rhagoletis cerasi* (L.), is a major pest of sweet cherry crops throughout Europe and western Asia. This invasive pest was first reported in North America in 2016. Accurate identification of the pest is important to confirm its current distribution and detect evidence of its movement and host use. Identification of damaged adults and immature life stages are not reliable using morphology. In this case, the use of molecular diagnostic tools may be able to provide an identification. Molecular identification tools that are available based on DNA sequencing are time consuming, expensive, and need expensive specialized equipment. In this study, a Loop-mediated isothermal amplification (LAMP) assay was developed for the identification of *Rhagoletis cerasi*. LAMP technology allows a very specific and rapid molecular identification that is straightforward, user-friendly, requires the use of simple and affordable equipment, and is easy to interpret. In the development of this assay, nuclear ribosomal internal transcribed spacer 1 (ITS1) DNA sequence

region was cloned from *R. cerasi* to select species-specific primer pairs. In addition, using the 18S ribosomal RNA (rRNA) gene, a control LAMP primer set was developed to serve as an indicator of sufficient sample quality. Both the ITS1 and the 18S LAMP protocols were optimized and combined to develop a test to identify *R. cerasi* in North America. Overall, as a result of its simplicity and speed, this LAMP assay should provide a timely and a promising new tool for precise molecular diagnostics of this insect pest.

ENT37

Determining relative infectivity/virulence (= efficacy) of selected entomopathogenic fungi against ACP using spray exposure bioassays

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As part of an integrated pest management strategy against the Asian citrus psyllid (ACP), Diaphorina citri Kuwayama (Hemiptera: Liviidae), a screening protocol was developed to determine infectivity, virulence, and overall mortality of commercialized entomopathogenic strains of fungi. Fourteen strains sourced from commercial formulations of blastospore and conidiospore suspensions have been tested against a standardized strain in a controlled setting. A statistical ranking system was established in which top performing pathogenic strains are selected for further screening and field trials. Potter spray towers are utilized to deliver a range of doses of viable spores per milliliter on adult D. citri in spray exposure bioassays. Spores/mm^2 deposited during the bioassays are consistent with the deposition rates observed in real-world spray applications (spores/hectare)(Fig.5). After a 7 day incubation period at climate parameters selected to reflect the conditions in the Lower Rio Grande Valley (LRGV), mortality rate of the D. citri is assessed and the collected data is analyzed against a standard, PFR-97® (Isaria fumosorosea; Apopka97 strain). The resulting data is analyzed in terms of infectivity and virulence, providing insight into mortality, rate of infectivity, and susceptibility to infection. Of the strains tested, 8 have shown significantly greater efficacy when evaluated against the standard. This protocol is being adapted for both primary and secondary acquisition of adult D. citri as well as primary acquisition of nymphs, and will elucidate potentially effective strains to be selected for field testing and application on citrus across the LRGV and other citrus growing areas.

ENT38

Unmanned Aerial Systems (UAS) SWARM release of marked Mexican Fruit Fly (*Anastrepha ludens* Lowe) adults for trap recapture in citrus orchards in south Texas

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The Mexican fruit fly (MFF), *Anastrepha ludens* (Loew), is an invasive species of great economic importance with a potential to cause millions of dollars in damage to the citrus industry in south Texas. An alternative method of sterile

fly release was tested using UAS (drones). Unmanned Aerial Systems (UAS) SWARM releases (having multiple drones in the air at one time) were conducted on 13 March 2019 in a ~140 acre citrus orchards in south Texas. The SWARM technique was demonstrated in this test when all three drones were in the air at the same time with each drone releasing a different color of dyed flies. Unfortunately, one of the drones sustained a mechanical problem with a motor. Two of the drones flew in tandem releasing sterile Mexican fruit flies across the field. Recaptures for the first drone were 295 flies at 24 hours, 54 flies at 72 hours, and 2 flies at 6 days for a total of 351 flies, with a recapture rate of 351 flies/ 39,457 flies = 0.0089 or ~0.89%. Recaptures for the second drone were 220 flies at 24 hours, 45 flies at 72 hours, and 3 flies at 6 days for a total of 268 flies, with a recapture rate of 268 flies/ 42,763 flies = 0.0062 or ~0.62%. Recaptures for the third drone release were 221 flies at 24 hours, 51 flies at 72 hours, and 2 flies at 6 days for a total of 274 flies, with a recapture rate of 274 flies / 38,201 flies = 0.0072 or ~0.72%. The release container using the belt drive successfully dispersed over 98% of the dyed flies.

ENT39

Effects of watering schedule on production of *Tamarixia radiata* and *Murraya paniculata* health

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Huanglongbing (HLB) also known as Citrus Greening is considered to be the most damaging citrus disease globally. The invasive species Asian citrus psyllid (ACP), *Diaphorina citri*, vectors the bacterium "*Candidatus* Liberibacter asiaticus" that is linked to this destructive citrus disease. A parasitoid known as *Tamarixia radiata*, having the highest host specificity, has been introduced and mass produced to control the population of *D. citri* in the Lower Rio Grande Valley. Observations have shown that the production of *T. radiata* decreases during high heat months. Varying watering schedules were investigated to determine the difference in production of adult *T. radiata* and overall health of the orange jasmine plant. A total of 27 plants were set up in nine cages and monitored over a period of eight weeks. Three groups of three cages were divided by watering schedules and greenhouse placements, while volumetric water content, salinity, and soil temperature were recorded to analyze plant health. In addition *T. radiata* collections were recorded daily to assess effect on parasitoid production. The outcome of the study showed that plants watered three times a week had better long-term health but had no impact on production of *T. radiata*. However, an increase of *T. radiata* was observed in cages located closest to ventilation fans. Cages nearest to fans produced an average of 597 *T. radiata* while cages that had the least access to airflow only produced an average of 143. These results revealed that cages placed closest to fans have an increase of *T. radiata* production, where watering schedules have little impact on mass rearing.

ENT40

Change of flush growth in Orange Jasmine (*Murraya paniculata*) plants after additional dose of liquid fertilizer

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Tamarixia radiata is a parasitoid wasp of the citrus pest, *Diaphorina citri*, which vector the bacteria responsible for Huanglongbing or Citrus Greening disease. Sixteen bug dorms were set up in a greenhouse used for *T. radiata* production for the biological control of *D. citri.* In order to improve the development of new flush needed by *D. citri* to lay their eggs on, three *M. paniculata* plants per bug dorm were retreated with liquid fertilizer. Each bug dorm was infested with *D. citri* every third day, allowing them to produce offspring. *T. radiata* production was logged daily using hand tally counters. Previous studies have shown that as more flush is present, the more eggs *D. citri* lay. As such, a positive correlation exists between *D. citri* egg quantity and *T. radiata* production. In this study, an

additional 200 mL of liquid fertilizer were added seven days after inoculation on half of the cohort while the other half was used as control. Four days after the secondary fertilization, seven out of eight bug dorms with treated plants had more developing flush than those that went untreated. In addition, production of *T. radiata* in five out of eight bug dorms with treated plants had higher emergence than those untreated. These findings revealed that an increase in fertilizer indirectly resulted in a net increase in *T. radiata* production.

ENT41

X-ray irradiation to reduce *Pseudomonas aeruginosa* in mass rearing facilities

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A test was conducted to observe the efficacy of irradiating a known culture of the bacterium *Pseudomonas aeruginosa (P. aeruginosa)* from a Mexican fruit fly (*Anastrepha ludens*, Loew) (Mexfly) mass rearing facility. The control and suppression of yield-reducing and often lethal microbial organisms is a major factor in the efficient production of high-quality insects in mass rearing facilities. Maintaining production of the Mexfly allows for a successful Sterile Insect Technique program that protects citrus fruits in the lower Rio Grande Valley. *Pseudomonas aeruginosa* was found in an adult Mexican fruit fly egging panel and identified to species by Accugenix® at Charles River Lab. Isolates were streaked onto Pseudomonas Fluorescens (PF) agar plates and irradiated in a Radsource 2400V X-ray machine at increasing doses (0, 70, 700, and 1000 Gy). Irradiated colonies were incubated at 28°C and observed at 24 hours, 48 hours, and 96 hours. No bacterial colonies were seen at the 1000 Gy level at any time post inoculation and irradiation. Bacterial growth increased noticeably as dosage decreased. Future tests will be performed to determine the most effective dose for inactivation of *P. aeruginosa* below 1000 Gy, with the goal of developing a suitable dose to irradiate Mexfly diet ingredients for control of harmful bacteria.

ENT42

Comparison of two Brewer's yeasts vs current hydrolyzed yeast in Mexican fruit fly adult rearing cages

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A study was conducted comparing the efficacy of two new formulas of Brewer's yeast, Amberex AX1003 (lot number 3036041) and Amberex AX695 (lot number 2260092), against the current yeast (Lallemand lot number FNI210ag) being used in the Mexican fruit fly adult diet. Brewer's yeast provides additional protein content in the diet of the adult Mexican fruit fly. Protein enriched diets reduce time to reach sexual maturity from approximately fourteen days to ten days. Decreasing the time in which an adult fly reaches sexual maturity helps to maintain

required production levels at the mass rearing facility. In order to determine if the two new formulations of Brewer's yeast are acceptable replacements for the current yeast, egg quantity and quality were measured and analyzed across time. Adult colony cages were set up with the current yeast, AX1003 yeast and AX695 yeast. Eggs were collected and measured daily to determine egg production over a period of four weeks. Collected eggs were sanitized and bubbled twice weekly in order to determine final eclosion (percent egg hatch). Egg eclosion data provide insight into the quality of the eggs based upon the percentage of total hatched eggs. Results indicated little variability amongst the three yeasts tested. Flies from the AX1003 yeast diet produced more eggs per week at a higher percent eclosion indicating improved egg quality.

ENT43

Testing LAMP method targeting the Internal Transcribed Spacer 1 (ITS1) region for detection of *Rhagoletis cerasi*

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Recently, a molecular diagnostic tool was developed at the APHIS-PPQ Mission Laboratory for the identification of *Rhagoletis cerasi*. This fruit fly is a pest native to Europe but has recently been detected in Canada and parts of New York State. The molecular diagnostic tool uses two LAMP (Loop-Mediated Isothermal Amplification) reactions to identify the pest. One reaction targets the internal transcribed spacer 1 (ITS1) DNA region of *R. cerasi*. The ITS1 diagnostic reaction is run alongside a control reaction which amplifies the highly conserved 18S rRNA gene. In this study, the species-specific molecular diagnostic tool was tested on a total of 312 *Rhagoletis* fruit flies to estimate the rate of false positive and negative reactions. The flies used to test the tool included eight species that were identified prior to testing using adult morphology or a DNA barcoding method. These flies consisted of 98 larvae and 214 adults. There were no false positives (i.e., incorrectly identifying a fly as *R. cerasi*) observed in the study. For the larvae data set, one *R. cerasi* specimen resulted in a false negative on the first run. For the adult data set, two *R. cerasi* specimens generated false negatives on the first run. Repeating the reactions resulted in no false negatives in the data set. In conclusion, the LAMP molecular diagnostic tool for identifying *R. cerasi* performs well as a screening technique but a low rate of ITS1 reaction failure is possible from random, technical errors. Repeated runs of fly specimens is one approach to reduce this error rate.

Horticulture

HORT44

-Withdraw-

HORT45

Developing sustainable sweet potato for South Texas: an opportunity to diversify agricultural production and promote soil health

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Bringing sweet potato to South Texas (So-TX), particularly the Rio Grande Valley (RGV), as a summer crop offers the potential for profit while supporting food and nutritional security. The high nutritional value of the sweet potato and its capacity to thrive under a So-TX summer presents opportunity to augment grower earnings and diversify Texas' specialty crop offerings. South Texas is located in a subtropical semi-arid climate due to its high temperatures and irregular precipitation; as a result of this, farmers opt to leave their fields fallow during the summer months, jeopardizing overall soil health (i.e., organic matter depletion, shutdown of soil biological activities, and particle losses through erosion). Thus, before committing acreage, farmers would benefit from tailoring crop and soil management towards maximum efficiency and sustainability. This research aims to (i) identify sweet potato varieties for the RGV with improved adaptability for our climate and soil conditions and (ii) identify the best fertilization practices that support both plant and soil health as well as efficient use of resources. We carried out a 150-day field trial testing organic and synthetic fertilizers on three sweet potato varieties (i.e., Bonita, Beauregard, and Vardaman). Soils were sampled every 30 days and agronomic performance was assessed at harvest. The trial implemented drip irrigation to minimize sodium build up and maximize water conservation. Following field trial, laboratory activities assessed soil available nutrients (N, P, K), moisture, pH, and salinity. We will present performance of each variety as well as the dynamic and use efficiency of each fertilization management identifying the best fertilization management and timing to meet the plants requirements, especially for sweet potato's limiting nutrients such as P and K.

HORT46

Weed management systems in imidazolinone tolerant sorghum (*Sorghum bicolor*) in south Texas

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With limited herbicide options and the lack of herbicide tolerance in sorghum (*Sorghum bicolor* [L.] Moench), weed management remains a challenge. Recent developments of imidazolinone-tolerant hybrids created an opportunity for exploring the uses of herbicides previously unavailable for production. Field trials were conducted in 2019 to investigate: 1) the efficacy of imazamox applied preemergence (PRE) and postemergence (POST) together with a diverse herbicide program for controlling weed species, 2) examine weed competition on grain yield. Twelve treatments were placed into a RCBD study with four replicates (3.9 × 9.1 m plots, 4 rows, 96 cm centers). This study was conducted across three locations in South Texas; Beeville, Corpus Christi, and Kingsville. Treatments included single or different combinations of five modes of action: 2 ALS inhibitors, PSII Site A, Mitosis, 4-HPPD, and PSII Site B. Making a total of five herbicides: Imazamox, Prosulfuron, Atrazine , Dimethenemid, Pyrasulfatol+Bromoxynil respectively. Imazamox at either 53 or 79 g ai ha^{-1} applied POST to grass weeds 5 to 8 cm in height resulted in some of the lowest amounts of late-season weed biomass. The same rates of imazamox applied to grass weeds 13 to 15 cm in height were not as effective. Atrazine PRE followed by atrazine + prosulfuron POST had little effect suppressing *Urochloa texana* but with the addition of imazamox POST, *U. texana* density was significantly reduced at both 14 and 28 DAT. Grass control was greatest with atrazine + dimethenamid PRE followed by atrazine + imazamox POST. Atrazine + dimethenamid PRE followed by atrazine + imazamox POST resulted in the highest yields, while the lowest yielding were the NTC and atrazine PRE followed by atrazine + prosulfuron POST.

HORT47

Using different adhesive agents to inoculate Sunn hemp (*Crotalaria juncea*) to ensure maximum biological nitrogen fixation

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Legumes used in cover-cropping contribute largely to sustainable nitrogen management through biological nitrogen fixation. Legumes grow a new organ called 'root nodule' in association with Rhizobacteria which convert inert nitrogen to ammonia in soil readily available to be used by crops. This benefit can be harnessed when appropriate live *Rhizobium* in soil contact legume roots to form nodules. Many times, this interaction fails to occur. Apart from factors like optimum soil moisture and temperature, inoculation of legume seed is one simple practical approach to

ensure nitrogen fixation. The objective of this study is to find out cost effective ways to bind peat- based inoculum (*Bradyrhizobium japonicum L.*) to Sunn hemp (*Crotalaria juncea*) seeds to ensure successful nodulation. Field experiment conducted from 18th September -10th December 2019 in 2200 sq. ft plot, Agro-ecology garden UTRGV using four adhesives, water, oil, 10% jaggery solution and 40% gum arabic solution in completely randomized design with 7 replications produced differing results. Gum arabic treatment showed better nodulation with maximum adherence of inoculant in the first four weeks. However, most active nodules were found in jaggery treatment as the weeks progressed. Oil treatment had highest germination rate but bound less inoculant, had lesser nodules but consistently active. Water treatment usually a weak adhesive, produced nodules but there was rapid loss of active nodules with time. Oil treatment could be seen appropriate for field trials due to its easy handling and smooth passage through seed hoppers unlike Gum arabic which tended to be sticky and clog the seed hoppers frequently. Further effect of the amount of nitrogen fixed through treatments would be checked with the follow up cash crop sown after the crops are terminated on the experimental plot.

HORT48

Effect of Kernel Size on Germination, Growth, Development and Yield of Peanut *(Arachis hypogea)*

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Peanut (*Arachis hypogaea L.)* is one of the most important food and oilseed crops cultivated and consumed in most parts of the world. The use of high-quality seeds is key for successful crop production and food security. Distinct seed sizes have different concentrations of starch and other energy reserves which may be an important factor in improving the germination, growth and development of the species. For instance, large seeds have higher amount of starch reserves than smaller seeds. The size of a seed could be an indicator of the fitness of the plant growing from it, thus larger seeds often have better growth and development fitness. Peanut seeds of one species and variety often have different size variations. This study investigates the effects of seed size on germination, growth, development and yield of a peanut species and variety (var. Valencia). The seeds were grouped into four size classes based on their weight in grams(g). They are as follows: 0.50-0.55g, 0.56-0.59g, 0.60-0.65g, and 0.70-0.75g, and grown in a completely randomized block design in the greenhouse. First five pots in each treatment of the seed classes were inoculated with *Bradyrhizobium* sp. to enhance nodulation. Statistical test ANOVA is used to analyze the data. Preliminary results indicate that root, pod, seed, and shell dry biomass are statistically significantly different ($P < 0.0255$), while shoot dry biomass and plant survival per pot are not significantly different ($P > 0.03079$). The study is continuing, and more data is required to elucidate this finding.

HORT49

The Response of Cotton Varieties to Bacterial Blight in South Texas

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Bacterial blight of cotton caused by *Xanthomonas citri* pv. *malvacearum* (Xcm), is a pathogen of concern in cotton production. Bacterial blight has been controlled effectively for more than 50 years by breeding for host plant resistance, however recent outbreaks of the disease have raised concerns among scientists about the possible development of new Xcm races that could cause disease in commercially available resistant varieties. The objective of this study was to evaluate cotton varieties for disease incidence and severity when inoculated with Xcm isolated

from the Texas cotton fields. The study consisted of one resistant variety, NG 5711, two susceptible varieties NG 3406 and DP 1725, followed by four moderately susceptible varieties NG 3729, DP 1646, DP 1845 and DP 1948. All the varieties were inoculated with Xcm at match head and candle stages at a concentration of 10^6 per ml and Silwet L-77® (.25%v/v), using a CO2 backpack spray. Data was collected on disease incidence and severity at 7 days after inoculation. A significant overall F test on the null hypothesis of variety equality was followed with a protected LSD test. A statistically significant difference was observed between the varieties both for disease incidence and severity. Results indicate that varieties known to be resistant were resistant and those known to be susceptible were susceptible against Xcm. Susceptible varieties had up to 100% more disease incidence than those resistant ones. The results found in this study indicate that the commercial cotton varieties tested remain resistant to Xcm.

HORT50

Interaction of Bradyrhizobium and Trichoderma in the growth, development and yield of *Arachis hypogea* (Peanuts)

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Peanut (*Arachis hypogea*), is an important legume crop in the world. Continuous cultivation of the species in the same land contributes to the accumulation of root exudates leading to increased soil pathogens and decreased yield. Endophytic fungi have been known to possess the ability to metabolize soil phenolic acids (Harman et al., 2004; Zhang et al., 2010). *Trichoderma harzianum* are naturally occurring plant growth promoting (PGP) endophytic fungi that are capable of enhancing plant growth, nutrient uptake and tolerance to biotic and abiotic stresses. Separately, *Bradyrhizobium arachidis* a biological nitrogen fixing (BNF) bacteria interacts with peanut roots in nodule formation, thereby promoting nitrogen fixation. The dynamics of symbiotic association between these two organisms was evaluated in the lab and greenhouse conditions. Peanuts were cultivated in pots inoculated with *Bradyrhizobium* and *Trichoderma* in four replicated treatments to evaluate growth, development and yield. The in vitro study results indicate that seeds treated with *Bradyrhizobium* and *Trichoderma* separately had better growth compared to the co-inoculations. Also, results of greenhouse studies showed that seeds inoculated with both microbes, and those inoculated with *Bradyrhizobium* alone had higher dry biomass ($P<0.0001$) as well as higher chlorophyll content ($P<0.0001$) compared to the other treatments. The results also showed that seeds treated with *Trichoderma* alone had better germination and initial growth. Understanding the interaction of fungal endophytes and rhizobial bacteria will help in both nutrient and disease management of *Arachis hypogea*.

HORT51

Interaction between peanut (*Arachis hypogea*) and tomato (*Lycopersicon esculentum*) in an agriculture intercropping system.

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Peanut *(Arachis hypogea)* is an agricultural leguminous crop consumed worldwide but can also be used in crop rotation because of its natural nitrogen fixing ability. Tomato (*Lycopersicon esculentum*) is also a widely grown crop and ranks high in world production and consumption. These two species were intercropped in a greenhouse condition to study the effects and benefits of legume-*Solanaceae* interaction in the growth and development of the two species. Comparable growth, development and yield of unfertilized peanut-tomato interaction in intercropping agriculture relative to these growth parameters obtained from individual cropping of the species in regular cropping practices would reduce fertilizer use in agriculture as well as the environmental consequences associated with fertilizers in the soil. Preliminary results of the study indicate that peanut shoot, root, pod, seed, and shell dry biomass are statistically significant different ($P < 0.0201$) while tomato shoot and root dry biomass are not significantly different ($P > 0.3634$). The study is continuing and more data is required to substantiate the initial results.

HORT52

The Effects of Simulated Soil Salt on Seed Germination, Growth and Development of Sorghum (*Sorghum bicolor*)

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Sorghum (*Sorghum bicolor)* is well known for its strong resistance to abiotic stress and wide adaptability to varying soil conditions including salt tolerance. Agricultural crops are usually not grown in soils with any measurable salt content but irrigation and evaporation leave significant levels of salt in the soil. Elevated levels of soil salt generally cause reduction in growth and yield of crops by limiting their ability to absorb water. Also, soil salt content affects seed germination and early establishment of crops. While seed germination and seedling establishment are important positive attributes of growth, development and yield, soil salt content can lead to reduction in crop yield especially if the crop is salt sensitive. A study of simulated irrigation-evaporation soil salt concentration was conducted to understand the effects of irrigation-evaporation salt residue in soil on growth and development of sorghum. Soil salt content in the range of 0.01–0.3% (1.96-46.24 mS/cm) was used in a randomized complete block study. Preliminary results indicate that dry leaf biomass is statistically significant smaller with increase in soil salt content ($P<0.0001$). Also, chlorophyll synthesis of the species showed a similar result (P<).0001). The study is continuing both in the laboratory and greenhouse conditions to understand the effects of soil salt due to irrigation and evaporation on sorghum seed germination and early establishment.

HORT53

Monitoring the Impact of Cover Crops on Soil Moisture in South Texas

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Cover crops are a conservation agriculture practice with demonstrated abilities to suppress weeds and improve soil health. The Lower Rio Grande Valley is a major agricultural region in Texas that might benefit from this practice yet cover crop adoption rates remain low. Soil moisture is a major obstacle to cover crop implementation in semi-arid regions like the LRGV, especially for growers without irrigation access. Producers hesitate to integrate cover crops into crop rotations due to concerns that cover crops will use soil moisture and result in poor yield of subsequent crops. Season one of on-farm cover crop trials confirmed that without careful management, cover

cropping can result in serious soil moisture deficits in cover cropped areas and significant yield losses can occur. In the second year of trials, a longer break between cover crop termination and cash crop planting as a soil moisture recharge period helped avoid yield drops in the sorghum cash crop. In season three, 25 soil moisture sensors are providing fine resolution insights into moisture patterns during cover crop, recharge, and cash crop periods. As expected, moisture levels were highest in the controls during the cover crop period and densely cover cropped plots dried out more quickly after rains in proportion to their biomass. This data will also help address questions about how much rain is required to compensate for cover crop moisture deficits and whether cover crop residues and organic matter increases result in slower drying rates after the cover crop season. These insights are an important component of our efforts to address the tradeoffs between heavily-promoted soil conservation practices and the biological and economic realities of agriculture in this region.

HORT54

Unmanned Aerial System (UAS) framework to Assess Citrus Orchards replanting

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One of the main problems for the long term of sustainability and profitability of citrus orchards is the decision whether to replace old, sick, and dead trees for new trees. The objective of the project is to develop an Unmanned Aerial Systems (UAS) assisted framework to manage citrus orchards and plan the replacement of trees for the long-term viability of the farm. UAS equipped with multiple sensors will be used to collect aerial images of a citrus orchard, and the collected data will be further processed to assess the health of the citrus orchard and to plan the replanting of new trees. An economic simulation model will be used to calculate economic projections if the trees are or are not replanted. The information and knowledge gained in this project will be utilized to provide recommendations for farmers on how to manage their farm, how to plan efficient and timely management strategies, identify, and monitor trees with diseases that affect citrus production in Texas. The water use efficiency will also be determined for these two management options.

HORT55

Development and efficiency of prototype RGB sensor for detection of daylight fluorescent dyes

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The current estimated market value of UAS Aerial applications in 2020 is approximately $62 million. To ensure that there are no added costs for the use of aerial applications, all UASs are required to first undergo a spray pattern test each season. In order to determine liquid spray deposition patterns is with the use of the new string analysis system (NSAS). This study aims to test the effectiveness of a newly developed, cost-efficient prototype that will help aid in the verification of a uniform spray pattern for UAS aerial applications using a fluorescent dye tracer in a controlled environment. The tracer was applied in 1 meter section intervals along a cotton string and was run through a single blue LED and RGB sensor that measured and recorded the exact spatial distribution and the intensity of tracer that was applied. The use of an RGB sensor eliminates the need for a high-powered optical camera for a more energy efficient and manufacture ready device. The RGB sensor and blue LED could be replaced with a UV Sensor and

LED to meet current National Agricultural Aviation Research and Education Foundation (NAAREF) standards for spray pattern testing, with minimal changes to the prototype.

Pathology

PATH56

Potyvirus in *Ipomoea setosa virus study*

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The genus *Potyvirus* contains several viruses that infect sweet potato, such as *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus C* (SPVC), *Sweet potato virus G* (SPVG), *Sweet potato virus 2* (SPV2), and potentially *Sweet potato latent virus (SwPLV).* SPFMV is the most infamous and ubiquitous that causes vein clearing or necrosis of the leaves. SPFMV is transmitted by the cotton aphid (*Aphis gossypii* Glover*)* and the green peach aphid (*Myzus* persicae Sulzer) and can cause significant yield loss. A way to diagnose SPFMV is to graft the infected *Ipomoea batatas* (sweet potato) onto the indicator plant Brazilian morning-glory, (*Ipomoea setosa*). To verify that viral transmission was successful on *I. setosa*, symptomatic leaf tissue can be confirmed by running a PCR reaction and an ELISA test. This SPFMV project tested three different grafting methods: the top wedge graft, lateral big graft (older plant), and lateral small graft (younger plant). After ten days of allowing the graft to fuse, which should allow for viral transmission of most if not all viruses, weekly ratings were done to ensure the graft was successful by symptomatology. We then conducted further testing to ensure the *I. setosa* was positive for SPFMV. Indirect ELISA tests were used to confirm the presence of the virus. We concluded that the grafting of *I. batata* to *I. setosa* did mechanically spread the virus.

PATH57

Complete genome characterization of diverse begomoviruses (family *Geminiviridae*) from Texas Lower Rio Grande Valley whiteflies

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Begomoviruses are economically important, whitefly-vectored ss(+)DNA viruses consisting of one or two circular genome segments. A recently conducted study into the begomovirome of whitefly samples collected from San

Benito, Cameron Co., TX revealed the occurrence of four bipartite viruses based on analysis of the core coat protein (CP) region of begomoviruses. While the CP of begomoviruses is useful for diagnostic purposes, definitive identification of species in the genus require determination of the complete genome sequence of the virus based on established criteria. To further characterize each of the four viruses detected in the study area, DNA extract from each of the five samples was subjected to rolling cycle amplification (RCA) and the RCA products were used as templates in PCR with abutting primers newly designed from the partial core CP gene specific sequences of each of the four viruses. The complete DNA-B genome of each virus will be similarly amplified. The expected 2.5–2.7 kb virus-specific DNA-A and DNA-B amplicons will be cloned individually into pJET1.2 vector and two plasmids per virus genome segment carrying the correct size inserts will be Sanger sequenced bidirectionally. Additional overlapping primers will be designed from the initially obtained sequences to walk the genome segments of each virus. All plasmid-specific sequences will be bioinformatically analyzed for contig assembly, gene annotations, and analyses using the SDT1.2 tool recommended by the *Geminiviridae* subgroup of the International Committee on the Taxonomy of Viruses. The sequences will also be scanned for recombination using the RDP4 program. The results will advance our knowledge on the extent of, and the factors shaping, the genome diversity of begomoviruses circulating in the Texas Rio Grande Valley area landscape.

PATH58

Development of Loop-Mediated Isothermal Amplification (LAMP) assay for the detection of '*Candidatus* Liberibacter asiaticus', a causal agent of Citrus Huanglongbing (HLB)

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Huanglongbing (HLB, Citrus greening disease) is one of the most destructive disease that undermines the production of citrus around the world. The presumed causal organism, '*Candidatus* Liberibacter asiaticus*'* (CLas), is a non-culturable, fastidious and phloem limited α-proteobacterium. Due to the lack of commercial citrus cultivars resistant to HLB, the current strategy to control the spread of HLB relies on the use of disease-free bud wood, removal of infected trees, and suppression of psyllid vector populations. The most common diagnostic method for HLB detection is based on real-time PCR. However, low titer and uneven distribution of the bacteria inside the infected plant can make the reliable detection difficult. The current study exploited an isothermal amplification strategy, Loop Mediated Isothermal Amplification (LAMP), aiming at the development of HLB diagnostic method that can be used in the field condition without the need of real-time PCR instrument. We designed primers for the LAMP assay based on the CLas five copy gene, *nrdB*, encoding the β-subunit of ribonucleotide reductase (RNR), an enzyme for multiplication of bacteria. The preliminary data showed that the optimum temperature for the LAMP assay using primers targeting *nrdB* gene was 67^0C. The results obtained with CLas positive and negative leaf and root DNA fractions indicated that CLas could be detected within 30 min, compared to ~1hr with real-time PCR. The newly developed LAMP assay for CLas detection will provide a more rapid and cost-effective method that can be conducted in the field.

PATH59

Investigation of '*Candidatus* Liberibacter asiaticus' Prophages in Texas and Florida

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Huanglongbing (HLB) disease has been one of the most disastrous diseases for citrus production worldwide. The presumed causal agent vectored by Asian Citrus Psyllid (ACP), '*Candidatus* Liberibacter asiaticus' (CLas) contains prophage-like regions within its genome. It has been shown that the presence of prophage sequences on CLas

genome can contribute to bacterial virulence. Given the difference in viral reproduction cycles that may be induced based on the genetic makeup of the prophage types, knowledge on the distribution of the different prophage types is essential in understanding how the bacterial infection and presence thereof unfold throughout the year within the plant. Preliminary data obtained with Texas CLas positive samples indicated a potential variation in prophage types depending on location and season. Since no study has been done about prophage types in Texas, the current study examined and compared prophage types present in leaves and root samples collected in summer (Jul. and Aug.) and winter (Nov. and Dec.) months in Texas and Florida. Results show that while Type 3 prophage was not detected in the current study, CLas strains in both states predominantly carry both Type 1 and 2 prophages in their genomes. Interestingly, there is a higher incidence of Type 1 only prophage in Texas leaf samples compared to roots in both summer and winter months, suggesting that it is not likely due to seasonal variation, unlike Florida samples. Prophage typing of ACP collected in Texas showed that more than 92% of psyllid samples carried both Type 1 and 2 prophages, which is consistent with the data obtained with plant tissue. Statistical significance is still to be confirmed for prophage type variation determined in the present study.

PATH60

Plant disease control via nanoparticle therapy

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The spread of vector-borne pathogens by arthropod hosts continue to cause human, animal and plant diseases of public health and economic importance. Using the integrated vector management approach, this study utilizes nanoparticles as potential targeted anti-pathogenic agents for the control of citrus greening disease. *Candidatus* Liberibacter asiaticus, the bacterial pathogen of citrus greening disease is spread by the Asian citrus psyllid (*Diaphorina citri Kuwayama*). Symptoms of the disease include blotchy mottle, yellow shoots, and improperly developed fruits. The significant decrease in edible fruit production caused by the disease has led to decreased productivity of citrus farms which in turn causes negative significant economic loss to the citrus industry. Silver and copper nanoparticles were synthesized and functionalized with surface capping agents to give them unique surface properties; polyvinylpyrrolidone (PVP), cetyltrimethylammonium bromide (CTAB), and citrate (Cit) coated silver nanoparticles as well as PVP, CTAB and ascorbic acid(AA) copper nanoparticles were synthesized. The synthesized nanoparticles were exposed to Gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* bacteria to assess antimicrobial sensitivity of the nanoparticles using the disk diffusion method. The results show that AA coated copper nanoparticles and PVP coated silver nanoparticles had the highest mean zones of inhibition against the growth of *E. coli* (11.56 mm, 10.44 mm respectively) and *S. aureus* (17.33 mm, 14.67 mm respectively) in comparison to other nanoparticles after 24 h of incubation. The Cit coated silver nanoparticles showed no effects on inhibition of both gram-negative and gram-positive bacteria growth. These results illustrate the efficacy of zerovalent nanoparticles as antimicrobial agents and their potential application in the field of agriculture as an alternate pest/pathogen control tool.

PATH61

Evaluation of Thermotherapy on HLB-affected citrus trees in Texas

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Huanglongbing (HLB) is a disease that severely affects citrus production worldwide. HLB is associated with phloem-limited bacteria, *'Candidatus* Liberibacter asiaticus,' (CLas) and is vectored by Asian citrus psyllid. The use of antimicrobials and heat therapies are currently used in Florida to mitigate disease. However, heat therapy has not

been tested in Texas citrus orchards. In this study, thermotherapy treatments were applied to evaluate the recovery of HLB affected citrus trees and assess the effect on CLas titers and other foliar diseases of citrus. Heat treatments were applied on 6-year old Rio Red grapefruit orchard located at the Texas A&M University-Kingsville Citrus Center using randomized split block design. Eighteen HLB positive and 18 HLB negative trees were randomly selected for evaluation from each experimental block. To evaluate tree recovery of HLB-affected citrus, canopy density, leaf surface area, bloom count, fruit set, fruit drop and yield were measured for all treatments. CLas titers, greasy spot and melanose severity were monitored throughout the year. Our results show that heat treatment did not affect the leaf area, canopy density, fruit set, and fruit drop in HLB affected trees. Treatments did not affect the susceptibility to greasy spot or melanose, as the disease index was not statistically different throughout the year. This study will provide Texas citrus growers with local information regarding the effectiveness of heat therapy for HLB management in the region.

PATH62

Delivery, Persistence, Viability, and Translocation of Non-Native Bacteriophage Viruses in Citrus

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Economically disruptive, the citrus greening disease, Huanglongbing (HLB), causes substantial and compounding damage to the citrus industry worldwide. While chemical and biological control methods as well as quarantine protocols impede the insect vector, *Diaphorina citri*, elimination of the bacterial pathogen, *Liberibacter asiaticus* (Las), remains elusive due to its fastidious nature and mortality outside of hosts. Prior to discovering antibiotics in the early 20th century, researchers investigated natural viral predators of bacteria known as bacteriophages for combating diseases. Leapfrogging antibiotic resistance and leveraging the motile, targeted nature of bacteriophages, our team plans to tackle *Las* using bacteriophage-vectored CRISPR/cas9 antimicrobial therapies. As a preliminary candidate screening, this study exposes non-native model bacteriophages to citrus foliage to optimize virus delivery and survival in and on plants. Representing a broad range of structure and functionality, our bacteriophage collection received specialized purification, propagation, concentration, and storage methods prior to inoculation in *Citrus x paradisi,* var. Rio Red, for greenhouse *in planta* assays, as well as tailored qPCR detection methods. Following foliar introduction (delivery) we measure the amplification of nucleic acids recovered at time intervals (persistence), the growth inhibition by recovered viral particles on bacterial host, *Escherichia coli* (viral viability), and viral particle recovery location in the plant, especially outside of inoculated areas (translocation). These canonical bacteriophages span morphologies in the Caudovirales, Inoviridae, and Leviviridae, including candidates adaptable for therapies. Bolstering knowledge on the impact of bacteriophage phenotypes on invasion, translocation, viability and persistence within citrus tissues allows this work to benefit future interventions against other phloem/xylem-limited bacterial pathogens.

PATH63

Using Insects to Deliver Nanocides for Therapeutic Treatment of Plants

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Huanglongbing (HLB), also known as Citrus Greening, is a disease of citrus caused by a vector-transmitted pathogen. The bacteria, *Candidatus* Liberibacter asiaticus, originated in Asia but has spread around the world and

caused substantial economic losses in citrus producing regions. Hence, it poses significant economic, regulatory, and scientific challenges. In an effort to control this disease, researchers are working to develop new forms of therapeutics. Many are “molecular”-based therapeutics, such as antimicrobial peptides or antisense RNAs. Delivery of such therapeutics is a challenge, however. Environmental exposure, such as UV radiation or desiccation during delivery, can damage such therapeutics, reducing or eliminating effectiveness. Nanoparticles can function as carriers of “molecular” therapeutics, offering protection and improved uptake. To evaluate the utility of nanoparticles as carriers, we produced functionalized nanoparticles varying in physicochemical properties. We asked whether the HLB disease vector, *Diaphorina citri*, could acquire and transmit nanoparticles to citrus plants. Further, we asked whether the plant could uptake nanoparticles for insects to acquire and spread. This approach may offer a unique therapeutic delivery system which allows the pathogen vector to directly assist the plant.