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Project (1): Integrated Weed Management Plans for Florida Cabbage Production

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Project Partners

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Project Summary

At the time of the original proposal, there was a total of 9,900 acres of cabbage planted on an annual basis in Florida. Unlike most vegetable crops, cabbage is typically grown on raised beds that are not covered with plastic mulch. Consequently, weeds are a significant problem throughout the growing season. They reduce crop yields, crop quality, farm income and hinder harvest operations. There have been several cases where entire shipments of cabbage heads were rejected because lamb's quarter seeds fell into the developing head and caused discoloration and the onset of disease. Growers rely on a limited number of preemergence herbicides, cultivation, and hand weeding to control weeds. There is a great need to identify alternative weed management options for cabbage growers including herbicides that can be applied after crop transplant and herbicides with activity on nutsedge species.

This proposal is timely because many of the cabbage growers in the state of Florida are struggling due to increased input costs and low cabbage prices. This problem is compounded by severe weed pressure. In recent years, weed pressure in cabbage fields in central Florida has become so severe that some fields have been abandoned. In other fields, the crop wasn't harvested or a large portion of the crop was left in the field due to poor quality and size due to heavy weed pressure. There is a great need for effective and economically viable weed management plans for cabbage growers.

Project Approach

The first step in the project was the evaluation of pre- and post-transplant herbicides applied alone and in tank mixes. Six trials were conducted at the Gulf Coast Research and Education Center and in Citra and two additional trials were conducted on a commercial farm in Parrish, Florida. Sulfentrazone was identified as an alternative pre-transplant herbicide for cabbage and registration has subsequently been achieved. Sulfentrazone is an effective herbicide for yellow nutsedge for which there was no previous management option available. It also is effective on a wide range of broadleaf weeds. We were also able to determine that clopyralid could be used post-transplant to control some common problem weed species of cabbage fields. We were unable to identify viable herbicide options for purple nutsedge or several other broadleaf weeds.

The second step in the project was the evaluation of a plasticulture production system for cabbage. Two trials were conducted at the Gulf Coast Research and Education Center and we were able to illustrate that plasticulture production significantly reduced weed pressure and weed management costs. We were also able to show that the adoption of a plasticulture production system would increase overall yield, yield consistency, and cabbage quality.

The third step in the project was the evaluation of fallow programs to determine if the use of managed cover crops would reduce weed densities within the crop. Four trials were conducted over two years at the Gulf Coast Research and Education Center and in Citra, Florida. Nitrogen inputs enhanced cover crop growth but the addition of S-metolachlor had minimal effects on weed density within the cover crop. Cover crops during the fallow period had low to no effects on weed densities within the crop. We conclude that the cover crops evaluated are not an effective weed management tool for growers in central Florida and this is in part due to the fact that many problematic weed species that emerge in the crop do not emerge in the summer when the cover crop is grown.

Goals and Outcomes Achieved

A. The number of growers that modify broadleaf weed management based on results obtained.

The goal was to identify an alternative herbicide program that could be adopted by growers. We were able to identify one new pre-transplant herbicide (sulfentrazone) and broaden the use of a post-transplant herbicide (clopyralid) option for growers. However, we were unable to develop an overall management program that was substantially better then the programs growers have currently adopted. On-farm evaluations were hindered because one of the commercial farms we were working with went out of business during this project. Thus far, sulfentrazone has not been adopted by growers but on-farm extension trials will continue in 2018-2019 and we are hopeful that adoption will occur.

B. Publication, presentation, and distribution of verbal, electronic and printed information pertaining to potential fallow programs for nutsedge management in brassicaceae crops.

As previously noted, the use of managed cover crops did not reduce weed densities within the crop. Results were presented at field days but adoption was not measured as we could not recommend the use of cover crops as a weed management tool.

C. Publication, presentation, and distribution of verbal, electronic and information pertaining to plasticulture cabbage production.

The results of our research on plasticulture cabbage production were presented at a cabbage field day and via printed handouts distributed to growers at the field. We are also currently working on a publication for a peer reviewed journal. The results were very promising with substantially greater yields and reduced weed management costs with plasticulture production. Thus far, growers have not adopted this new technology primarily because it represents a substantial shift from current practices. However, some growers have expressed interest and we plan to conduct some on-farm extension trials beyond this proposal to illustrate on-farm how this approach could benefit the growers.

Beneficiaries

There are 90-100 cabbage growers in the state of Florida and they were the primary beneficiaries of this research. Farms that grow other cruciferous crops could also benefit. The results of our research were presented at a cabbage field day and handouts were provided. In addition, we conducted on-farm field trials so that growers could see the results themselves. The primary benefits for the growers were: (1) the registration of a new herbicide that controls a broad spectrum of broadleaf weeds and yellow nutsedge and (2) the development of a plasticulture production system for cabbage growers that substantial increases crop quality and yield. Neither of these management tools have been broadly adopted yet but we are confident that adoption will occur over time. We are confident because yellow nutsedge is a serious weed problem in some commercial fields and sulfentrazone is the first herbicide registered in Florida that is safe for use in cabbage and controls yellow nutsedge. The potential economic impact is unknown but likely to be highly significant because yellow nutsedge significantly reduces crop yields and in some cases in central Florida fields were removed from production due to severe crop losses. Plasticulture production has also not been widely adopted yet but our results clearly show a yield and quality benefit as well as a substantial reduction in herbicide inputs and labor for weed removal. We had hoped that adoption would occur during this project but given that this has not happened we will continue to work with the growers following the end of this project to demonstrate how the management tools developed through this research could benefit their operation.

Lessons Learned

All of the proposed work was completed. We did obtain an extension to complete the research but this was primarily due to the fact that the production season for cabbage did not line up well with the timing of the receipt of the funds more so than any problems or complications. No changes were made to the work plan and all objectives were achieved. The project successfully identified multiple management options that should enhance weed management in cabbage fields.

Initially we were very confident that managed cover crops would be an effective weed management tool and were very surprised when this did not occur. In fact, we saw minimal to no benefit of cover cropping. As a research team, we do not wish to discourage the use of cover crops as they provide many benefits but in the case of cabbage production in Florida they do not appear to be an effective weed management tool

We did learn that on-farm trials can be very effective if you work closely with the growers but complications do arise (last minute production changes, harvest before you obtain yield data, etc). These difficulties can hinder data collection but we still believe on-farm trials are the best way to encourage adoption. The biggest difficulty was obtaining a measure of adoption by the growers or an increase in knowledge. This was complicated by a turn-over in growers and by the lack of adoption thus far. A change in production practice generally occurs over very long time periods and a two year project is not adequate to measure change. In addition, growers are generally reluctant to participate in questionnaires or quizzes that measure a change in knowledge. Although this type of information is desirable for funding agencies and university faculty it is difficult to obtain, results are likely inaccurate, and growers do not like to participate. Alternative methods are needed to measure a change in knowledge or practice.

Total Funding Expended: $60,470.93

Percentage of Project Completed: 100

Project (2): Advancing integrated practices for managing bacterial spot in tomato transplants

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Project Summary

Bacterial diseases are a major challenge for the commercial production of high quality transplants for Florida's vegetable industry. High plant density, frequent overhead irrigation, high temperatures and humidity are conducive for the rapid development and spread of bacterial pathogens in commercial transplant operations. These disease outbreaks have large financial implications for commercial transplant operations, as diseased transplants are frequently rogued in an effort to slow disease spread and provide a healthier looking product. However, excess rogueing can lead to inadequate transplant numbers to meet requested orders and poorer transplant quality than desired; which can jeopardize future orders from vegetable growers. More importantly, infected seedlings introduces the bacterial pathogen to the field potentially leading to even greater production losses. Focusing on bacterial spot of tomato, this project aimed at developing integrated management strategies to limit transplant losses and subsequent spread of bacterial pathogens in commercial transplant facilities.

Florida accounted for over half of U.S. tomato production based on yields (57%) and market value (58%) in 2013. Unfortunately, Florida's lucrative winter tomato production has suffered in recent years due to increased competition from foreign imports and increased production costs. This project is a direct result of tomato grower and transplant producers need for additional research to limit production losses to common foliar diseases. Bacterial spot, mainly caused by the bacterium Xanthomonas perforans, is a common disease of tomato in Florida that affects transplant production. We hypothesize that improved control of bacterial pathogens during transplant production will reduce the amount of bacteria being introduced into field production sites and improve field management of bacterial diseases. Furthermore, growers have relied on copper-based fungicides for disease management in transplant and field production. However, state-wide surveys for X. perforans revealed the prevalence of copper-resistant

strains across tomato production areas and have compromised the efficacy of copper-based fungicides. Alternative compounds are either not labeled or little information is known for their usage in transplant production. Therefore, this project addresses the need of growers for alternative and effective spray programs in tomato production. Transplants throughout the state are typically grown in protected structures with open sides which results in highly conducive environmental conditions favorable for bacterial outbreaks in these facilities. Therefore, this project tackles the need to understand how bacterial outbreaks occur in these facilities in order to limit disease spread and improve rogueing practices.

Project Approach

Per objective 1, we conducted a total of 8 separate trials to evaluate 13 different conventional and biological-based compounds for managing bacterial leaf spot (BLS) on tomato transplants. Stand-alone applications of Actigard, Cueva, Quintec, Innovotech A, and Innovotech B significantly reduced disease compared to the inoculated controls. Programs that included Actigard + Tanos + Agriphage and Tanos + K-Phite + Cueva also significantly reduced disease compared to the control. The antibiotics, oxytetracycline and streptomycin, and Agriphage were ineffective at reducing disease severity under high disease pressure. Severe phytotoxicity was associated with Quintec at rates greater than 1 fl. oz. The copper formulation in Cueva performed better than the copper hydroxide standard on seedlings.

Per objective 2, this objective is a follow up for objective 1. We conducted and selected two transplant trials from objective 1 and carried out two field trials to evaluate the reduction of bacterial disease severity on pre-treated infected seedlings into the field. Transplants were sprayed with 13 different treatments including a non-treated control. Transplant were evaluated for disease severity and three best performing treatments were selected in addition to the grower standard (copper) and non-treated treatments were transplanted into the field in a split-plot design. Transplanted seedlings from the five selected treatments were further treated with Actigard and Kocide in the field. Data analysis showed that transplants treated with Actigard or Kocide were not significantly different than non-treated control. However, the transplants treated with silver oxynitrate showed the best disease suppression followed by Actigard and Tanos+Cueva. Based of this experimental data, we recommend pre-treating transplants with Actigard or silver oxynitrate or Tanos+Cueva which leads to significant disease reduction throughout the growing season.

Per Objective 3 we completed a total of 4 small scale and 2 large commercial scale trials to evaluate the movement of Xanthomonas relative to BLS development on tomato seedlings. Initial transplant studies performed at GCREC in January and February 2016 found little to no movement of X. perforans beyond initial source plants; even though source plants exhibited symptoms of BLS. A third study performed at GCREC in October recorded 5 day lag period after inoculation until the visible development of water

soaked lesions, which didn't become clearly visible until 9 to 10 days after inoculation. By 14 days after inoculation, X. perforans could be recovered at high levels from asymptomatic transplants 1.3 meters away from the inoculation point. Similar trends were observed in the fourth trial with less amount of disease pressure compared to trial 3. In order to understand the cause of the limited movement observed in trial 1 and 2, we evaluated the movement of X. perforans using an artificial system by placing plates with selective media adjacent to infected plants in two different trials. Pathogen dispersal was monitored over a period of two weeks starting at 1 days after inoculation. We found that X. perforans moves rapidly within one day after inoculation and reaches a population peak about 7 to 10 days after inoculation depending on environmental conditions. Results suggest that conducive environmental conditions and overhead irrigation are critical for pathogen dispersal.

Large-scale movement studies were performed at a commercial transplant facility in May 2016 when weather conditions were favorable for rapid disease development. These large commercial studies found X. perforans could be detected at around 0.5± to 1.6± m away from the initial source plants 12 days after inoculation. By 26 days, X. perforans could further disperse to distances greater than 2.6 m with disease symptoms appearing only on those neighboring plants nearest to the pathogen source. Results from movement studies suggest that transplant operations would need to rogue at least 1 meter in all directions from an outbreak source under low disease pressure (equating to 1 to 2 speedling trays) when conditions are not favorable for rapid disease development. Under favorable conditions or with high disease pressure, operations would need to aggressively rogue 1.5 to 3 meters (equating to 2 to 4 speedling trays) to remove infected plants. Results and observations suggest that aerosols likely account for rapid strain movement.

We completed the whole-genome sequence analysis for 67 strains previously collected from two separate tomato transplant/field growers. We conducted core-genome and single-nucleotide polymorphism (SNP) analysis to evaluate relatedness between strains collected from transplant operations and field sites. We evaluated a number of pathogenicity effectors involved in exacerbating disease and host range expansion. We also evaluated the race structure of the strains and their sensitivity to copper and streptomycin. We found that all 67 strains belong to X. perforans race T4. All strains are copper tolerant but streptomycin sensitive. We found a high genetic diversity between bacterial populations recovered from transplant and grower sites but low to moderate intragenic diversity within the population in each locality. We also found that outbreaks in the field are likely caused by transplant outbreaks based on phylogenetic and distance-based analysis using SNP data with at least 80% of the strains recovered from the field showing high homology to strains recovered from transplant production facilities. Several pathogenicity factors were also identified and others were absent in the population most likely associated with overall improvement of pathogen fitness, survival and dissemination.

Per objective 4, research results were presented to growers and other relevant industry stakeholders during the 2016 Florida Seed Association Meeting and the Florida AgExpo and also at the Southern Division-American Phytopathological Society (College Station, TX) and at the 2017 Annual Meeting of the

American Phytopathological Society (San Antonio, TX) and at the 32nd Annual Tomato Disease Workshop (Balm, FL) and at the 2017 Tomato Institute (Naples, FL), and the International Congress of Plant Pathology (Boston, MA). We also carried out a field site demonstration (from objectives 1 and 2) for local growers at the Florida AgExpo. Findings were also shared during several meetings with local transplant producers. Research manuscripts are currently being prepared and ready to be submitted to peer-reviewed journals.

Goals and Outcomes Achieved

Outcome 1. The evaluation of Actigard, kasugamycin, oxytetracycline, and 2 additional non-copper alternatives to reduce the incidence and severity of bacterial spot alone or in rotation with other products during transplant production. The relative efficacy of all products will be compared to a standard copper bactericide, and a non-treated control.

The above mentioned treatments excluding Kasugamycin were evaluated in addition to eleven other non-copper alternatives to reduce bacterial spot severity on tomato transplants. We initially evaluated products as single mix application. After we identified products with superior performance we established additional trials to evaluate different spray programs by rotating and mixing different products. As a result, we developed an improved spray program that can limit severity and incidence of bacterial spot in commercial transplant operations.

Outcome 2.Evaluate how transplant programs for managing bacterial spot impact the subsequent incidence and severity of bacterial spot in the field under prescribed field management programs.

Results from outcome 1 were utilized for establishing trials to identify the potential impact of transplanting pre-treated seedlings with the best performing copper-alternative products. We identified 3 products that gave superior results and as a result we saw a significant reduction in disease severity when these plants were later transplanted regardless if they were sprayed or non-sprayed in the field.

Outcome 3. Measure temporal spread of X. perforans, as a function of distance over time, during transplant production with unidirectional overhead watering.

We conducted small and large-scale trials to simulate transplant production practices. We monitored the spread of X. perforans from a single foci at different time intervals and at different distance from the infection foci. As a result of our trials we recommend different roguing practices to limit bacterial spot outbreaks in transplant facilities.

Outcome 4.Characterize changes in Xanthomonas populations during transplant and field production using whole-genome sequencing strategies.

We characterized X. perforans populations at two major transplant producers in the state. We characterized the whole-genomes of 67 strains. Based on single nucleotide polymorphism analysis, we identified transplant operations as a critical point in the outbreak of X. perforans in the field. We recommend that practices and efforts to limit disease outbreaks in the transplant facility would eventually lead to lower disease severity in the field.

Beneficiaries

This project directly benefited the Florida tomato industry that includes several large packing house operations encompassing 35,000 acres of tomato production, accounting for 18% of all vegetable acreage in Florida. Based on prior studies (Van Sickle and Weldon, 2009), improvements in bacterial spot management could save Florida tomato growers over $3,000 per acre in lost productivity every season, which translates to approximately $100 million per year (NASS, 2014). The outcome of this project was delivered during various local extension meetings, grower meetings, national scientific meetings, EDIS publication and workshops.

Lessons Learned

We did not encounter any problems or delays throughout the project period. We did not make changes to the work plan.

Total Funding Expended: $132,420.25

Percentage of Project Completed: 100

Project (3): Alternative weed management options for leafy greens to mitigate loss of hand labor

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Project Summary

Leafy greens particularly lettuce are important cool-season crops grown in south Florida mainly for consumption in eastern United States during the winter season. However, current production and further expansion of the acreage to meet growing local and regional demand is limited by lack of effective weed management programs, particularly chemical control. As a result, costly intensive hand labor has become a major component of weed management in these crops. Leafy green production is also vulnerable to changes in immigration policies since most of the hand labor is based on seasonal migrant workforce. Therefore, it is important to develop weed management programs for leafy greens that are less dependent on hand labor to mitigate labor shortages. Hand labor shortage is further exacerbated by a structural shift in low-skill labor in rural communities resulting in fewer workers taking low-skill agricultural jobs.

This project was conducted to evaluate efficacious weed management programs critical for leafy green production, particularly chemical control that would be less reliant on hand labor. Evaluation of chemical control programs for leafy greens based on herbicide selectivity, tolerance, and persistence in soils was conducted to help develop an effective weed management program. Studies were conducted to evaluate effect of preemergence herbicides (imazethapyr, pronamide, bensulide, and oxyfluorfen) applied alone or in combination with a postemergence herbicide (imazethapyr) for weed control in leafy greens (iceberg and romaine lettuce). The field component of the study was conducted on organic soil used for leafy green production in south Florida. Application of imazethapyr, pronamide, and bensulide preemergence resulted in transient stunting of lettuce, indicating that the crop had acceptable tolerance to these herbicides. Preemergence imazethapyr, pronamide, and bensulide provided acceptable broadleaf weed control (mostly common lambsquarters, spiny amaranth) initially following planting but did not have long residual effect for the entire season indicating that their persistence in organic soil is

short-lived. Oxyfluorfen applied preemergence provide complete weed control but resulted in complete stand loss showing that lettuce is not tolerant to the herbicide. Postemergence application of imazethapyr at the cotyledon, two-leaf, and four-leaf stage of development of broadleaf weeds showed acceptable control at the cotyledon to the two-leaf stage compared to later stages of development. However, injury from imazethapyr was more pronounced at the cotyledon stage of lettuce compared to the later stages of development. This research showed that preemergence herbicides on leafy greens on organic soils do not have a long residual effect on broadleaf weeds and must be followed by postemergence imazethapyr applied by the two-leaf stage of the crop and weeds to control weed escapes. This program resulted in better lettuce yield compared to preemergence only treatments indicating a yield benefit by having a preemergence and postemergence weed control program in lettuce. Such a program will significantly reduce the need for additional hand labor to supplement weed control particularly of common lambsquarters and Amaranthus species. However, these herbicides were not able to control late emerging common purslane which is now becoming an emerging weed issue for leafy greens although not as problematic as common lambsquarters.

Project Approach

The main goal of this project was to evaluate chemical control options for weed management for leafy greens based on herbicide selectivity and tolerance. In addition, persistence of herbicides in the soil and subsequent residual weed control in leafy greens was also evaluated.

The first component of the project was accomplished by determining efficacious rates of herbicides for weed management in leafy greens (iceberg and romaine lettuce, the main leafy greens cultivated in south Florida) and promoting their use to sustain and expand production. In 2016, visits and meetings with leafy green growers were conducted to formulate field trials that would address weed management issues in the crop in south Florida. The visits and meetings were with growers who represent >75% of the leafy green acreage. Production fields were surveyed to provide information on problematic weed species which affect leafy green production and a questionnaire ('Initial Survey of Everglades Agricultural Area Leafy Green Growers') was sent to all the five big growers who cultivate approximately 12,000 acres of leafy greens in the region. The initial survey indicated that broadleaf weeds and sedges were the major weed management concerns. The problematic broadleaf weeds which were listed included common lambsquarters, common purslane, ragweed parthenium, spiny amaranth, and smooth pigweed, while sedges included yellow and purple nutsedge. Common lambsquarters was the most problematic. The growers only used imazethapyr for selective postemergence control of broadleaf weeds with no preemergence herbicide options to use. The growers reported that hand labor used to supplement chemical weed management represented 35 to 81% of the total cost of weed management with an approximate total cost of weed management from $150 to $360/acre. Also, the growers reported concerns with increasing hand labor costs for weed management and were concerned about losing or having inadequate hand labor in the future. Field

surveys conducted in growers' field corroborated the initial survey results with regard to problematic weeds. The surveys showed that leafy green growers in the region were in need of alternative weed management options including use of preemergence and postemergence options or a combination of both to reduce current weed management costs and mitigate loss or shortage of hand labor.

Based on the field visits, meetings, and initial survey, field studies were initiated in spring 2016 to evaluate imazethapyr, oxyfluorfen, pronamide, and bensulide for weed control in iceberg and romaine lettuce. The major weed evaluated for control was common lambsquarters, the most problematic broadleaf weed. The initial results showed that preemergence pronamide, bensulide, and imazethapyr followed by postemergence application of imazethapyr provided acceptable common lambsquarters control in lettuce. The results of the initial field trial were presented at the Florida Lettuce Advisory Committee Meeting in 2016 attended by leafy green growers or their representatives followed by a visit to the research plots by some of the growers. These results were then used to formulate further field studies conducted in 2017 and early 2018 to address weed management issues.

These field trials evaluated the effect of preemergence herbicides applied alone or in combination with a postemergence herbicide for weed control in iceberg and romaine lettuce. Sixteen treatments including 14 herbicide treatments, a hand weeded check, and an untreated weedy control were established. Experimental plots were 6 feet wide by 25 feet long arranged in a randomized complete block design with four replications of each treatment and repeated in time. Herbicide treatments included preemergence imazethapyr (2 and 4 fluid ounces/acre or 0.0313 and 0.0626 lb ai/acre), pronamide (9.6 and 14.4 pints/acre or 3.96 and 5.94 lb ai/acre), bensulide (5 and 9 quarts/acre or 5 and 9 lb ai/acre), and oxyfluorfen (1 pint/acre or 0.5 lb ai/acre). Preemergence treatments were either applied alone or followed by postemergence application of imazethapyr at 2 fluid ounces/acre (0.0313 lb ai/acre). Crop injury and weed control were evaluated between 14 and 42 days after postemergence treatment. A second study was conducted to evaluate the effect of timing of postemergence imazethapyr (2 fluid ounces/acre or 0.0313 lb ai/acre) on weed control and lettuce tolerance. Timing of application was based on the cotyledon, 2-leaf, and 4-leaf stage of development of both weeds and lettuce. Emergence and stages of growth of both weed species and lettuce are similar early in the growing season. Experimental plots were 6 feet wide by 25 feet long arranged in a randomized complete block design with four replications of each treatment and repeated in time. Preemergence oxyfluorfen resulted in crop stand loss (>95%) while pronamide, bensulide, and imazethapyr resulted in transcient crop stunting (<10) and no stand loss. Imazethapyr resulted in 85% common lambsquarters control as a preemergence treatment at 35 days after treatment. Both rates of pronamide and bensulide provided 75 to 85% common lambsquarters control at 14 days after treatment, however control decreased to <60% at 35 after treatment. Oxyfluorfen applied preemergence provide complete weed control at 14 days after treatment and thereafter. Application of postemergence imazethapyr at the two true-leaf stage of common lambsquarters following preemergence application of all the herbicides resulted in >85% common lambsquarters control at 35 DAT. Postemergence imazethapyr resulted in <8% injury of both romaine and iceberg lettuce. However, these herbicides were not able to control late emerging common purslane which is now becoming an emerging weed issue (not as competitive as common lambsquaters) for leafy greens on organic soils. Postemergence application of imazethapyr at the cotyledon to two-leaf stage of development of broadleaf weeds (common lambsquarters, Amaranthus species) showed acceptable control compared to later stages of development. However, there was more pronounced injury from imazethapyr at the cotyledon stage of lettuce compared to the later stages of

development. Preemergence imazethapyr, pronamide, and bensulide followed by postemergence imazethapyr resulted in better lettuce yield compared to preemergence only treatments indicating a yield benefit by having a preemergence and postemergence weed control program in lettuce grown on organic soils. Imazethapryr and recently pronamide are available for use in lettuce in Florida. These results showed that preemergence herbicide application (imazethapyr, pronamide, and bensulide) followed by postemergence imazethapyr provides acceptable weed control and can be used to mitigate hand labor shortages without growers getting a yield penenalty from weed interference.

The persistence of herbicides in soil and subsequent residual weed control in leafy greens was also evaluated between 2016 and 2017. The herbicides were applied on bare organic soil with no crop cover. Six herbicide treatments were established including imazethapyr (2 fluid ounces/acre or 0.0313 lb ai/acre), pronamide (9.6 and 14.4 pints/acre or 3.96 and 5.94 lb ai/acre), bensulide (5 and 9 quarts/acre or 5 and 9 lb ai/acre), and oxyfluorfen (1 pint/acre or 0.5 lb ai/acre). Experimental plots were 6 feet wide by 25 feet long arranged in a randomized complete block design with four replications of each treatment and repeated in time. Following application, soil samples were collected immediately and thereafter weekly for a period of seven weeks and stored at -20 C prior to analysis. The research showed that preemergence herbicides with the exception of oxyfluorfen on leafy greens on organic soils did not have a long residual effect to be used alone thereby validating the need for sequential postemergence imazethapyr application. Although oxyfluorfen had a longer residual activity, it was phytotoxic to the crop as shown in the efficacy and tolerance study and thus cannot be used in the crop. Based on the initial grower survey, leafy greens are majorly followed by sugarcane in south Florida. Because sugarcane is vegetatively propagated, the preemergence herbicides currently available for use in Florida (imazethapyr, pronamide) would have no plantback concerns on the main rotational crop.

Laboratory and greenhouse experiments were to be conducted for herbicide absorption, translocation, and metabolism to evaluate lettuce tolerance to the herbicides evaluated. But, there we issues with our biological oxidizer to finish the C-14 based herbicide absorption, translocation, and metabolism studies within the specified project period. This was the only component of the project not competeted. However, the field studies on herbicide efficacy and persistence provided most of the information that we required for successful completion of the project (i.e. to make recommendations on efficacious weed management on leafy greens). In addition, the field based tolerance component indicated that only oxyfluorfen was phytotoxic to lettuce resulting in total crop failure.

Following presentation of the research findings to growers at the Florida Lettuce Advisory Committee meeting and subsequent interviews, half the growers indicated that they would evaluate preemergence herbicides available for use in Florida followed by postemergence imazethapyr for their weed control programs.

Goals and Outcomes Achieved

Goal 1 was to evaluate chemical control options for weed management for leafy greens based on herbicide selectivity and tolerance so that growers would have efficacious rates of safe herbicides to use in the crop. Weed management programs emanating from adapting new herbicide options would sustain and enhance expansion of acreage and mitigate concomitant hand labor shortages while reducing the overall cost of weed management. Initial grower survey indicated reliance on

postemergence use of one herbicide to control broadleaf weeds, which are the most problematic, in combination with hand labor. Results from the project showed that preemergence herbicide application (imazethapyr, pronamide, and bensulide) followed by postemergence imazethapyr enhanced weed control to acceptable levels compared to using a postemergence herbicide (imazethapyr) program alone. The combination of these preemergence herbicides with timing of the postemergence application at the 2-leaf stage of weeds and the crop can be used to reduce reliance on hand labor without growers receiving a yield penenalty from weed interference. This information was conveyed to growers at meetings, during interviews, and was presented to a national audience at a profesional meeting. In addition, these results will be published as a free online University of Florida extension article. Interviews with leafy green growers indicated that at least half of them would start evaluating combinations of preemergence and postemergence herbicides for efficacious weed control in the crop.

Goal 2 was to evaluate persistence of herbicides in soil and subsequent residual weed control in leafy greens and rotational crop choices. This is because of limited information on residual activity of the aforementioned herbicides and rotational crop choices and restrictions following use in leafy greens in organic soils. The results from the project indicated that preemergence herbicides with the exception of oxyfluorfen on leafy greens did not have a long residual effect on organic soils. This implies that preemergence herbicides (imazethapyr, pronamide, and bensulide) with crop safety on leafy greens cannot be used alone to provide season-long weed control and must be followed by postemergence application of imazethapyr to provide control of weeds that escape the initial treatment. The initial grower survey showed that leafy greens are majorly followed by sugarcane in south Florida. The preemergence herbicides currently registered for use in Florida (imazethapyr, pronamide) would have no plantback concerns on the main rotational crop because it is vegetatively propagated and these herbicides did not persist in organic soil for an extended period of time that would be injurious to sugarcane. Use of these preemergence herbicides would still provide leafy green growers with the ability to continue and maintain rotational crop choices and flexibility following adaptation of the weed management program recommended by this project.

Beneficiaries

The primary beneficiaries of the project were five main leafy green growers in the Everglades Agricultural Area in south Florida, which is the main production area of the crop. These growers have approximately $70 million in farm income from approximately 12,000 acres. The project provided the growers with information on efficacious chemical weed management options derived from science-based research to reduce their dependence on contract hand labor for weed management, which is currently vulnerable to changes in immigration policies. Safeguarding and increasing leafy green production in south Florida through the weed management program recommended in this project will enable growers to continue deriving farm income from production. The end product (or results) of this project was disseminated in grower and professional meetings and will be become available online in extension publications for not only growers but also for crop consultants in the region.

Lessons Learned

The main core of the project on herbicide efficacy and tolerance was accomplished using field studies. However, there were issues with growing leafy greens in the greenhouse for scheduled components of the project in the summer months in south Florida. The iceberg and romaine lettuce used in the studies did not grow well in the summer because of relatively high temperatures and relative humidity in our greenhouses. Such greenhouse-based experiments with leafy green should be conducted during the relatively cooler months (from late October) in the subtropical climate of south Florida to ensure growth of healthy plants. Also, issues with our biological oxidizer limited conducting herbicide absorption and translocation studies for the compounds evaluated in the project. We were content that field studies on herbicide efficacy and persistence provided most of the information that we required for successful completion of the project to make recommendations to growers.

Total Funding Expended: $52,789.10

Percentage of Project Completed: 100

FINAL PERFORMANCE REPORT

FINAL PROJECT REPORT TEMPLATE

Final Performance Reports must illustrate the completion of each project within the grant agreement. Each project shall be outlined as separate project profiles. You will report on projects in the same order they were submitted in the approved State Plan and subsequent amendments.

FDACS CONTRACT NUMBER:

FDACS 022909

PROJECT TITLE

Reducing waste of specialty crops by developing a new approach to determine the impact level of each step along the supply chain on the quality

PROJECT IMPACT AND FINDINGS

The Food Quality Laboratory at the University of South Florida in collaboration with The Iluminate Group developed a novel approach to determine the impact level of each step along the supply chain on blueberry, strawberry, tomato and pepper quality through focusing on the impact level of each step along the supply chain, from the farm to the consumer, on the quality of these specialty crops, and to identify critical supply chain steps where the decline in quality was highest. Researchers tested the impact level of each step of the supply chain on quality of the selected specialty crops. A control at constant optimum conditions plus 16 to 19 time-temperature supply chain scenarios were conducted simultaneously. Sensory quality was determined subjectively, and color, texture, weight loss, acidity, soluble solids, anthocyanins, carotenoids, sugars, and ascorbic acid contents were determined by quantitative analysis. Results from this study showed that maintaining constant optimum temperature throughout the supply chain is paramount to reducing losses in quality, particularly in appearance, texture, weight loss, sugars, and bioactive compounds. Non-optimum conditions encountered during storage at the grower, shipping to stores, storage at the distribution center, retail display and storage at the consumer level had the greatest impact on the overall quality, in comparison to maintaining optimum conditions, and were considered critical supply chains steps. These results were disseminated via a presentation at the FSHS Annual Meeting, a poster presentation at the USF Graduate Symposium, a publication in a scientific journal and via a webinar presented to retailers

BENEFICIARIES

Number of project beneficiaries: 233

ACTIVITIES PERFORMED

OBJECTIVES

Provide the approved project's objectives.

#	Objective	Completed?	
		Yes	No*
1	Determine the losses in the overall quality and quantify the waste of specialty crops (blueberry, strawberry, pepper, and tomato) at each step of the supply chain, from the farm to the consumer.	X	

2	Recommend new approaches that will help each participant in the FFVs supply chain identify where the significant loss in quality occurs and take the necessary actions to reduce waste.	X	

**If no is selected for any of the listed objectives, you must expand upon this in the challenges and lessons learned sections.*

ACCOMPLISHMENTS

List your accomplishments for the project's period of performance, including the impact they had on the project's beneficiaries, and indicate how these accomplishments assist in the fulfillment of your project's objective(s), outcome(s), and/or indicator(s).

Accomplishment/Impact	Relevance to Objective, Outcome, and/or Indicator
Developed a new approach to evaluating the impact of supply chain conditions on the quality of blueberry, strawberry, tomato, and peppers.	We developed a novel approach to determine the impact level of each step along the supply chain on blueberry, strawberry, tomato and pepper quality through focusing on the impact level of each step along the supply chain, from the farm to the consumer, on the quality of these specialty crops.
Recommended new approaches that will help each participant in the specialty crop supply chain identify where the significant loss in quality occurs and take the necessary actions to reduce waste.	We identified critical supply chain steps where the decline in quality was highest. 1) Strawberry: storage at the distribution center; shipping to the store; storage at the consumer without refrigeration. 2) Blueberry: shipping to the distribution center; display at the stores; storage at the consumer without refrigeration. 3) 2) Tomato: storage at the grower; shipping to the stores; storage at the consumer inside the refrigerator. 4) Peppers: storage at the distribution center; display in the stores; consumer with refrigeration.
Estimated waste reduction if supply chain conditions are improved (i.e., better temperature management throughout the supply chain).	We estimated that losses of selected specialty crops could be reduced and quality consistency and availability increased at the retail level: 1) Strawberry: 10% 2) Blueberry: 5% 3) Tomato: 13% 4) Pepper: 8%

CHALLENGES

If you experienced any challenges during the project's period of performance, provide a listing of them below. Also, provide the corrective actions you took to address these issues. If you did not attain the approved outcome(s) and indicator(s), provide an explanation in the Corrective Actions column.

Challenge	Corrective Actions
The experimental setup was too ambitious regarding time, the number of specialty crops tested and resources (personnel and budget).	Reduced data point collection for each specialty crop tested without changing the project objectives.
Dissemination of the results to larger non-scientific audiences.	Webinar to nationwide retailers.

LESSONS LEARNED

Recommendations:

1) *Reduce the number of specialty crops tested and focus on those with higher economic impact (for example, strawberries).*
2) *Develop a way to communicate between growers and consumers.*

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

1) *Presentation at the AGRITECH organized by the Florida Strawberry Growers' Association is planned for next year.*
2) *Presentation of the results at the Institute of Food Technologists.*
3) *A project based on the results of this project and other conducted previously by our team is being implemented to test the impact of pre-harvest conditions on waste and quality of strawberries. This project is funded by the European Union, and the results will be disseminated in the U.S. and Europe.*

OUTCOME(S) AND INDICATOR(S)/SUB-INDICATOR(S)

Provide the results of the project outcome(s) and indicator(s) as approved in your State Plan and project proposal. The results of the outcome(s) and indicator(s) will be used to evaluate the performance of the SCBGP on a national level.

OUTCOME MEASURE(S)

Select the Outcome Measure(s) that were approved for your project.

- ☐ **Outcome 1**: Enhance the competitiveness of specialty crops through increased sales
- ☐ **Outcome 2**: Enhance the competitiveness of specialty crops through increased consumption
- ☐ **Outcome 3**: Enhance the competitiveness of specialty crops through increased access
- ☑ **Outcome 4**: Enhance the competitiveness of specialty crops through greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources
- ☐ **Outcome 5**: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems
- ☐ **Outcome 6**: Enhance the competitiveness of specialty crops through increasing the number of viable technologies to improve food safety
- ☐ **Outcome 7**: Enhance the competitiveness of specialty crops through increased understanding of the ecology of threats to food safety from microbial and chemical sources
- ☐ **Outcome 8**: Enhance the competitiveness of specialty crops through enhancing or improving the economy as a result of specialty crop development

OUTCOME INDICATOR(S)

Provide the indicator approved for your project and the related quantifiable result. If you have multiple outcomes and/or indicators, repeat this for each outcome/indicator.

1) Improving the time-temperature conditions throughout the supply chain can help improve overall quality and reduced waste of specialty crops by up to 13%, depending on the crop.
2) Recommending new approaches that will help each participant in the specialty crop supply chain identify where the significant loss in quality occurs and take the necessary actions to reduce waste. Results from this project indicate that conditions at the retail store (DC, transport to stores and display) and consumer are critical supply chain steps, and therefore future recommendations should target these two segments.

DATA COLLECTION

Task 1: Supply Chain Simulations for Blueberries, Strawberries, Yellow Peppers and Pink Tomatoes
First Year: Blueberries and strawberries were obtained from local growers in Florida the day of harvest and were brought to the laboratory in Tampa with minimal delay after harvest. Upon arrival to the laboratory, the fruits were selected for uniformity of and freedom from defects. For the blueberry experiments, 4 small clamshells (170 g) were used per evaluation (a total of 680 g of blueberries per evaluation). For the strawberry experiments, 4 clamshells containing 15 fruit each were used per evaluation (a total of 60 strawberries per evaluation). The fruit was then exposed to typical time-temperature conditions normally encountered throughout the supply chain. The time-temperature scenarios were chosen based on real time-temperature and humidity (RH) profiles previously measured by our team during field-to-store trials (Nunes et al., 2009; Lai et al., 2010; Pelletier et al., 2011). The time chosen to simulate shipping from the grower to the DC was based on the farthest distance from the specialty crops production areas to the U.S. Midwestern States or Eastern Canada.

A typical supply chain for *blueberry* includes the following steps: harvest → grading → cooling → storage at grower → transport from grower to distribution center (DC) → storage at DC → transport from DC to stores → display at the store → purchase by the consumer. To quantify the impact level of each step of the supply chain on the loss of quality and waste, a control (optimum conditions; 1°C and 90% RH) plus 18 supply chain scenarios were conducted simultaneously. In summary, the supply chain simulation for blueberries took a total time of 278 h (≈ 12 d) and consisted of the following independent but simultaneous experiments: a) grading delays: 2 or 4 h at 30°C; b) grading: low temperature, 5°C for 4 h or high temperature, 25°C for 4 h; c) cooling: low temperature, 2°C for 2 h or high temperature, 5°C for 2 h; d) storage at the grower: low temperature, 2°C for 48 h or high temperature, 5°C for 48 h; e) shipping to distribution center (DC): low temperature, 2°C for 72 h or high temperature, 5°C for 72 h; f) storage at DC: low temperature, 2°C for 48 h or high temperature, 5°C for 48h; g) shipping from DC to stores: low temperature, 2°C for 8 h or high temperature, 8°C for 8 h; h) display in the store: low temperature, 2°C for 48 h or high temperature, 15°C for 48 h; i) consumer: low temperature, 4°C for 48 h or high temperature, 20°C for 48 h. A typical supply chain for *strawberry* includes the following steps: harvest → cooling → storage at grower → transport from grower to the distribution center (DC) → storage at DC → transport from DC to stores → display at the store → purchase by the consumer. To quantify the impact level of each step of the supply chain on the loss of quality and waste, a control (optimum conditions; 1°C and 90% RH) plus 16 supply chain scenarios were conducted simultaneously. In summary, the supply chain simulation for strawberries took a total time of 178 h (≈ 7.4 d) and will consist on the following independent but simultaneous experiments: a) cooling delays: 2 or 4 h at 30°C; b) cooling: low temperature, 2°C for 2 h or high temperature, 5°C for 2 h; c) storage at the grower: low temperature, 2°C for 24 h or high temperature, 5°C for 24 h; d) shipping to distribution center (DC): low temperature, 2°C for 72 h or high temperature, 5°C for 72 h; e) storage at DC: low temperature, 2°C for 24 or high temperature, 5°C for 24 h; f) shipping from DC to stores: low temperature, 2°C for 8 h or high temperature, 8°C for 8 h; g) display in the store: low temperature, 2°C for 24 h or high temperature, 15°C for 24 h; i) consumer: low temperature, 4°C for 24 h or high temperature, 20°C for 24 h.

Second Year: For the first set of experiments/harvests, we obtained yellow peppers and pink tomatoes from a new local greenhouse grower, Red Barn Hydroponics, located in Dove, Florida. However, there were many issues with this grower and later their facilities closed which forced us to delay the second part of the experiment/harvest until we could find a grower with similar characteristics. First, the grower was asked to provide us with green bell peppers, but on the day scheduled for the experiments (April 26, 2017) we received sweet yellow peppers (cv. Cornito Giallo) instead of green bell peppers. The quality was very good, and thus we decided to proceed with the experiments. Pink tomatoes (cv. Resolute) for the first experiment/harvest were also obtained from the same grower on April 5, 2017. Afterward, the grower had problems with fungal contamination, and we had to cancel the second part of the experiments for both tomatoes and peppers, and then they closed the facilities. Therefore, for the second set of experiments/harvest, pink tomatoes (cv. Resolute) were obtained from another grower, Hydro Harvest Farms, located in Ruskin, Florida. The pint tomatoes for the second experiment were harvested on November 29, 2017. This same grower will provide the sweet yellow pepper for the second set of experiments. The fruit was harvested and on the same day brought to the laboratory in Tampa with minimal delay after harvest. Upon arrival to the laboratory, tomato and peppers were selected for uniformity of and freedom of defects.

For the pepper and tomato experiments, a total of 9 fruit per treatment (control + 18 simulated supply chain steps) were used. The whole experiments will be repeated twice (two harvests) during the season.

The major supply chain steps for *peppers* include the following: harvest → grading → cooling → storage at grower → transport from grower to distribution center (DC) → storage at DC → transport from DC to stores → display at the store → purchase by the consumer. To quantify the impact level of each step of the supply chain on the loss of quality and waste, a control (optimum conditions; 7°C and 90% RH) plus 18 supply chain-scenarios will be conducted simultaneously. In summary, the supply chain simulation for green bell peppers will take a total time of 278 h (≈ 12 d) and will consist on the following independent but simultaneous experiments: a) grading delays: 2 or 4 h at 35°C; b) grading: a low temperature of 10°C and a high temperature of 30°C for 4 h; c) cooling: low temperature, 2°C for 2 h or high temperature, 25°C for 2 h; d) storage at the grower: low temperature, 2°C for 48 h or high temperature, 25°C for 48 h; e) shipping to distribution center (DC): low temperature, 2°C for 72 h or high temperature, 20°C for 72 h; f) storage at DC: low temperature, 2°C for 48 h or high temperature, 15°C for 48 h; g) shipping from DC to stores: low temperature, 2°C for 8 h or high temperature, 8°C for 8 h; h) display in the store: low temperature, 2°C for 48 h or high temperature, 20°C for 48 h; i) consumer: low temperature, 4°C for 48 h or high temperature, 20°C for 48 h. The major supply chain steps for *tomatoes* include the following: harvest → grading → room cooling → commercial ripening (ethylene applications) → storage at grower → transport from grower to distribution center (DC) → storage at DC → transport from DC to stores → display at the store → purchase by the consumer. However, since we will be using pink tomatoes, we will skip the ethylene treatment step. To quantify the impact level of each step of the supply chain on the loss of quality and waste, a control (optimum conditions; 13°C and 90% RH) plus 18 supply chain-scenarios will be conducted simultaneously. In summary, the supply chain simulation for pink tomatoes will take a total time of 278 h (≈ 12 d) and will consist on the following independent but simultaneous experiments: a) cooling delays: 2 or 4 h at 35°C (average field temperature during summer season); b) grading: low temperature, 10°C for 4 h or high temperature, 35°C for 4 h; c) cooling: low temperature, 10°C for 2 h or high temperature, 25°C for 2 h; d) Storage at the grower: low temperature, 10°C for 48 h or high temperature, 25°C for 48 h; e) shipping to distribution center (DC): low temperature, 10°C for 72 h or high temperature, 20°C for 72 h; f) storage at DC: low temperature, 2°C for 48 h or high temperature, 15°C for 48 h; g) shipping from DC to stores: low temperature, 2°C for 8 h or high temperature, 8°C for 8 h; h) display in the store: low temperature, 2°C for 48 h; high temperature, 20°C for 48 h; i) consumer: low temperature, 4°C for 48 h or high temperature, 20°C for 48 h.

Ten temperature and humidity controlled chambers (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) were used to conduct the different supply chain simulations. The temperature was monitored throughout the study using Stow Away® XTI02 temperature loggers (-5 °C to +37 °C) (Onset Computer Corporation, Pocasset, Mass). RH was monitored with Stow Away® RH loggers (10 to 95% RH) (Onset Computer Corporation, Pocasset, Mass.).

Task 2: Sensory Quality and Physicochemical Analysis
First Year: Sensory quality of strawberries and blueberries were determined subjectively. Surface color measurements and firmness were evaluated using quantitative methods as described below. Weight loss, moisture content, acidity, pH, soluble solids content, sucrose, glucose, fructose, and ascorbic acid were determined by quantitative analysis as described below. Second Year: Sensory quality of tomatoes and peppers were determined subjectively. Surface color measurements and firmness were evaluated using quantitative methods as described below. Weight loss, moisture content, acidity, pH, soluble solids content, total carotenoid content, sucrose, glucose, fructose, and ascorbic acid were determined by quantitative analysis as described below.

- *Visual Quality.* Subjective quality attributes, namely color, shriveling and decay were determined subjectively using a 1 to 5 visual rating scale, and firmness was determined subjectively based on the whole specialty crop resistance to slightly applied finger pressure and recorded using a 1 to 5 tactile rating. These data will be primarily used to determine the end of strawberry shelf life and to quantify postharvest losses.
- *Instrumental Color (L*a*b*) Analysis.* A total of two color measurements was taken on the opposite sides of the specialty crops at the equatorial region. A hand-held tristimulus reflectance colorimeter

(Model CR-400, Minolta Co., Ltd., Osaka, Japan) equipped with a glass light-projection tube (CR-A33f, Minolta Co., Ltd., Osaka, Japan) was used. The color was recorded using the CIE-L*a*b* uniform color space (CIE-Lab), L* (lightness), a* (redness), and b* (yellowness) values. Numerical values of a* and b* were converted into hue angle using the Konica Minolta CR-400 Utility software CR-S4w (2002-2010 Konica Minolta Sensing, Inc., Osaka, Japan).

- *Texture Analysis.* Before texture is analyzed, fruit from each treatment were conditioned at room temperature for approximately one hour. After that, firmness was measured using a TA.XT Plus Texture Analyzer (Texture Technologies Corp., NY, USA) equipped with a 50 kg load cell. For blueberry, three replicated samples of 30 g each were placed into three-100 mL plastic beakers and the fruit compressed to a depth of 30 mm using a 38 mm diameter and 20 mm high acrylic cylinder probe. The probe will then be driven at a crosshead speed of 1 mm s^{-1}, and the compression force will be recorded after the probe had compressed the fruit by 30 mm (Sanford et al. 1991). Strawberry firmness was measured at the equatorial part of the fruit using a 7.95 mm stainless convex probe. The probe was driven with a crosshead speed of 1 mm s^{-1}, and the compression force was recorded at 3.0 mm deformation (Whitaker et al., 2012). Peppers were first cut transversely, approximately three-quarter way from the shoulder end and then placed, with the cut end down, on the flat platform of the texture analyzer. A 7.95 mm stainless convex probe was centered on a shoulder lobe with two measurements taken on opposite lobes. The probe was driven with a crosshead speed of 1 mm s^{-1}, and the compression force was recorded at 5.0 mm deformation (Nunes et al., 2012). Tomato was placed on the flat surface of the texture analyzer with stem-end down, so the pressure was applied to the blossom-end part of the fruit. The instrument was fitted with a 76.2 mm diameter stainless compression plate and the probe was driven with a crosshead speed of 1 mm s^{-1}, and the compression force was recorded at 10.0 mm deformation (Chilson et al., 2011).
- *Weight Loss and Moisture Content.* The weight was measured using a precision balance with an accuracy of ±0.01g (Denver Instruments, Timberline Series Model TP-3102, CO, USA) as described by Proulx et al. (2011). Moisture content was determined by the standard gravimetric method. A 10 g homogenized sample will be spread evenly over the bottom of a metal dish, weighed, and dried 24 h at 80°C in a laboratory oven (Model 40GC, Quincy Lab Inc., Chicago, IL). Dry samples were cooled in desiccators then weighed, and the final weight will be subtracted from the initial weight to obtain the moisture content.
- *pH, Acidity and Soluble Solids Content (SSC).* A 50-g aliquot of the tissue slurry was centrifuged at 6,000 × g for 20 min. The clear juice was decanted and the pH, titratable acidity and SSC of the clear juice will be determined as described by Nunes et al. (2006).
- *Total Carotenoid Content.* The total carotenoid content of tomato and yellow peppers was calculated following the method described by Talcott and Howard (1999). Briefly, 2g of tissue was extracted in the darkness for 1 hour at 5°C. The extract was filtered, and absorbance was read at 470 nm.
- *Total and Individual Sugar Contents.* Total sugar analysis was conducted using a Hitachi HPLC system with an RI- refractive index detector and a 300 mm × 8 mm Shodex SP0810 column (Shodex, Colorado Springs, CO) with an SP-G guard column (2 mm x 4 mm). Isocratic solvent delivery of water was set at 1.0 mL/min. Standards including sucrose, glucose and fructose were used to identify sample peaks. After comparison of retention time with the standards, the peaks were identified. The amount of total sugar in strawberries was quantified using calibration curves obtained from different standard concentrations.
- *Total Ascorbic Acid Content.* 2g of homogenized fruit was mixed with 20 ml metaphosphoric acid mixture (6% HPO_3 containing 2 *N* Acetic acid) and filtered through a 0.22μm filter before HPLC analysis. The ascorbic acid analysis was conducted using a Hitachi LaChromUltra UHPLC system with a diode array detector and a LaChromUltra C18 2μm column (2 × 50 mm) (Hitachi, Ltd., Tokyo, Japan). The analysis was performed under isocratic mode at a flow rate of 0.5 mL/min with a detection of 254 nm. Mobile phase was buffered potassium phosphate monobasic (KH_2PO_4, 0.5%, w/v) at pH 2.5 with metaphosphoric acid (HPO_3, 0.1%, w/v). After comparison of retention time with the ascorbic acid standard, the peaks were identified. The amount of total ascorbic acid content in strawberries was quantified using calibration curves obtained from different concentrations of ascorbic acid standards.

Task 3: Data Analysis

First Year: The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data collected. Overall, a completely randomized design was used with a factorial arrangement of two harvests, 17 or 19 supply chain treatments (control plus impact level of: delays before cooling/grading, grading, precooling, storage at grower's, shipping to distribution center, storage at distribution center, shipping to stores, display in-store, consumer), three evaluation times (at harvest, after supply chain step, end of supply chain) and three replicated samples of each specialty crop used. Second Year: Overall, a completely randomized design was used with a factorial arrangement of one harvest, and 19 supply chain treatments (control plus impact level of: delays before cooling/grading, grading, precooling, storage at grower's, shipping to distribution center, storage at distribution center, shipping to stores, display in-store, consumer), two evaluation times (at harvest and at end of supply chain) and three replicated samples of tomato and peppers (9 fruit per treatment/supply chain). The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of data. The data from each specialty crop was treated by a three-way analysis of variance (ANOVA), with harvest, supply chain treatments, and evaluation times as factors. The General Linear Model procedure of SAS was used to identify significant main effects and interactions between treatments. Significant differences between treatments were detected using the least significant difference (LSD) test at the 5% level of significance.

CONTACT PERSON

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FEDERAL PROJECT EXPENDITURES TO DATE

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$70,766.00	$69,761.79
Fringe Benefits	$5,790.00	$2,511.94
Travel	$7,083.00	$3,611.86
Equipment	$0.00	$0.00
Supplies	$53,694.00	$60,874.99
Contractual	$0.00	$0.00
Other	$11,114.00	$11,544.05
Direct Costs Sub-Total	$148,477.00	$148,304.63
Indirect Costs	$0.00	$0.00
Total Federal Costs	$148,477.00	$148,304.63

PROGRAM INCOME

Source/Nature (i.e., registration fees)	Amount Approved in Budget	Actual Amount Earned
N/A	N/A	N/A
Total Program Income Earned		

ADDITIONAL INFORMATION

Please see Appendix (includes detailed expenditures; copy of publication, poster, presentation and webinar information).

Project (5): Establishing a Hops Production System for Florida Growers

Contact Person

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Project Partners

Primary organization: University of Florida/IFAS/Gulf Coast Research and Education Center. University of Florida/IFAS/Mid-Florida Research and Education Center.

Partner organizations: Florida Specialty Crop Foundation, Hillsborough County Economic Development/Agriculture Industry Development.

Project Summary

Many Florida specialty growers have expressed keen interest in growing and producing hops in Florida. Numerous Florida brewers have asked us to establish a hop production system that can allow Florida growers to supply locally produced hops for brewing beers for their customers and businesses. Within the U.S., the states of Washington, Oregon, and Idaho produce about 99% of all hops, with only 1% of hops production occurring in the remaining 47 states. Until 2013, hops had not been cultivated within the state of Florida. Given a strong interest in hops cultivation, Co-PI Pearson cultivated several cultivars of hops beginning in spring of 2013. After two consecutive years of cultivation, Co-PI Pearson determined that hops cultivation in Florida was possible; however, yields were much lower than those reported for the Pacific U.S. region.

Commercial hop production in other states requires high trellises, selection of locally adapted varieties, proper plant spacing, and adequate fertilization level. Florida growers are in urgent need of answers to these questions to make decisions as for whether or not engaging in commercial hop production in Florida. This project is designed to test 15 hop varieties in high trellis hop yards at two locations in Florida and evaluate the effects of planting spacing and nitrogen fertilization level on hop cone yield and quality.

The project was both timely and important to the state and its agricultural producers. Demand for information about hops cultivation in Florida was high at the initiation of the project and has increased throughout the study period. Demand increased due to reductions in citrus production and a simultaneous increase in craft beer demand. Production of craft beer, in Florida alone, increased 3-fold between 2013 and 2016. The completion of this 2-year project has resulted in much needed data for hop cultivar selection, horticultural practices, as well as hop yard construction and pest management. The data collected on hop plant growth, cone yield, and chemical analysis are very valuable information

for Florida growers, craft brewers, and other interested parties, and also serve as an indispensable guide for the next phase trials.

Project Approach

Objective 1. Evaluate the adaptation and cone yield potential of major hop varieties under Florida's climatic and soil conditions utilizing a high-trellis production system.

1. Hop variety trials at the GCREC:

One typical high-trellis hop yard was constructed in spring 2016, using pressure-treated wooden poles 25 feet tall and high tension cables. Thirteen hop varieties were planted in three blocks, and each plot consisted of 4 hills of plants. Hop plant heights were measured and main bines and lateral bines of each plant were counted on multiple dates. Hop cones were harvested from early June to late August 2016. After the last cone harvest, all plants were cut back to the ground for regrowth and the FL AgExpo. In 2016, the 13 hop varieties showed drastic differences in plant height, lateral shoot production, and cone yield. The average plant height ranged from 67.4 to 155.4 inches, and the average number of lateral shoots per plant ranged from 2.3 to 11.0 per plants. Hop varieties Willamette and Nugget had the tallest plants (155.4 and 140.6 inches, respectively). Cascade had a moderate plant height but it produced the largest number of lateral shoots. Based on fresh cone weights, the 13 hop varieties could be separated into four groups: Group 1 (0.75 to 1 pound of fresh cones per hill) – Cascade; Group 2 (0.25 to 0.5 pounds of fresh cones per hill) – CTZ and Nugget; Group 3 (less than 0.25 pounds of fresh cones per hill) – Centennial, Chinook, Crystal, Fuggle, Magnum, and Willamette; Group 4 (did not produce cones) – Perle, Sorachi Ace, and Tettnanger.

In spring 2017, tissue culture hop plantlets were available only for three varieties, Cascade, Centennial, and Chinook. Tissue culture liners of these varieties were transplanted to the hop yard on May 30. Plant growth (bine height, number of bines, and number of laterals) were recorded on multiple dates, hop cones were harvested in late August 2017. Plants were cut back to the ground level in early September 2017, and the second crop of hop cones were harvested in early December 2017. Plants of Cascade and Chinook were 1 to 3 ft taller than those of Centennial. Plants of Cascade have 0.5 to 1 more bines and 3 to 4 times more lateral shoots per hill than Centennial or Chinook. Cascade yielded the highest per acre, 251 lb of fresh hops. The three cultivars showed a dramatic difference in regrowth. Cascade regrew quite well, while Centennial and Chinook had little regrowth. As the fall crop, Cascade plants produced 15 to 20 grams of fresh cones per hill, which is equivalent to 32 to 42 lbs of fresh hop cones per acre.

To increase the number of hop varieties for trials in the GCREC hop yard in 2017, the Hillsborough County Economic Department's Agribusiness Development Manager secured tissue culture stock for 14 hop varieties from Hopsteiner. Hop cultivars from Hopsteiner came in as plantlets in test tubes (about 1 to 2 inches long). These varieties were transplanted to the hop yard in mid-September. These plants will be used in a continuation hop project funded by the FDACS SCBG project.

2. Hop variety trials at the MREC (Mid-Florida Research and Education Center):

During Year 1, Co-PI Pearson oversaw construction of a traditional, tall-trellis hop yard at the MREC in Apopka, FL. Using tissue culture propagated plant material, 20 different hop cultivars were transplanted into the soil and allowed to reach establishment (when significant root mass is produced that allows plants to avoid drought throughout periods without rainfall). Insects of hops were identified and pest management strategies were established. At the end of 2016, it was determined that greatest hop cone yields were harvested from 'Brewers Gold', 'Comet', Teamaker', 'Cascade', and 'Chinook'; however, yields amongst all cultivars examined were significantly lower than those cultivated in the Pacific Northwestern U.S.

Research during Year 2 began once hops emerged from winter dormancy and were trained onto the hop yard infrastructure (April 2017). Co-PI Pearson assessed growth and performance of all 20 hop cultivars throughout the growing period (April through September 2017). In early September, Hurricane Irma passed through Central Florida and severely damaged hops plants and caused defoliation of 90% of hops in cultivation. Although plants produced new leaves in mid-October, it was too late in the season for plants to produce additional cones. Growth and yield results differed from results observed in Year 1. Differences were expected given hops were undergoing establishment during Year 1. Eighteen of the 20 cultivars in our trial had greater yield during Year 1 than Year 2. 'Cascade', 'Neo1', and 'Cluster' produced the greatest yields; however, yields still remained ~1/3 of yields reported for the Pacific Northwestern U.S.

Objective 2. Evaluate the effects of horticultural practices on hop yield and quality.

Half of this hop yard at the GCREC was used for production trials in 2016 and 2017 to evaluate two N rates (0.75 and 1 lb/acre/d) and three in-row plant distances (30, 36, and 42 inches) in factorial combinations for two cultivars (‘Cascade’ and ‘Chinook’). In the first year, rhizomes were used to establish the trials. Bine growth was similar in terms of height regardless of cultivars and other treatments. By contrast, branching was 186% greater for ‘Cascade’ than ‘Chinook’ (8.23 vs. 2.88 bines/plant) at the mid harvest. Cone yield showed significant cultivar × N rate interaction effects: ‘Cascade’ increased yield with increasing N rate by 33% (297 vs. 394 lb/acre, fresh weight), whereas yield of ‘Chinook’ was very small regardless of N rates (40 kg/ha). In both cultivars, bine growth was unaffected by in-row plant spacing. Although not statistically significant, decreasing in-row distance from 42 to 30 inches increased yield by 40% (155 vs. 217 lb/acre). The results suggest that ‘Cascade’ has a much higher yield potential than ‘Chinook’ in Florida, and that branching is an important yield-related trait. Furthermore, the first season yield of ‘Cascade’ can be maximized by optimizing N fertilization rate and implementing narrow plant spacing.

In the second year (2017), tissue culture plants were used to reestablish the trials. Tissue culture seedlings from two commercial nurseries demonstrated different plant performance. Compared to seedlings from Michigan, seedlings from Florida grew 114% taller (9.0 vs. 4.2 ft) and produced 155% more branches (2.6 vs 1.0). Although seedlings from Michigan were not able to produce any cones, seedlings from Florida produced 288 pounds of fresh hop cones per acre. Plant growth was not affected by in-row spacing, although this result was expected as plants in the first season were not fully established and had little plant competition. Compared to the rhizomes used in Year 1, tissue culture plants from Florida had similar height growth (9.4 vs. 9.0 ft) but 46% less branching (4.8 vs. 2.6) and 17% less cone yield (345 vs 288 lb/acre). More vigorous growth of rhizome plants in the first season may be due to the stored carbohydrate that can support quick establishment. However, drawbacks of rhizomes are high risks of virus infection and relatively low emergence compared with tissue culture seedlings (65 vs 98%).

Objective 3. Assess the chemical and sensory qualities of locally produced hops for use in craft beer brewing.

Hop cones harvested at the GCREC were provided to 3 Daughters Brewing, Cigar City Brewing, and Coppertail Brewery for sensory quality assessment by their staff brewmasters. One of the brewers used the provided hops in dry-hopping of their beer products. Feedback indicated that the hop cones produced in Florida hop yards produced quality beers. As most of the hop cones harvested from the GCREC hop yard were sent to local craft breweries, there were only three dried hop samples for chemical analysis. The alpha acid content ranged between 4.9% and 10.9%, and beta acids varied from 2.9% to 5.0%. Total oil content was between 0.3% and 0.4%. As for oil profile, 23.0% to 52.1% was myrcene, 3.4% to 10.1% caryophyllen, 5.4% to 11.3% farnesene, and 7.3% to 20.7% humulene.

Samples of hop cones harvested from the MREC hop yard were sent to a commercial laboratory (Kar Labs) for quantification of alpha and beta acids (ASBC Hops-14 method) as well as full volatile oil profile (ASBC Hops-17) where sample sizes were sufficient. Harvested hop cones possessed good quality with differences amongst cultivars. Average alpha and beta acid concentrations were 4.6% and 3.1%, respectively.

Objective 4. Disseminate results to Florida growers, beer brewers, and the general public (Agehara, Deng, & Pearson).

In 2016, one hops field day was held at the GCREC on June 9 and it attracted some 30 Florida microbrewers and brewing educators, and four presentations were made to the participants. One hop yard tour was given at the GCREC on July 29, 2016, and 12 local produce producers visited the hop yard and trials, and handouts were distributed. One statewide field day event was held at the MREC on September 27, 2016 with 62 participants in attendance. The field day event included a field tour, research update, as well as guest lectures from supporting programs at the center. Two hop yard tours were given at the GCREC on November 2, 2016 during the FL AgExpo. Posters were displayed in the hop yard, more than 200 copies of handouts were distributed, and participants visited the hop yard and

trials. One presentation was made to the general session of the AgExpo with several hundred participants. Together, these events attracted more than 250 visitors from the Florida microbrewery industry, produce industry, allied industries, grower associations, research institutions, etc. and Six presentations were made at these events to educate the participants and to disseminate results from this project. We have contributed information on hops and this project to numerous local and regional news agencies and trade magazine staff. More than a dozen of newspaper articles, trade magazines, websites and several TV stations used our results in their reports. It is estimated that results from this project have been disseminated to tens of thousands of Floridians.

In 2017, PI Deng participated the following major extension and outreach events (Brewing students on 3/4/17, Warner University students 4/21/17, Epcot students 6/1/17, the 2017 Master Brewers Conferences 10/12-14/17, FL AgExpo on 11/8, and Epcot students 12/14/17, and disseminated research results and findings to 338 individuals. Co-PI Pearson organized several field day events, 167 individuals attended these events to learn about hops cultivation at MREC. With the project complete, findings will be disseminated through scientific manuscripts and in Extension publications. People benefitting from the work are estimated at several hundred within the next couple of years. Co-PI Agehara presented research findings from the project to about 50 participants at Fruit Crop Management Short Course in Lake Alfred on Nov 7 and to about 120 participants at AgExpo in Balm on Nov 8. Participants were mostly growers but also included industry personnel, and extension agents. Production trials were also shown to about 60 participants during the field tour at AgExpo. There are many growers who are highly interested in growing hops, and the information from this project provides basic production guides that can help them select better management options.

Hops are a specialty crop only. Research conducted in this project benefitted only specialty crops.

Significant contributions and roles of project partners:

Sonia Tighe at the Florida Specialty Crop Foundation managed this project and helped us connect with Florida specialty crop growers. Simon Bollin at the Hillsborough County Economic Development/Agriculture Industry Development assisted us in selecting hop varieties for trials, securing planting stock and strong support from local brewers, and evaluating hop plant growth and yields. 3-Daughters Brewing, Cigar City Brewing, Coppertail Brewing, Hopsteiner supported this project and tested our hops.

Goals and Outcomes Achieved

Goal 1: Collect data on plant growth, disease severity, cone yield, and cone quality of major hop varieties under Florida's climatic and soil conditions and typical horticultural practices.

Two replicated hop variety trials were conducted in Florida, one at the GCREC, and one at the MREC, for two years. Collectively more than 20 hop varieties were tested in these trials, that is, at least five more varieties were tested compared to the original goal established for the project. Prior to this project,

some information was available for two to three hop varieties. Now data are available for the plant growth, cone yield, and cone quality for more than 20 hop varieties.

The collected data suggest that hop variety selection will be extremely critical for hop growing and production in Florida. The data have allowed for identification and recommendation of the highest yielding and best quality hop cultivars for Florida growers. A number of hop varieties produced significantly more hop cones than others. Among them, Cascade showed the best potential. Other varieties that may have potential are Cluster, CTZ, Neo1, and Nugget. While Cascade, Neo1, and Cluster produced the greatest yields at the MREC, their yields still remained ~1/3 of yields reported for the Pacific Northwestern U.S.

Expected outcomes for the production trials were plant growth and yield data of two hop cultivars to assess the effects of N rate and in-row plant spacing. To obtain the outcomes, we built a 20-ft high hop trellis system, established and conducted production trials for two growing seasons from 2016 to 2017, and collected plant growth and yield data. Based on our data described under the Project Approach section, we were able to develop some basic production guidelines. First, Cascade has a much higher yield potential than Chinook in Florida, and that branching is an important yield-related trait. The first season yield of Cascade can be maximized by optimizing N rate and in-row plant spacing. Our fertility recommendation is 130-150 lb/acre or 1 lb/acre/d.

Although not listed as a component of Goal 1, Co-PI Pearson and staff developed Integrated Pest Management (IPM) Practices for several hop pests.

Goal 2: Assess the chemical and sensory qualities of locally produced hop cones for use in craft beer brewing.

Prior to this project, there were no data available for the chemical and sensory qualities of Florida produced hops. Now chemical and sensory assessment was completed for 16 of the 20 hop cultivars grown at MREC. Four hop cultivars did not produce enough cones for chemical and sensory assessment. With the exception of one hop cultivar, 'Comet', all hop cultivars produced lower alpha and beta acid concentrations than similar cultivars grown in the Pacific Northwestern U.S. Hops cultivated during Year 2 were used in sensory trials and allowed for identification of hop cultivars that possessed superior aromatic traits. Harvested hop cones possessed good quality with differences amongst cultivars. Average alpha and beta acid concentrations were 4.6% and 3.1%, respectively.

From the GCREC hop yard, there were only three dried hop samples that had adequate amounts for chemical analysis. Their alpha acid content ranged between 4.9% and 10.9%, and their beta acids varied from 2.9% to 5.0%. Total oil content was between 0.3% and 0.4%. As for oil profile, 23.0% to 52.1% was myrcene, 3.4% to 10.1% caryophyllen, 5.4% to 11.3% farnesene, and 7.3% to 20.7% humulene. Feedback from local craft breweries indicated that the hop cones produced in Florida hop yards produced quality beers.

Compared to the goal established in the project, at least one more hop variety was tested for chemical qualities.

Goal 3: Develop and disseminate articles on hop varieties and production to Florida growers, beer brewers, and the general public.

Efforts by Co-PI Pearson resulted in >150,000 individuals viewing research results on hops. Additionally, >300 farmers and brewers visited the MREC hop yard and met directly with Co-PI Pearson. Several commercial beers were brewed with hops cultivated at MREC. Multiple media outlets visited the hop yard and provided newspaper and television coverage of the research. Moreover, Co-PI Pearson worked with FDACS Division of Marketing and Development staff to establish a new logo for beer produced with Florida hops. Co-PI Pearson and staff traveled to Tallahassee, FL in March 2017 to showcase the release of this new logo and provided one-on-one extension and outreach efforts to the public and legislators during the Taste of Florida Agricultural Reception event.

PI Deng and Co-PI Agehara participated in many extension and outreach events held at the GCREC, the Citrus Research and Education Center, and other sites, made numerous presentations at these events to educate the participants and to disseminate results from this project, and their presentations and handouts were distributed to more than 600 visitors from the Florida microbrewery industry, produce industry, allied industries, grower associations, research institutions, etc. and

The research team has contributed information on hops and this project to numerous local and regional news agencies and trade magazine staff. More than a dozen of newspaper articles, trade magazines, websites and several TV stations used our results in their reports. It is estimated that results from this project have been disseminated to tens of thousands of Floridians.

Beneficiaries

Primary beneficiaries identified in the project included Florida growers and craft brewers. More than 600 growers and brewers visited the MREC and GCREC hop yards and interacted directly with Co-PI Pearson, PI Deng, and Co-PI Agehara. These growers and brewers benefitted from learning about how hops are cultivated, which cultivars perform best in Florida, and how to manage pests effectively. They also benefitted through learning about hop quality assessment techniques and how to interpret and use hop chemical data for brewing. Both growers and brewers were able to learn about research findings through online extension documents, oral presentations, and newspaper and television coverage of the research. As a result, several commercial hops yards are now in operation throughout Florida and multiple breweries have showcased craft beer made with Florida grown hops.

Secondary beneficiaries identified in the projected included hop processors and dealers. Several hop dealers are now established in the state and have benefitted from one-on-one discussions and meetings with Co-PI Pearson. Two hop processors are now located in the state and have been instrumental in assisting hop harvesting efforts in smaller farms that do not have the capital to purchase hop harvesting equipment. Both hop processors and dealers learned about hop harvesting and processing through oral presentation and meetings with Co-PI Pearson.

Tens of thousands of Florida consumers and the general public learned about Florida-produced hops from newspapers, TV, and magazine articles. These consumers and general public may help increase the consumption of locally produced hops, which may increase the market demand for Florida-produced hops.

Lessons Learned

Within less than a month after planting, some of the hop plants at the GCREC began to show symptoms of Apple Mosaic Virus (ApMV). ELISA assays confirmed the incidence of ApMV. This virus is common in the Pacific Northwest hop production area; it is highly likely that the planting stock used at the GCREC hop yard were infected with ApMV. Because of the incidence of ApMV, we decided to reestablish the trials at GCREC using tissue culture seedlings from two commercial nurseries (Florida and Michigan). Because of this unexpected problem, we were not able to get the second season data from the initial trials established with rhizomes. Instead, we were able to assess the plant performance of both rhizomes and tissue culture plants.

The most important lesson learned is that virus-free planting stock is essential and critical for successful establishment of hop yards in Florida. Hop plants are perennial, and their cone yields in the first and second year are generally low. Hop plants need long days to produce the maximum amount of biomass and the highest cone yields. To increase hop cone yields, extended day lengths or day-neutral varieties are needed.

Total Funding Expended to Date: $154,130.48

Percentage of Project Complete: 100

Project (6): Control of Guignardia citricarpa (Kiely) to Slow the Spread of Citrus Black Spot

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Project Partners

Southwest Florida Research and Education Center, University of Florida - IFAS

Partners: 3 commercial citrus growers

Florida Specialty Crop Foundation

Project Summary

Background

Citrus black spot (CBS), first identified in Collier County, FL, in 2010 (Schubert et al., 2012), is an economically devastating disease of citrus worldwide. The control and management of CBS is of primary concern to Florida citrus growers, as the acreage placed under quarantine has expanded each year. As of February 10, 2015, the Animal and Plant Health Inspection Service (APHIS) had 48,449.5 acres of citrus groves under quarantine for CBS. Of this, approximately 30,000 acres are 'Valencia', 13,000 acres are 'Hamlin' and 1,200 acres are grapefruit.

In 2010, cost estimates of additional sprays to combat CBS ranged from $100 per year in groves where spray programs already existed to combat HLB and citrus canker to $200 - $300 per acre per season. The cost of adding two to four additional sprays per season increased the overall spray costs by 136 to 155% -$478/acre for 'Valencia' to $928/acre on grapefruit (Muraro, 2012). Based on 2012 spray costs and 2015 acreage under quarantine, growers were expected to spend $14.34 M on 'Valencia', $5.93M on 'Hamlin' and $1.11 M on grapefruit in the next citrus season (2015-2016) on preventative fungicide sprays.

Presence of CBS on trees in groves reduces the ability of growers to export or move fresh fruit within the state, nationally, and internationally. Citrus black spot is considered an A1 quarantine pest and as such trade restrictions are levied on countries where the disease is present. As of July 1, 2013, the European Union mandated that groves in a county with a CBS quarantine either have a Harvesting Permit declaring the grove as visually free of both canker and CBS, or fruits be inspected and found free of CBS and canker in the packinghouse. Growers have also been given the option to submit a statement that the source grove has been, "subjected to appropriate treatments against CBS" (in place of a pre-harvest survey of the grove) in addition to inspection and certification in the packinghouse.

Citrus black spot can cause premature fruit drop. Up to 80% yield losses have been seen in Florida groves (Roberts et al., unpublished) and in other countries (Kiely, 1948; McOnie, 1964; Reis et al., 2006). Premature fruit drop can occur in severely infected groves, especially under environmental conditions favorable to disease development in late harvested fruit such as 'Valencia' oranges. Collier and Hendry counties produced 26% of the total 'Valencia' oranges in Florida during the 2012-2013 season. The majority of CBS infected trees are located in both counties. An 80% loss due to fruit drop was estimated at $115 M loss in revenue based on the equivalent on-tree price ($8.33 per box) (NASS, 2013).

Importance and timeliness of the project

Despite the current quarantine and management practices implemented by the state, the number of groves with trees having CBS had increased since its detection in 2010. In 2009-2010 approximately 10,242 acres were under quarantine, compared to 48,449.5 acres of citrus groves at the 2015 survey. Knowledge on the rate of disease spread and effectiveness of current CBS management practices in slowing the disease progress is crucial in combating the disease.

The incidence of CBS in two citrus groves under study, designated as Grove I and II were 2.6% and 7.1% respectively for the 2013-2014 season. Preliminary spatial analysis of disease using the binomial dispersion index (D), revealed clustering of symptomatic trees for both groves in all quadrant sizes tested (2x2 up to 10x10). Monte Carlo simulations for the L function in R confirmed the non-random pattern of the disease within each grove (Hendricks et al., 2017). However, in order to assess movement over time, we monitored disease spread within groves for 4 consecutive years (3 consecutive citrus season).

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The proposed research assessed the specific issue of spatial distribution of CBS within affected groves to determine the rate of disease spread under the unique conditions in Florida and to monitored movement of the disease to previously asymptomatic trees within three commercial groves under current management conditions.

Project Approach

Objective 1: Determine spatial distribution of CBS in three affected groves over two consecutive citrus seasons to determine rate of spread and severity in Florida groves.

Activities performed

Citrus black spot distribution maps were produced in two of three infected groves between January and April 2016 (Year 1) and November 2016 and April 2017 (Year 2), and data on the incidence and severity of symptoms on fruit were also collected during these periods. Initial visits to the groves to determine color break within the study area were conducted. These are essential as CBS symptom expression is more clearly seen following color break and progresses as the season advances; although in "hot spot" areas (heavily infected areas of trees within a grove), symptoms can be seen on green fruit.

Plot maps. Initial visits consisted of a walk-through of select rows on the ends and middles of the study area. Once satisfactory color break had occurred physical surveys of Groves I, II and III were completed within one to three visits and plot maps prepared. Plot maps are prepared to ascertain any changes in the tree numbers and layout which may occur due to tree removal or planting of (new) resets.

CBS distribution, incidence, and severity in groves. Over the subsequent visits, the distribution of CBS positive trees was recorded and the position of the tree identified within the plotted area of study. These visits involved a thorough examination of each tree in the study area (Grove I – 2,266 trees, Grove II – 4,572 trees and Grove III – 2,853 trees). This was an intensive part of the study, with each row taking an hour to an hour and a half to complete, resulting in groves being visited a minimum of 6 times initially plus additional follow-up visits to confirm the preliminary distribution finds for each year. From this data the incidence and severity of CBS within the trees was determined in Grove I and II in Years 1 and 2 and in Grove II in Year 1 only. Spatial distribution parameters describing the pattern of distribution were analyzed using the statistical programs R and SAS.

Incidence and severity of CBS on fruit. Once accurate CBS distribution maps for the year were produced, a subset of CBS positive trees were selected using a random number generator and examined to determine the incidence and severity of hard spots on fruit. Two methods were used to determine the incidence and severity of CBS on fruit in Groves II and III in Year 1.

Significant results

(i) The incidence of CBS continues to increase at each study site from year to year despite implementing control regimes.

Table 1. The percent change in trees with fruit expressing CBS within the surveyed area of Grove I and II for the 2015-2016 and 2016-2017 citrus seasons (see word document)

(ii) For Grove I and II, Ripley's K indicated statistically significant clustering above 4.08 m and 6.5 m, respectively, regardless of year. This is within the 6.77 x 3.71 m and 6.70 x 3.36 m tree spacing in each grove, respectively.

Conclusions

(i) Citrus black spot disease is clustered in the groves evaluated in this study in Florida, traveling over short distances to infect citrus fruit (tree to tree).

(ii) The disease progressed under the optimal environmental conditions present in Southwest Florida.

Recommendations

Further research is needed to understand how the inoculum in the grove is spread from tree to tree in order to devise better management systems to reduce further spread within infected groves.

Unusual developments

(i) Climatic conditions during the 2015-2016 season (Year 2) lead to an early harvest in Grove III to avoid fruit drop and increase yields, therefore only a partial severity rating was able to be completed.

(ii) The final year's data was not collected from one of the grove sites due to interference of treatments applied to rows in our project assessment area and the resulting lack of uniformity of treatments to plot area and inability to obtain cultural conditions of the trees.

Objective 2: Relate disease spread within individual groves to their current management practices.

Activities performed

Data corresponding to the spray program from Grove I was obtained for baseline year 2014 and study years 2015 and 2016. These indicated that preventative fungicide sprays for CBS were applied in the Spring and Summer 2014, Summer 2015, and Spring 2016. The chemical applied was pyraclostrobin, a strobilurin.

Information on sprays was not made available for Grove II.

Weather data was collected from the Immokalee weather station, which is closest to all three study sites. Weather data consisted of 15 minute data on soil temperature; air temperature at 0.6, 2, and 10 m; relative humidity (RH) at 2 m (%); dew point at 2 m; rainfall (mm); wind speed at 10 m; and solar radiation.

Estimated periods when conditions for infection were likely in the study groves were generated from this data, along with data obtained from the Flower Bud Induction Overview and Advisory web page and from the Citrus Flowering Monitor model, along with published data on the susceptibility period for fruit and the production and germination of ascospores and conidia. These periods were defined for the fruit and for all susceptible citrus tissues other than fruit.

Significant results

Defining fruit/citrus tissue (twigs, leaves and fruits) susceptibility as days with temperatures between 15 and 30°C and RH ≥ 90% for 8 hr (with fruits, IN2; with or without fruit, IN7) or total daily rainfall ≥0.25 mm (with fruits, IN3; with or without fruit, IN8):

(i) There were greater than 100 days where the weather conditions were conducive for infection of citrus tissue.

(ii) 2013 had the greatest number of days when the fruits were susceptible to infection (IN2=160 days), and conditions were optimal for tissue infection 72% of the year (IN7=261 days).

Figure 1. Weather and Susceptibility Days: Number of days that weather conditions were favorable for infection with Phyllosticta citricarpa, the fungus causing citrus black spot on (A) fruit and (B) citrus tissue of 'Valencia' oranges trees surveyed in Florida (see word document).

Conclusions

(i) Weather conditions for infection must be taken into consideration when deciding on the length of time required for the application of fungicides to prevent and suppress the disease.

Recommendations

(i) Fungicide application year round;

(ii) Or extend the current spray period recommendations to include both local climatic conditions (favorable to conidial release, germination and infectivity) and the susceptibility period for the fruit to significantly reduce the inoculum and disease pressure and significantly slow the spread of the disease while other modes of control are being developed.

Unusual developments

(i) See above.

Table 2. (see word document)

Objective 3: Relate CBS incidence and severity with tree health of mature trees within each grove.

In the literature, CBS symptom development and severity of the disease is greatest in aged, diseased and/or stressed trees. There is no data on the likelihood of disease symptom expression for Florida citrus trees positive for canker and/or HLB at this time.

Activities performed

(i) Age

Grove II. For the 2015-2016 season, trees were categorized into five groups based on approximate age and fruiting status. Tree were classified as indicated: 4 = trees >4 years in the grove, fruiting during the 2013-2014 citrus season at the beginning of the study; 3 = fruiting resets, resets fruiting for the first time in the 2014-2015 citrus season (2-4 years); 2 = Resets, not yet fruiting (1.5 - 2 years); 1 = new resets and newly planted resets (1-1.5 years).

Significant results

(i) Grove II – Tree age and CBS: One hundred and twenty-two (8.3%) of these newly fruiting resets contained fruits positive for CBS in Year 2, with the majority located in areas with the highest disease pressure (the northern portions of the study area).

(ii) Fruiting resets with CBS positive fruit demonstrated significant clustering between 4 and 15 m (nearest-neighbor, F, G and J functions) or between 4 and 35 m (Ripley's K and Besag's Lfunctions)

Conclusions

(i) Young reset fruits are not excluded from disease due to age and the disease is clustered in newly fruiting resets, similar to that in trees that have been fruiting for a number of years.

(ii) Health

Data on canker and HLB (citrus greening) was collected; however, 100% HLB of trees in surveyed sites and low canker incidence led to a change in the approach for assessing tree health and its impact on CBS.

Grove III. During the 2014/15 citrus seasons fruiting trees were classified as either healthy or declining. Citrus tree were defined as declining if they exhibited a combination of the following canopy features, either sectored within the canopy or as a whole – thinning, reduced growth flushes, dieback, reduced fruiting and/or vegetative sprouts on the trunk or on larger interior branches. This data formed a baseline for the 2015/16 citrus season.

Significant results

(i) There was a tendency for diseased trees to have a higher incidence of hard-spot lesions of CBS on fruit.

(ii) Health did not affect the severity to which the fruits were affected.

Conclusions

(i) Once fruit are infected with CBS the severity of the expression is not a health factor.

Recommendation

(i) A study on severity and inoculum numbers is needed. It seems intuitive that if there are a larger number of conidia present the severity of fruit symptoms on healthy and/or diseased trees will be greater than in an environment where the inoculum numbers are lower.

Objective 4: Provide extension program to extend the information to growers including updating recommendations for management of CBS.

Activities performed

Data collected was compiled and analyzed using statistical software and either condensed into manuscripts for publication or incorporated into power point presentations or posters.

(i) Early findings were incorporated into the February 11, 2016, "All You Need to Know About Scouting and Managing Citrus Fungal Diseases" program held at Southwest Florida Research and Education Center in Immokalee, FL. Presentation title, "Citrus black spot and Phytophthora management."

(ii) The spatial distribution findings were published in the peer-reviewed journal Scientific Reports under the title "Spatial and Temporal Patterns of Commercial Citrus Trees Affected by Phyllosticta citricarpa in Florida" in 2017.

Hendricks, K. E., Christman, M., and Roberts, P. D. 2017. Spatial and Temporal Patterns of Commercial Citrus Trees Affected by Phyllosticta citricarpa in Florida. Sci Rep-Uk 7:1641.

(iii) Results on the distribution of CBS in Florida groves were distributed to growers and citrus industry participants in the form of 250 fliers at the 2017 Citrus Expo at the Lee Civic Center in North Fort Myers, FL, on August 15th and 16th.

(iv) Research findings were shared with researchers, FDACS-DPI, USDA-APHIS and Florida growers attending the 2017 Citrus Black Spot Technical Working Group Workshop held on August 17-18, 2017.

(v) Individual consultations with citrus growers

(vi) A second manuscript has been drafted based on the disease incidence and severity of CBS on fruit based on tree health and tree age, and is currently under review by participating authors for submission to a peer-reviewed journal.

Goals and Outcomes Achieved

Goal 1

Generate spatial distribution maps of CBS in studied groves for 2015-2016 and 2016-2017 citrus season; percent change in incidence (trees with fruit expressing CBS) and severity of fruit symptoms on selected trees from one season to the next season.

Measure Produce maps of CBS infected trees in two groves (Grove I – 2,266 trees and Grove II – 4,572 trees), analyze data and identify percent change in trees with fruit expressing CBS from one citrus season to the next. Analyze disease severity in each grove (Grove I, II and Grove III – 2,853 trees) and generate data on changes in severity from one citrus season to the next.

Actual accomplishments: Spatial distribution maps of CBS were generated for Grove I and Grove II for 2015-2016 and for Grove I in the 2016-2017 citrus season. Data was analyzed to identify percent change in trees with fruit expressing CBS from one citrus season to the next in Grove I. Disease severity data was collected and analyzed for Groves II and III during the 2015-2016 citrus season. No data on severity was collected during the 2016-2017 citrus season in Grove II. Severity was so low in Grove I during the 2016-2017 season, a single fruit with a single lesion or historically positive trees having clean fruit (no lesions), that a severity rating for this grove was abandoned.

Table 1. The percent change in trees with fruit expressing CBS within the surveyed area of Grove I and II for the 2015-2016 and 2016-2017 citrus seasons (see word document)

Goal 2

Correlate CBS expression and severity with grower applied fungicide sprays and other cultural management practices, such as leaf litter decomposition, irrigation frequency, and sanitation using information on grove management practices supplied by growers.

Measure Data on applied fungicides and other management practices correlated to CBS control and/or spread.

Actual accomplishments Data from Grove I on spray regime and tree maintenance were collected and reviewed. Data from Grove II was not available.

Goal 3

Correlate CBS expression and severity with tree health. Tree health will be based on general health (healthy vs. declining) and specific disease(s) present (citrus greening and canker). CBS symptoms are

reported to be more severe on declining trees; however, in our evaluations declining trees produce less and smaller fruit, and therefore the disease was not as severe.

Measure Data on tree health with which to correlate CBS control.

Actual accomplishments Baseline data for Florida did not exist on the effect of tree health on CBS in affected groves. Year 1 (citrus season 2015-2016) baseline data was collected for Grove III on the severity of CBS on fruit but Year 2 (citrus season 2016-2017) data was not. The data indicated a tendency for trees classified as declining to be more likely to express CBS symptoms than healthy trees and the severity (percentage of fruits covered with hard spots) was not affected by health. A more extensive study of tree health would give more definitive answers.

Goal 4

Dissemination of research findings to growers including updating recommendations for management of CBS.

Measure Peer reviewed article(s); discussion in trade publications/associated websites and e-newsletters; presentation at growers meetings.

Actual accomplishments Data on the distribution characteristics and the incidence and severity of CBS in Florida groves studied have been published in a peer reviewed journal and a second manuscript is in the final stages of review among authors for submission to a peer reviewed journal. Data from this project has been presented at the growers meeting “All You Need to Know About Scouting and Managing Citrus Fungal Diseases” at SWFREC and at the 2017 Citrus Expo in North Fort Myers, FL. Findings have been presented to researchers, FDACS-DPI, USDA-APHIS and Florida growers attending the 2017 Citrus Black Spot Technical Working Group Workshop at SWFREC.

Beneficiaries

The specialty crop beneficiaries of the project were citrus producers and industry stakeholders who have been affected by citrus black spot and its associated mandated procedures for the quarantine and movement of affected citrus. The project provided much needed information on the spread of CBS within citrus groves in Florida. Research findings point to short distance spread of the disease within the groves, implicating conidial spread. This adds to the body of work by researchers who have found that the only spore found in Florida to date is the conidia, thus this research also had a benefit to researchers investigating ways and means of reducing the spread and managing the disease. Other implications of

short distance movement within groves are that spread across larger geographical areas such as across county lines would require either infected citrus groves spanning county lines or physical transport of infected material into clean groves or clean areas within a grove, not directly adjacent to infected trees. This research does not, however, speak to the implication of infection being spread over wide geographical areas by catastrophic weather events such as Hurricane Irma.

Findings of this study have been published and presented at citrus grower's meetings (approx. 51 persons) and the 2017 Citrus Expo as mentioned previously. Two-hundred and fifty fliers were prepared for the Citrus Expo. Additionally, the findings have been presented to researchers, FDACS-DPI, USDA-APHIS and Florida growers attending the 2017 Citrus Black Spot Technical Working Group Workshop at SWFREC.

Despite the management practices implemented, CBS continued to spread in the groves. This indicates that current management practices are not enough to stop the disease from spreading. This research did not look at the impact of recommended spray regime vs. current grower practices; however, the potential economic impact of the project leans towards an increase in spray applications to control the disease spread and perhaps a year-round fungicide cycle.

Currently, a second manuscript is being reviewed by contributing authors for submission to a peer reviewed journal.

The 2012 Census of agriculture indicates 1,201 operations with bearing acreage of grapefruit, tangerines, tangelos, or temples, which are Florida's primary exported fresh citrus products. Orange producers were not counted among the beneficiaries because over 90% of Florida oranges are processed as juice, and CBS poses a greater threat to fresh exported citrus. All of these 1,201 citrus growers can be expected to benefit from the results of this project.

Lessons Learned

Problems and Delays

In order to enter study groves, certain self-imposed phytosanitary conditions had to be met, such as entering when the tree canopies had dried to reduce the likelihood of spreading disease, which was doubly important when canker had been noted on trees. Secondarily, mornings were chosen to reduce the likelihood of heat stress, although there were days when data collection occurred throughout an 8-hr work day. Weather conditions such as afternoon rain prohibited movement in the grove. Management procedures such as spray applications (herbicides, insecticides or fungicides), mowing, and harvesting activities reduced the opportunities when data could be collected. Additional manpower to collect data would have greatly improved the efficiency and reduced the time spent in the grove.

A handheld GPS unit, while having the ability to give longitude and latitude, was not accurate enough to pinpoint exact trees when tree spacing was within the error limits of the unit. Had the incidence of trees

with CBS been randomly distributed throughout the grove the GPS would have been more useful; analysis for a point of reference was more useful in the analysis of the data.

Changes in canker evaluations and monitoring other diseases (canker and HLB) were due to the reduced incidence and severity of canker and the fact that all trees within the study area of each grove were positive for HLB. The alternative definition of healthy vs. diseased tree was more useful and provided some insight into tree health and CBS, however a larger number of trees needed to be assessed to give a clearer picture. This is also attributed to loss of valuable data from Grove II which was not accessible in Year 2, manpower limitations, and early harvesting due to weather conditions and a need to reduce the potential for yield losses due to fruit drop in Grove III.

Total Funding Expended to Date: $99,710.00

Percentage of Project Completed: 100

Project (7): First Coast Datil Peppers– Unique to St. Augustine

Contact Person

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Project Partners

First Coast Technical College (FCTC) has partnered with their agri-science program and the culinary program to implement the datil project. The agri-science department has been the primary grower and producer of the datil peppers. The culinary program students, who are our future chefs, have had the opportunity of cooking with the datil peppers and they have developed culinary recipes that promote the use of the datil peppers. In addition to this school partnership, FCTC has partnered with neighboring farmers to provide and sell the crop for their use and sale to the community. FCTC has also participated within community activities to include the Farmers Market, Culinary Arts Festival and the FCTC Annual Job Fair. These community events will allow FCTC to sell and showcase the datil peppers to include tasting of the recipes that have featured the datil peppers. Local markets have featured sauces that local chefs have develop from the datil peppers purchased from FCTC. FCTC also have developed relationships with different farmers who reside over 3 counties in Florida including; Bite Me Farms (St Johns County), Revels Pepper Company (Putnam County), Morgan family from Tallahassee, and a few others. The FCTC students volunteered and attended Farmers Markets in both Duval and St Johns County. The education that the students offered to the surrounding counties "spread the word" about the datil pepper specialty crop and the unique uses for the datil pepper. The community interest of the datil pepper, special crop has increased overtime. Local papers have included many articles, books and festivals that showcase food creations utilizing the datil pepper. Representatives of the Agricultural Center and community members presented the historical and cultural significance of the Datil Pepper within St. Johns County. The St. Johns County Board proclaimed the datil pepper as the "Official Plant" of St. Johns County. The St. Johns County Board also proclaimed the first Saturday in October as "Datil Pepper Day". FCTC is now known as the "Datil Pepper School" to include opportunities for students in agriculture to sow the seeds and cultivated this wonderful specialty crop. This also includes the culinary department of the school to specialize in creative foods utilizing the datil peppers. I have attached several articles, recipes and even a book that has been published about the datil pepper, which are all a direct result of the FCTC project initiative.

Project Summary

The datil pepper has a deep rooted history in St. Augustine and is unique to the region. It is a versatile crop that is commercially being developed but also has a tremendous potential to be a sizable agricultural crop for the state of Florida. The Agri-science program at First Coast Technical College (FCTC) has been including datil peppers as part of their curriculum and teaching ptrgram for several years. The limited production of the regular datil pepper and sweet datil peppers have been an ongoing issue here in St.

Augustine. The FCTC agri-science program currently produces a great number than in the past and the demand by local consumers and by businesses in the region has increased. FCTC would like to provide a wider availability of this specialty crop, as the market has a greater demand by chefs everywhere. The culinary program within FCTC operates an on-campus restaurant where the future chefs have incorporated datil peppers grown on campus by the Agri-science program, into their recipes. As datil peppers become more popular outside of the state of Florida, increased production within the state of Florida is important to meet this growing demand. The hot and sweet datil pepper has versatility and that is what makes datils poised to become an up and coming taste sensation, similar to the popularity of chipotle. Florida would profit from positioning itself to be the leading producer of datil peppers.
This projects targets to increase the sale of datil peppers each year. Please see the recent sales within our powerpoint attachment and our measurable goals attachment. Additionally, the project has increased awareness of datil peppers to encourage new growers and consumers. The St. Johns County Board proclaimed the Datil Pepper as the official plant of St. Johns County, and the first Saturday in October as "Datil Pepper Day". Representatives of the Agricultural Center and community members presented the historical and cultural significance of the Datil Pepper in St. Johns County. FCTC is now known as the "Datil Pepper School" to include opportunities for students in agriculture to sow the seeds and cultivated this wonderful specialty crop, which also includes the culinary department of the school. I have attached several articles, recipes and even a book that has been published about the datil pepper, which are all a direct result of this project initiative. I have attached a time-line of the importance of the learning and growing process for the datil project. This time-line also outlines the challenges that have occurred to include Hurricane Matthew, which caused a loss in the crop and also the need to have the datil plant analyzed because of a discoloration of the datil leaf plant. I have outlined how the student learned valuable lessons about these events and the steps the school went through to "save the crop".

.

Project Approach

Timeline of Work Plan:

NOVEMBER 2015

- FCTC students Sowed seeds collected from prior FCTC crop, UF Research Facility, Revels Pepper Company (Wendy Revels) , and “Bite Me Farms” (Joe Hill).
- Students planted approximately 25 to 35 seeds per 4-inch pot in Fafard Germination Mix.
- Twelve flats were planted first week of November
- Seeds were set in greenhouse and watered in.
- Germination began in 14 to 16 days.

DECEMBER 2015

- Transplanted individual seedlings into 4-inch pots.

- Approximately 5000 Plants were planted.
- Weekly fertilizing began with Peters 20-20-20, typically on Fridays.
- Students sowed another 10 flats of datil seeds approximately 25-50 seeds per 4” Pot in Germination mix.

January 2016-

Newspaper article (see attached)

- Students pinched/cut off all Terminal Buds on 4” pots.
- Fertilizing continued weekly.
- Marathon 1%-Granular Systemic insecticide was applied to 4” pots.
- Seedlings planted prior to winter break are planted in 4 “pots.

FEBRUARY 2016

- Sell 50% of plants- Pre-orders of plants from farmers began in February Bite Me Farms (St Johns County), Revels Pepper Company (Putnam County), Morgan family from Tallahassee, and a few others.
- Shift up 50% to 1 gallon pots- Several 4” were transplanted into 1 gallon pots. These will be sold to the public and used for FCTC.
- Make sales at Farmers Markets-Robert and volunteering students attended Farmers markets in Duval and St Johns County.
- Collect Sales Data- $373.00 in plant sales.

MARCH/ APRIL 2016

- Sell 1 gallon pots - Sales of 4” and 1 gallon datil plants is in full swing.
- Bite Me Farms 1000 4”, Revels Pepper Co. 800, Williams 400. Several persons purchased plants.
- Shift 1,000 plants to 15 gallon pots-Six hundred 1 gallon plants were shifted into 15 gallon pots at FCTC Bite Me Farms planted 1000 in 15 gal. Wendy Revels grew hers hydroponically in 3 gallons. Williams said they planted theirs in the ground.
- Plants planted in 1 gallon and 15 gallon pots have a Soil Ph 5.5 - 6.5 with minor's package soil was purchased from C & C Peat Co.
- Holganix company donated soil nourishing root stimulating microbes.
- Advertisement- Students, Marketing Dept., Instructors, and Word of mouth advertised datils.
- Sales Data- March $2584.0 in sales. April sales was approximately $3600.00 in plants. Sales conducted by students.

Administration discontinues FCTC's agriculture programs participation in farmers markets

MAY 2016

- Marketing & Promotion of Datil Peppers- Was not necessary in May. FCTC datil peppers were almost sold out.
- Peppers are fertilized weekly, blooms are prevalent and fruit is beginning.
- Sales data- Approximately $1900.00 of datil pepper plants were sold. These were mainly one and three gallon plants.
- Students and Instructors conducted daily walk thru on peppers checking for insects, i.e. aphids, white flies, and stink bugs.

JUNE 2016

- Harvest- FCTC Students conducted first harvest of datils.
- A few red peppers were noticed in harvest, a discussion with Holganix Geneticist took place.
- Peppers were used in Culinary, seed collection, sold to local bottlers, and shipped to at least six different states.
- Recipes- Began inquiring as to Recipes from Culinary, locals, and companies.
- Sales data- Plant sales at a minimum, approximately $300.00.

JULY 2016

- Harvest- Continues, Peppers going to Culinary, seed, Restaurants, and companies.
- Farmers Market-Has not been reinstated.
- Sales Data- Approximately $1500.00 in sales
- Monitor Recipes - Datil Dave - datildave.com, developed several recipes.
- Seminar on Datil Pepper and The Project- Was conducted with students.
- “Heat Stall” was reported by student.

AUGUST 2016

- Harvest continues
- Farmers Market-Interim administration set up at FCTC via St Johns County School Board, Farmers Market was discussed for 2017.
- Sales Data-$335.00 were received in datil sales.
- Jelly/Sauces (FCTC Culinary)-Datil Jellies were sold in FCTC book store. Cookies were also made.
- Sales Data (FCTC Culinary)
- Marketing & Promotional Activities-Limited to businesses and Farmers concerned with FCTC datil pepper production under interim administration.
- Heat Stall continues production harvesting is limited.

SEPTEMBER 2016

- Harvest- Peppers harvested mainly for seed production. Summer has been very hot and production was very low “Heat Stall” was reported.
- Sales Data-$88.00 in peppers were sold.
- Recipes
- Collect seeds for next cycle
- Several calls from Bottlers, sauce makers, Restaurant's, and Individuals. We notice a severe shortage in datil peppers.

OCTOBER 2016

- Harvest- Was limited to seed only, Hurricane Matthew caused damage to crop, and green house.
- Students- Lectured in datil pepper production, the lack of peppers, business and industry shortages.
- Datil Pepper farmers and persons interested in growing datils do several visits to discuss procedures in growing, soil mixes, fertilizers, irrigation and Insect control. Dehydrated datil peppers that are ground into a fine aromatic and spicy dust. Add to salsa's, sauces, chili. This concentrated spice will heat it up instantly.

NOVEMBER / DECEMBER 2016

- Several Thousand Datil seeds were planted week before Thanksgiving.
- As anticipated several persons interested in pepper plants.
- Germination and moving up to 4" pots began first week of December.
- Several thousand more datil seeds planted second week of December.
- Farmers begin orders approximately 18,000 plants are wanted.
- Students are excited by the number of visitors wanting datil.
- Discussions/Lectures on Germination, watering, Fertilizing, disease, insects, stepping up, temperature (highs & lows), pricing, business, commodities, and products

JANUARY 2017

- Datils are moved into 4" pots
- 2016 shortage of peppers has encouraged larger production of Datils
- Chris Barnes-Barnes Farms is requesting up to 18,000 plants
- "Wanda" Cultivar seeds are gathered
- Revels Pepper Company donates more seeds

FEBURARY 2017

- Orders for datil plants from growers increases.
- Revels Peppers Co. - 1400 plants
- Students reported odd markings on leaves of datils.
- New Administration reinstates Farmers Markets

MARCH 2017

- Called in Bonnie Wells (UF and IFAS) to discuss leaf issue.
- Gathered samples of leaves and photo's and sent to University of Florida- Plant Diagnostic Center
- Report came back negative for: Fungus, Bacteria, and Virus.
- Plant Specimen Diagnostic Report # 2017-287
- Growers and Students discuss hypothesis as to how leaf tissue was damaged.
- Robert & Adult students attend River Side Arts Market, a few datil plants sold.

GROWERS CONTINUE 2017

- Jarrett Manucy, 19 picks up 400 1 gallon plants orders another 140. First time grower of Datil peppers he has arrangements for 1000 pounds of fruit.

- Chris Barnes- Who had stopped growing datils a couple years ago has ordered approximately 18,000 - 4" datils. For field growing.
- Chris Barnes also purchased 1000 1 gallon to grow hydroponically in his greenhouse and he is the largest commercial grower of datil peppers.

Goals and Outcomes Achieved

Goal 1: Increase demand for sweet and regular datil peppers to growers, consumers, and food industry

Progress toward goal: Sales of plants doubled, from $3,000 in 2013-14 to over $6,000 during the project year.

Goal 2: Increase popularity of regular and sweet datil peppers in food products and recipes

Progress toward goal: 6 additional recipes were developed (included in attachment), representing a 24% increase, only 1 percentage point short of the goal stated in the proposal.

Goal 3: Promote awareness of the datil pepper and the vendors who sell datil peppers and value added datil pepper products.

Progress toward goal: 4 growers made sales at the FCTC market and were featured in the project's marketing materials (see attachment).

This project has provided these students with a true entrepreneurial experience, which includes growing datil peppers to marketing and selling the specialty crop to the public and businesses. The production cycle of datil peppers falls within the completion of agri-science program hour requirement. Students have had the experience of the entire process of sowing the seeds, growing and producing the datil pepper product. They have also had the opportunity to experience a natural disaster of Hurricane Matthew, and learn first hand how a crop disease may effect the production of the final product. The students learned the process for sending a possible disease plant to be analyzed by the UF Plant Diagnostic Center and the findings of the plant analysis is attached within the Power-point. The students have also learned about the plant from an academic aspect which will prepare them to not only grow datils, but will also learn how to promote the crop, from a specialty crop within the region they live in. The work has been completed by the students and instructors from the preparation of the land for the plants to the picking and selling of the plants and fruit. The culinary students have also benefited from this project by having the opportunity to include the datil specialty crop into their recipes for the Reef Restaurant and the recipes for community fairs ect. The agri- science department has also had the opportunity to work with FCTC marketing department to include datil peppers in the marketing and promotional activities. I have attached the articles and newspaper features that FCTC marketing have facilitated. The agri-science department have hosted educational booths at which time they disseminated information about datil peppers and its growing popularity, including its history and how it could be the next big crop for Florida. Please see the articles attached and also the other community activities that promoted and showcased the agri-science

student and culinary students.

Datil peppers are certainly growing in popularity as you can see from the orders placed and the attention to developing sauces, creating delicious recipes and the increased press about this speciality crop. The local farmers and datil peppers growers have increased their request for datil peppers from FCTC. Similarly, chefs and other community members within the food and beverage industry have developed sauces and recipes that include the datil pepper. Please see the attached recipes and the latest book about datil peppers entitled; Chasing Chiles; Hot Spots Along the Pepper Trail, by Gary Nabhan, Kraig Kraft, and Kurt Friese.

Beneficiaries

The impact of growing the datil peppers had several positive outcomes. First and foremost, the growing of the datil pepper impacted directly to the enriched learning experiences that our agri-science students received. This hands on learning experience provided them the opportunity of growing to the final stages of selling the product. The hands on experience of sowing, replanting to extend the plant and then the actual sale of the crop is a "real-life" experience of being an entrepreneur. The students from both agri- science and culinary arts benefited directly to this specialty crop. As I said, the agri-science students hands on education and training with specialty crop, specifically growing datil peppers include also operating a supply chain, marketing products for wider use of the product for sustainable growth of datil peppers. This entrepreneurs experience for our students provides them the understanding of making the necessary purchases to sow the seeds and bring the product to the market. The instructors included the students in every aspect of purchasing and the financial decisions in what they needed to buy and how to determine the selling of the datil pepper. The students also participated within the community Farmers Market, Job Fair and meetings with local farmers interested in purchasing the datil peppers. In addition, the students learned a great lesson in what may happen to a crop, as we had with the discovery of discoloration on the leaves of the datil peppers. The students again learned the valuable experience of having to send the plant leaves out to be analyze at the University of Florida Plant Diagnostic Center. We were extremely fortunate that their was not any virus or bacteria found in the plants. During the waiting process, the students learned the possibility of crop disease. Again, the instructors included this learning experience into the curriculum for teaching and learning opportunities for the students. The impact on the local consumers who frequent our "Hidden Nursery" on a monthly basis, also received the benefit of learning new knowledge of growing datil peppers. Local Farmers benefited directly from the datil pepper crop and the farmer can sell the crop for $7.00 to $10.00 a pound. Pre-orders of plants from farmers began in February; Bite Me Farms (St Johns County), Revels Pepper Company (Putnam County), Morgan family from Tallahassee, and a few others. Recent sales include March sales-

The culinary students at FCTC also had a direct benefit from the datil pepper crop that the agri-science students grew.
The "chefs to be" introduced datil peppers specialty to many recipes. Please see attached recipes, which include datil pepper ice cream, datil pepper jelly, datil pepper shortbread cookies, datil pepper mullet dip, sumer sausage, and datil pepper creole gumbo. The students made these fantastic recipes for cooking competitions, Job Fair and weekly recipes in the Reef Restaurant, the on campus restaurant within the culinary program. The students also attended the St. Johns Datil Pepper Festival which celebrates the datil pepper and its growing in St. Augustine Florida.

In total, the project benefitted 6 new growers of datil peppers, dozens of students who learned about the commodity, and several hundred consumers who were introduced to the datil pepper.

Timeline of Project- February 2016 - Sell 50% of plants- Pre-orders of plants from farmers began in February Bite Me Farms (St Johns County), Revels Pepper Company (Putnam County), Morgan family from Tallahassee, and a few others.

- Shift up 50% to 1 gallon pots- Several 4” were transplanted into 1 gallon pots. These will be sold

to the public and used for FCTC.

- Make sales at Farmers Markets-Robert and volunteering students attended Farmers markets in Duval and St Johns County.
- Collect Sales Data- $373.00 in plant sales.

March April 2016- Sell 1 gallon pots - Sales of 4" and 1 gallon datil plants is in full swing.

- Bite Me Farms 1000 4", Revels Pepper Co. 800, Williams 400. Several persons purchased plants.
- Shift 1,000 plants to 15 gallon pots-Six hundred 1 gallon plants were shifted into 15 gallon pots at FCTC Bite Me Farms planted 1000 in 15 gal. Wendy Revels grew hers hydroponically in 3 gallons. Williams said they planted theirs in the ground.
- Plants planted in 1 gallon and 15 gallon pots have a Soil Ph 5.5 - 6.5 with minor's package soil was purchased from C & C Peat Co.
- Holganix company donated soil nourishing root stimulating microbes.
- Advertisement- Students, Marketing Dept., Instructors, and Word of mouth advertised datils.
- Sales Data- March $2584.0 in sales. April sales was approximately $3600.00 in plants. Sales conducted by students.

May 2016- Marketing & Promotion of Datil Peppers- Was not necessary in May. FCTC datil peppers were almost sold out.

- Peppers are fertilized weekly, blooms are prevalent and fruit is beginning.
- Sales data- Approximately $1900.00 of datil pepper plants were sold. These were mainly one and three gallon plants.
- Students and Instructors conducted daily walk thru on peppers checking for insects, i.e. aphids, white flies, and stink bugs.

June 2016-Harvest- FCTC Students conducted first harvest of datils.

- A few red peppers were noticed in harvest, a discussion with Holganix Geneticist took place.
- Peppers were used in Culinary, seed collection, sold to local bottlers, and shipped to at least six different states.
- Recipes- Began inquiring as to Recipes from Culinary, locals, and companies.
- Sales data- Plant sales at a minimum, approximately $300.00.

July 2016- SJCSD takes over FCTC- Please note for funding purposes especially note the funds receivable from sales moving forward. Harvest- Continues, Peppers going to Culinary, seed, Restaurants, and companies.

- Farmers Market-Has not been reinstated.
- Sales Data- Approximately $1500.00 in sales
- Monitor Recipes - Datil Dave - datildave.com, developed several recipes.
- Seminar on Datil Pepper and The Project- Was conducted with students.
- "Heat Stall" was reported by student.

August 2016-Harvest continues

- Farmers Market-Interim administration set up at FCTC via St Johns County School Board, Farmers Market was discussed for 2017.
- Sales Data-$335.00 were received in datil sales.
- Jelly/Sauces (FCTC Culinary)-Datil Jellies were sold in FCTC book store. Cookies were also made.
- Sales Data (FCTC Culinary)
- Marketing & Promotional Activities-Limited to businesses and Farmers concerned with FCTC datil pepper production under interim administration.
- Heat Stall continues production harvesting is limited.

September 2016-Harvest- Peppers harvested mainly for seed production. Summer has been very hot and production was very low "Heat Stall" was reported.

- Sales Data-$88.00 in peppers were sold.
- Recipes

- Collect seeds for next cycle

Sales requests begin to come in over the next 3 months-
October 2016-Harvest- Was limited to seed only, Hurricane Matthew caused damage to crop, and green house.
November and December 2016 we needed to make up on the product lost due to Hurricane Matthew and sales requests were still coming in.

- Several Thousand Datil seeds were planted week before Thanksgiving.
- As anticipated several persons interested in pepper plants.
- Germination and moving up to 4" pots began first week of December.
- Several thousand more datil seeds planted second week of December.
- Farmers begin orders approximately 18,000 plants are wanted.
- Students are excited by the number of visitors wanting datil.
- Discussions/Lectures on Germination, watering, Fertilizing, disease, insects, stepping up, temperature (highs & lows), pricing, business, commodities, and products

January 2017-Datils are moved into 4" pots

- 2016 shortage of peppers has encouraged larger production of Datils
- Chris Barnes-Barnes Farms is requesting up to 18,000 plants
- "Wanda" Cultivar seeds are gathered
- Revels Pepper Company donates more seeds

February 2017-Orders for datil plants from growers increases.

- Revels Peppers Co. - 1400 plants
- Students reported odd markings on leaves of datils.
- New Administration reinstates Farmers Markets

March 2017- Brown disease noted on the crop, need to send out for analysis• Called in Bonnie Wells (UF and IFAS) to discuss leaf issue.

- Gathered samples of leaves and photo's and sent to University of Florida- Plant Diagnostic Center
- Report came back negative for: Fungus, Bacteria, and Virus.
- Plant Specimen Diagnostic Report # 2017-287
- Growers and Students discuss hypothesis as to how leaf tissue was damaged.
- Robert & Adult students attend River Side Arts Market, a few datil plants sold.

Lessons Learned

Unexpected problems and delays are noted to include the sales of the datil peppers happening prior to St. Johns School District taking over FCTC. Prior to the July 1 2016 date sales totaled

February 2016- Collect Sales Data- $373.00 in plant sales.

March 2016 Sales Data-$2584.0 in sales. April sales was approximately $3600.00 in plants. Sales conducted by students.

May 2016- Sales data- Approximately $1900.00 of datil pepper plants were sold. These were mainly one and three gallon plants.
June 2016- Sales data- Plant sales at a minimum, approximately $300.00.
Total sales prior to St. Johns County taking over FCTC= $ 5158.00

After St. Johns County Schools takes over FCTC. July 2016 -Sales Data- Approximately $1500.00 in sales
August 2016- Sales Data-$88.00 in peppers were sold.

September 2016 - Sales Data-$335.00 were received in datil sales.

More unexpected challenges- Hurricane Matthew October 2016-Harvest- Was limited to seed only, Hurricane Matthew caused damage to crop, and green house. The students learned the valuable experience of having to send the plant leaves out to be analyze at the University of Florida Plant Diagnostic Center. We were extremely fortunate that their was not any virus or bacteria found in the plants. During the waiting process, the students learned the possibility of crop disease. Again, the instructors included this learning experience into the curriculum for teaching and learning opportunities for the students.

November 2016- Due to Hurricane Matthew we had additional costs to plant additional seeds to attempt to fill the needs for orders from farmers.

Several Thousand Datil seeds were planted week before Thanksgiving.

- As anticipated several persons interested in pepper plants.
- Germination and moving up to 4" pots began first week of December.

- Several thousand more datil seeds planted second week of December.
- Farmers begin orders approximately 18,000 plants are wanted.
- Students are excited by the number of visitors wanting datil.
- Discussions/Lectures on Germination, watering, Fertilizing, disease, insects, stepping up, temperature (highs & lows), pricing, business, commodities, and products

January 2017 Due to Hurricane Matthew there is a shortage of peppers which has encouraged larger production of Datils

- Chris Barnes-Barnes Farms is requesting up to 18,000 plants
- "Wanda" Cultivar seeds are gathered
- Revels Pepper Company donates more seeds

FEBURARY 2017 - Orders for datil plants from growers increases.

- Revels Peppers Co. - 1400 plants
- Students reported odd markings on leaves of datils.
- New Administration reinstates Farmers Markets

March 2017

3/12/17, Joe Hill lost 800 plants.

- Joe Purchased 1800 4" plants week of 3/22/17.
- Jarett Manucy purchased 500-1 gallon and 150 4".
- Wendy Revels plants are in green house doing very well.
- Chris Barnes continues to pick up plants, Currently 1000- 1 gallon and 5767- 4". Greenhouse has 7500- 4" plants which he will pick up next week.
- Dollars generated by sales is $9700.00 from these four growers.
- Adult students 3/29/17

Overall Progress and Funding Expended

Project was approximately 50% complete at the time of cancellation, and $8,791.08 was expended.

Program income totaled approximately $6,658.00, which amount was used to further the goals of the program through maintaining plants and producing additional planting stick for interested growers.

Florida Specialty Crop Block Grant Program
Project Final Performance Report

Note: Most sections of the final progress report relate directly to a corresponding section of the original project proposal or annual report. Please have these documents handy while writing the final report, so that you may refer to them where necessary. Some final report sections which relate to sections of other documents are:

Final Performance Report Section	Corresponding Section of Proposal (P) or Annual Report (AR)
Project Summary	Project Purpose (P)
Project Approach	Work Plan (P), Activities Performed (AR)
Goals and Outcomes Achieved	Expected Measureable Outcomes (P)
Beneficiaries	Potential Impact (P)
Lessons Learned	Problems and Delays (AR)

Project Title: Integrated Pest and Disease Management for Sustainable Florida Fresh Citrus Production Systems

Project Partners: The primary organization implementing the project is the University of Florida CREC/IFAS. Project partners include various commercial citrus growers and packing houses, including KLM Farms, Southern Fulfillment, Circle H Citrus, Dundee Citrus Growers Association, Precision Citrus, LLC.

Project Summary:

- ☐ Please refer to the "Project Purpose" section of your proposal when writing this section.
- ☐ Provide a background for the initial purpose of the project, which includes the specific issue, problem, or need that was addressed by this project.
- ☐ Describe the importance and timeliness of the project.
- ☐ If the project built on a previously funded project with the SCBGP or SCBGP-FB describe how this project complemented and enhanced, but did not duplicate, previous work.

- **What is the specific issue, problem, or need that the project will address?**

Endemic incurable Huanglongbing disease (HLB, "greening") in Florida makes it virtually impossible to profitably grow citrus. HLB is associated with the bacterium, *Candidatus* Liberibacter asiaticus (*C*Las) and is vectored by the Asian citrus psyllid (ACP, *Diaphorina citri*). The disease can lead to general canopy and root decline, asymmetric chlorosis of leaves, and fruit that is discolored, misshapen, drops off prematurely, and is otherwise not fit for sale. This is a particularly dire situation for Florida's fresh citrus fruit growers because fresh fruit products must maintain cosmetically appealing aesthetics throughout the entire production cycle. In the absence of other solutions for HLB control, some Florida fresh citrus growers are turning to screen house protected agriculture in partnership with UF/IFAS researchers, in order to physically exclude the psyllid insect vector and thus ensure long-term disease-free citrus trees. This project addresses the use of and development of sustainable fresh citrus growing systems based primarily on production blocks grown under large protective screen houses using novel integrated pest and disease management technologies combined with accelerated production techniques using high planting densities, selective pruning, and hydroponics.

- **Why is the project important and timely?**

Time is running out for Florida citrus because no long-term solutions for HLB control have been found or implemented yet, nearly all mature groves are affected, and statewide annual yields have dropped to historic lows of <50 million boxes. HLB was discovered in south Florida ten years ago, and in excess of $150 million have been allocated during that time to develop solutions. None are available yet. Florida's problems are echoed in the much larger Brazilian and Chinese citrus industries, which according to recent (2014-2015) news reports have recorded unprecedented citrus yield losses due to long-term endemic HLB disease. Other citrus producing states in the USA such as Texas and California are at risk of suffering a similar fate.

Since the discovery of "greening" disease in Florida, attempts to limit the spread of HLB have been via the extensive use of insecticides aimed at reducing the abundance and activity of the ACP vector. Unfortunately, this widespread, and almost exclusive, use of synthetic insecticides has led to the development of insecticide tolerant populations of ACP throughout the state. In order to preserve the utility of insecticide chemistries that limit the spread of HLB by controlling the ACP vector, integrated disease and pest management programs must be developed and are sorely needed by the fresh citrus fruit industry in Florida. This proposal seeks to promote novel tree production systems which double as truly integrated disease and pest management programs as well.

In 2013-2014 the PIs first began exploring the prospects of growing fresh citrus profitably under protective screen houses, and five grower groups also prepared to begin their own commercial covered production systems. The high cost of the screen house structure is the major concern of growers wanting to use this otherwise attractive off-the-shelf production system that is "shovel-ready". In this proposal we will continue to demonstrate the use of long term production systems under large protective screen houses but also will develop an alternative, lower cost method of growing trees without a screen house, but with the ability to eliminate the *C*Las bacterium from infected trees through whole-tree thermotherapy (WTT). WTT is made possible by growing the trees in suitable containers for the duration of the grove production cycle. Infected trees are moved to a steam-heated chamber for 48 hours, which eliminates the CLas from both roots and canopies on an as-needed basis. Additionally, this project seeks to investigate the efficacy of introducing bumblebees to the screen house to rapidly and significantly increase flower pollination and fruit yields, thus shortening the time to economic break-even point. Lastly, this proposal will determine the optimal and most environmentally responsible program of insecticide applications to control the ACP and manage HLB development within these novel tree cultivation systems.

- **If the project is a continuation of a project that the Specialty Crop Block Grant Program (SCBGP) funded previously, describe how the project differs from and builds on the previous project's efforts.**

The proposed SCBGP project is a continuation of a SCBGP project (contract 00092195) funded from 1 January 2014 to 31 December 2015. The title of the project is "Citrus Undercover Production Systems (CUPS): Keeping High-Value FL Varieties Competitive in an Era of Endemic Diseases", and focuses on off-the-shelf screen house technology to provide an insect-free environment for HLB-free fresh citrus cultivation enhanced by modern high density planting and hydroponics. Project 92195 has already generated new scientific data since January 2014, and interest by growers to adopt the CUPS growing system is growing rapidly. We hosted a grower's field day at the UF/IRREC site on November 5, 2014. This new project aims to build on our recently developed CUPS technology and experiences <u>by</u>

additionally developing and testing a less costly whole-tree thermotherapy (WTT) method at the UF-CREC site for controlling HLB in fresh citrus without screen houses.

Project Approach: (*Please also refer to the photos and figures attached to the end of this report*)

- ☐ Please refer to the "Work Plan" section of the proposal and the "Activities Performed" sections of your annual reports when writing this section.
- ☐ Briefly summarize activities performed and tasks performed during the grant period. Whenever possible, describe the work accomplished in both quantitative and qualitative terms. Specifically, discuss the tasks provided in the "Work Plan" section of the proposal. Include the significant results, accomplishments, conclusions and recommendations. Include favorable or unusual developments.
- ☐ If the overall scope of the project benefitted commodities other than specialty crops, indicate how project staff ensured that funds were used to solely enhance the competitiveness of specialty crops.
- ☐ Present the significant contributions and role of project partners in the project.

Focus Area 1: Disease Mitigation and Integrated Pest Management (IPM)

First Activity: ACP prevalence monitoring/HLB diagnoses; Where: CREC project site; When: semi-weekly (ACP prevalence **via sticky traps and visual inspection of citrus flush**) and annually (HLB diagnoses **via visual inspection for foliar symptoms, qPCR analyses of extracted DNA**); Who: All program staff; Date Accomplished: November 2017

Second Activity: WTT of HLB-positive trees; Where: CREC project site; When: WTT applications annually, each May and September (2016 and 2017) **via custom-built WTT mobile unit**; Who: AS; Date Accomplished: September 2016

Third Activity: Evaluation of chemigation-applied systemic insecticides: Where: CREC project site; When: Dosing trials of neonicotinoids every three to six weeks **via liquid injections of neonicotinoids to plants and subsequent LC/MS/MS analysis of leaf tissue for target parent compounds and metabolites;** Who: MR, AS: Date Accomplished: January 2017

Significant results, accomplishments, conclusions, and recommendations:

The screen house – grown citrus remained ACP-free for every weekly inspection during 2015 and 2016, except for a single psyllid that was found on a yellow sticky trap near the main service entrance. Consequently there were no detectable cases of HLB disease in the screen house, both by visual and PCR identification, after 3.5 years (August 2014 to December 2017). In major contrast, the outdoor-grown control trees were frequently infested with ACP, especially during the leaf-flushing periods. At most times of the year we were able to spray the outdoor trees with insecticide to kill ACP and manage the infestations, but it was impossible to achieve adequate ACP control during the prolonged bloom periods when insecticides were forbidden by label restrictions that were designed to protect bees. As a result, in mid to late 2016 a very high number of new HLB cases were identified in the outdoor block (as a result of infections during the spring 2016 bloom period). By beginning 2017, the HLB incidence in the outdoor block exceeded 50%, most fruit on the trees was severely compromised and fruit drop was excessive (>70%). During spring 2016 we successfully completed whole-tree thermotherapy on the few outdoor trees that were HLB-positive at the time. Most trees survived the 48-hour heat treatment at 42°C, and were healthy, symptom-free and with increasing PCR Ct values four months later. We moved

a few treated trees into the CUPS screen house for long-term observationin the absence of ACP and to date they have remained HLB-free for more than two years, with undetectable Ct by PCR testing. Unfortunately the outdoor trees rapidly became reinfected due to the high numbers of ACP, and by spring 2017 we discontinued the WTT experiments in the outdoor block because it was impossible to keep up with new HLB disease cases. We had hoped to deter ACP from actively feeding on the outdoor trees by frequently dosing the trees by chemigation with low split doses of imidacloprid. The split doses were calculated to total the annual labeled limit of imidacloprid per tree. Despite the verified existence of adequate imidacloprid residues in the leaf tissues by frequent sampling and LC-MS measurement, the psyllid numbers on outdoor trees remained high, especially in summers of 2016 and 2017. We discontinued the treatment of outdoor trees with imidacloprid in 2017 due to the risk of developing insecticide resistance in local ACP populations. *In conclusion, we can safely say that insecticide-based management of ACP for prevention of HLB is not feasible in Florida, and that the recommended method for growing HLB-free fresh fruit of high quality and value is under protective screen (CUPS).*

Focus Area 2: Horticultural Practices

First Activity: Establish and maintain insect pollination treatments with bumblebee hives; Where: CREC project site, inside the CUPS protective screen house structure; When: February to April, 2016 & 2017; Who: AS; Date Accomplished: April-May, 2016

Second Activity: Tree performance measurements, including WTT and pruning experiments **via canopy volume calculation, tree relative growth rate, leaf mineral nutrient quantification, and fruit set assessments**; Where: CREC project site; When: Quarterly for life of project, and each July for fruit set; Who: AS; Date Accomplished: November, 2017

Third Activity: Fruit harvest (**estimates kilograms of fruit per tree for replicated trial plots, and bulk fruit measurement in boxes for commercial harvest**); Where: CREC project site; When: January-February 2016 & 2017; Who: AS; Date Accomplished, November 2017

Significant results, accomplishments, conclusions, and recommendations:

We tested the performance of a large "class A" bumblebee hive inside the CUPS screen house during the main bloom period of 2016. The hive was placed in a cool location on the north end of the screen house, following all provided instructions. When there was a need to spray insecticides, such as during an outbreak of flower thrips, the hive was closed and removed from the screen house for protection of the bumblebees inside. After adequate ventilation and re-entry period had elapsed for the pesticides, the hive could be returned and opened. We visually observed any activities of the bumblebees around the hive and their forays into the trees inside the screen house. A wireless video camera with motion detection was also installed to more closely monitor the movements of the bumblebees around the hive. Our observations from this preliminary test indicated a relatively low activity of bumblebees in the citrus blooms of the CUPS, and suggested a low contribution to any pollination of citrus blooms. Despite these results, the 2016/2017 citrus fruit set and harvest was impressive, and confirms that most citrus varieties are successfully self-pollinated and perform very well in the total absence of pollinators. This was additionally confirmed in 2017, when we did not use any introduced insect pollinators during the spring bloom period, and fruit set was still excellent. The trees both inside the CUPS screen house and in the outdoor block were hedged immediately following the harvest in March 2017. There were large measurable differences in tree canopy growth rate between the screen house and outdoor trees, where the warmer, disease-free environment of the CUPS accelerated and sustained better tree growth than

outside. This was confirmed by the higher fruit yields picked from the trees in the screen house than from outside. The 'Honey' murcott average yield in the screen house was 680 boxes/acre at 2.5 years age. Packout in the CUPS was 99%, compared with 71% in the outdoor block, according to the commercial packing house. In 2017/18, at 3.5 years age, the average grapefruit yield inside the CUPS was 679 boxes/acre, and packout was 100%. The price per box was very favorable at $24, thanks to 60% of the fruit size falling in the premium 40-36-32 size classes that fetch up to $36 per box. In 2017/18, the outdoor grapefruit yield was only 80 boxes/acre (12% of the CUPS yield), and the size quality was so poor there was hardly anything to pack. *In conclusion, the rapid degeneration of the outdoor block in less than four years can be entirely attributed to the high incidence of HLB, while the protected CUPS trees in the screen house continued to produce increasing fruit yields of high quality due to the elimination of ACPS and prevention of HLB. We recommend that commercial growers proceed to cautiously adopt CUPS technology as the only known "cure" solution for preventing HLB in fresh fruit at this time.*

Focus Area 3: Financial and Outreach

First Activity: Economic analyses of protected screen house production and WTT production scenarios **via constructing a 10-year net present value model using production costs and harvested fruit values**; Where: CREC project site; Who: AS; When: Annually, December 2016 & November 2017

Second Activity: Develop Extension Educational Programming for Stakeholders; What: Live seminars and grower field days **implementing polling participants to determine changes in knowledge and behavior**, writing articles in trade periodicals, and articles for peer-reviewed journals; Where: CREC project site; When: At least one live seminar/field day per year (October-December, 2016 & 2017) at CREC, and one trade periodical article per year (November 2016 & 2017); Who: All program staff; Date Accomplished, November 2017

Significant results, accomplishments, conclusions, and recommendations:

Due to the relatively short duration of the CUPS research so far (3.5 years), and the lack of any prior experience with commercial growing of fresh citrus under screen, we could not conduct a comprehensive long-term economic analysis using NPV of the CUPS at this time. Instead, we reported "economic indicators" in the form of the high fruit yields of excellent quality, packout and high price (see above discussion and figures at end of report) so far as proof of concept for the CUPS to become a highly profitable citrus enterprise. We organized and participated in frequent extension educational programming activities for stakeholders during 2016-17. A well-attended field day was held at the CREC in December 2016, but the event was canceled in 2017 due to hurricane recovery activities. An extension information article about CUPS was published: http://edis.ifas.ufl.edu/pdffiles/HS/HS130400.pdf, and two articles on CUPS were published in the Citrus Industry magazine during 2016-17. In addition, presentations on CUPS were made to growers at the annual Citrus Expo, and Citrus Shows, as well as the Citrus Growers Institute meetings and smaller grower seminars at CREC and SWFREC. See 2016 field day program evaluation sheet at the end of this report. Two refereed journal articles were published in Horttechnology during 2017.

Significant contribution and role of project partners: The momentum gained by the CUPS research project would not have been possible without the enthusiastic participation of the project partners from the citrus industry. In particular, KLM Farms established 20 acres of their own CUPS in 2017 and are

currently constructing 30 more acres of screen houses. Ed Pines (Precision Citrus: http://www.precisioncitrus.com/) and Steve Callaham, Lindsey Raley and others from Dundee Citrus Growers Association have embraced the CUPS method by constructing more than 100 acres in 2017/18 (http://dun-d.com/citrus-under-protective-screen/) in Polk County. We hosted dozens of visits to our CREC CUPS by interested growers in 2016-17, and we reciprocated by field visits to grower sites. We established two weather stations and web sites to continuously monitor the climate in CUPS structures for commercial growers (example: http://166.130.8.210:4252/weewx/index.html). These site-specific data stations also feature accurate soil moisture sensors that assist the grower to improve their irrigation scheduling.

Goals and Outcomes Achieved:

- ☐ Please refer to the "Expected Measureable Outcomes" section of your proposal. List each expected measureable outcome from that section, and explain what progress you have made toward achieving each one.
- ☐ If outcome measures were long term, summarize the progress that has been made towards achievement. Every expected measureable outcome listed in your original project proposal must be addressed in this section.
- ☐ Provide a comparison of actual accomplishments with the goals established for the project.
- ☐ Clearly convey completion of achieving outcomes by illustrating baseline data that has been gathered to date and showing the progress toward achieving set targets.
- ☐ Highlight the major successes of the project in quantifiable terms.

Goal 1: Disseminate project progress and results to interested stakeholders in order to increase awareness regarding the CUPS covered production and whole-tree thermotherapy approaches.

Performance Measure 1: Performance measures include polling stakeholders who have been exposed to extension and outreach efforts relating to this program in order to determine their relative knowledge and behavior change(s). Additional performance measures could include UF-IFAS EDIS document downloads, CUPS web page hits, and the total number of stakeholders/acreage.

Benchmark 1: Because the CUPS approach for citrus is a new and emerging technology it will be assumed that stakeholder knowledge base will be zero percent. Thus, any positive increase in percentage knowledge and/or behavior change will be considered successful.

Target 1: Increase stakeholder knowledge base about these novel citrus cultivation practices by 25%. Also, ~1,000 stakeholders will physically attend industry seminars and field days at Lake Alfred. Trade magazine publications will also reach, conservatively, hundreds of stakeholders.

Progress made for goal 1: Please refer to the previous discussions above, of the successful outcomes of this project for disseminating project progress and results to interested stakeholders. Also refer to the field day evaluation form copied to the end of this report which shows the actual performance measures, benchmark and target metrics discussed in the proposal . 97% of respondents replied "YES" to the question: "2. Have you gained any knowledge or new information from this program?" 66% responded "YES" to "4. Will you change or improve your management practices because of the

information gained from this program?". We strive to teach the CUPS principles by example, and it appears that growers are learning rapidly from that approach.

Goal 2: The second goal is to establish and maintain a 0% Asian citrus psyllid (ACP) prevalence and 0.0% HLB incidence within the protective environments of the CUPS screen structure for the lifetime of the project.

Performance Measure 2: ACP prevalence will be monitored monthly-to-quarterly using insect counts in sticky trap cards and inspection of young leaf flush for adult, nymph and egg stages of ACP. HLB will be diagnosed using scouting for visual symptoms, followed by real-time polymerase chain reaction (PCR). Results expressed as percentage HLB incidence.

Benchmark 2: ACP prevalence in the standard outdoor grown trees is dynamic, according to seasons (10-100%), but is expected to average 1-20% over each year, as a result of the elevated ACP prevalence, despite pesticide use.

Target 2: The target for this goal is 0.0%-0.1% ACP incidence within the protective ACP screen structure annually.

Progress made for goal 2: Please refer again to the previous complete discussions (above) of ACP and HLB incidence in the CUPS screen house relative to the outdoor control block. We made excellent progress with this second goal since the outcome at 3.5 years is still 0% HLB and 0% ACP inside the CUPS structure.

Goal 3: Evaluate the effects of pruning treatments and develop water use consumption models for container-grown fruits

Progress toward goal 3: Carefully timed canopy management (pruning, hedging, topping), irrigation and fertilizer management, and crop thinning were found to reduce adverse alternate bearing, which presents a financial risk for growers.

Goal 4: Alternative IPM for outdoor, container-grown citrus trees using soil-drench and foliage-spray pesticides for psyllid control and a mobile steam chamber to achieve whole-tree thermotherapy for HLB elimination of trees that acquire the bacterial pathogen.

Progress toward goal 4: While indoor trees remained 100% HLB-free**, the outdoor control trees are nearly 100% HLB+, despite frequent pesticide spraying and soil drenching with systemic neonicotinoid insecticides. The IPM regimen tested was not effective at preventing infection.**

Major successes of the project: The CUPS research and extension project has succeeded to demonstrate that high quality fresh fruit can be grown multiple years for commercial markets by eliminating ACP and HLB using protective screen houses. Our business plan for final proof of concept is to demonstrate long-term success of the CUPS method for 10 years, at which time the complete financial analysis of the enterprise can be evaluated. There is no other comparable solution for growing fresh citrus in HLB-endemic Florida at this time, and CUPS is seen as a timely solution to prevent the complete disappearance of fresh citrus production in Florida due to HLB.

Beneficiaries:

- ☐ Please refer to the "Potential Impact" section of your proposal. Any beneficiary group mentioned in that section must be addressed here.
- ☐ Provide a description of the groups and other operations that benefited from the completion of this project's accomplishments. What specialty crop stakeholders benefitted from this project? **How did they benefit, and how were they made aware of project results?** How many of them are there?
- ☐ Clearly state the quantitative data that concerns the beneficiaries affected by the project's accomplishments and/or the potential economic impact of the project.

- **Who are the specialty crop beneficiaries of the project?**

The primary beneficiaries are the fresh citrus growers of Florida, but also the United States of America as a whole, since all citrus growing states are threatened by HLB disease. Secondary specialty crop beneficiaries could be strawberry, blueberry and papaya growers. Both strawberry and blueberry

growers and researchers have recently investigated using covered production to modernize their production systems and papaya growers would benefit from the insect-free environment of screen houses because the devastating papaya ringspot virus is spread by aphids.

- **How many specialty crop beneficiaries did the project benefit?**

There are approximately 50 fresh citrus growers in eastern Florida, representing about 25,000 acres, that benefitted from the project, and many more will potentially be reached in California, Texas, and Arizona, with smaller numbers in Louisiana, Georgia, Alabama and Mississippi. The CUPS technology developed and tested in this project is anticipated to significantly benefit those Florida citrus growers developing their own commercial CUPS at this moment. By the end of 2018 we expect there will be more than 350 acres of commercial CUPS in Florida. The timing of our research to precede the construction of commercial CUPS by Florida citrus stakeholders was perfect since it has served as a template for them to get started rapidly and to learn from our mistakes. Additionally, should CUPS demonstrate a longer-term HLB-free growing environment for citrus, it is anticipated that many more growers in Florida and the United States will adopt this approach.

- **How will the project benefit the specialty crop beneficiaries?**

The research and extension project was conducted with a very strong demonstration component which was showcased to growers through organized field days, individual grower visits, educational seminars, videos, and a dedicated web site. The main goal of this project is to help undertake the necessary risks using public funds which growers are reluctant to take with their own funds in order to advance the science of protected agriculture and thermotherapy. Although all the project's proposed techniques use off-the-shelf technology, the particular way in which they are combined and used is unconventional, untested and therefore still considered "risky" for growers. Our role is to reduce the perceived risk through carefully conducted, significant, convincing research and extension. In addition CUPS technology is anticipated to significantly reduce the amount of fruit drop and tree decline and decrease the amount of small unharvestable fruit due to HLB infection.

- **What is the potential economic impact of the project if it can be estimated?**

The combined on-tree value of Florida's grapefruit, tangelo, early tangerine, and honey tangerine (the most prevalent fresh fruit varieties) crops is approximately $165 million dollars, and is under threat of collapsing due to the prolonged impacts of HLB disease. In the United States, per capita fresh citrus fruit consumption is second only to fresh banana consumption. If conservatively 10% of the Florida growers successfully adopt the outcomes of this project within the next two years, the estimated economic impact could be at least $10 million dollars per year, increasing as the technology is more widely adopted.

- **If applicable, how will the project have a multi-state (benefitting two more or states) or national impact?**

HLB impacts nearly 100% of trees in all citrus-producing counties in Florida. HLB is spreading at an alarming rate in Texas, and the psyllid vector is rapidly infesting California and other citrus-producing states. The severity of HLB is strongly determined by the duration of the epidemic, which is why Florida is most severely affected (2005-2015). HLB was discovered later in the other states but likely they might

all suffer the same fate as Florida. It would be very timely to have the proposed alternative fresh citrus production methods in this project developed and ready for use in other states in a few years' time.

Lessons Learned:

- ☐ Please refer to the "Work Plan" and "Expected Measureable Outcomes" sections of your proposal, as well as the "Problems and Delays" sections of your annual reports. Any problems and delays, any changes which had to be made to the work plan, and any project goals which could not be achieved must be addressed in this section.
- ☐ Offer insights into the lessons learned by the project staff as a result of completing this project. This section is meant to illustrate the positive and negative results and conclusions for the project.
- ☐ Describe unexpected outcomes or results that occurred as a result of implementing this project.
- ☐ If goals or outcome measures were not achieved, identify and share the lessons learned to help others expedite problem-solving.
- ☐ Lessons learned should draw upon positive experiences (i.e. good ideas that improve project efficiency or save money) and negative experiences (i.e. lessons learned about what did not go well and what needs to be changed).

Problems and delays: The construction of citrus screen houses is fairly complex and labor-intensive, which in our case resulted in the delayed completion of our CUPS at the CREC. In conversations with citrus growers, we confirmed that they also encountered unexpected delays when constructing their own CUPS. Some growers opted for cheaper outsourced anti-psyllid screen by importing direct from overseas. There were reports of delivery delays as well as some quality issues for screen imported directly from China. Citrus nurseries may have substantial delays of more than 12 months for providing certain combinations of varieties and rootstocks. Orders for clean trees to be used for CUPS should be one of the first activities in a new enterprise.

Lessons learned: Hurricane winds can potentially devastate CUPS screen houses, as two of the commercial CUPS growers in Polk and Hardee Counties discovered during hurricane Irma (September 2017). The CREC CUPS suffered moderate to severe damage to screens and pole infrastructures but the damage was not catastrophic. At no time were there breaches or holes in the screen house that could allow uncontrolled entry of ACP and subsequent HLB disease entry. Our lab team immediately embarked on a salvage and recovery effort after Irma in order to stabilize the trees and shore up the breaches in the structure as rapidly as possible. We achieved that finally in December 2017 and to date we have not detected any ACP or HLB in the CREC CUPS. In future CUPS construction, more attention should be paid to reinforcing and strengthening the entire structure to better withstand storm winds and flooding rains.

Unexpected outcomes: The very high price that CUPS fruit fetched in 2017/18 harvest was not expected, and is seen as a consequence of critically short supply of premium grade healthy fruit in Florida. We don't expect the price of fresh fruit to drop in the near to medium term due to the ongoing struggle of Florida citrus growers with the HLB disease complex.

Contact Person:

- ☐ Name the Contact Person for the Project, including telephone number and email address.

Arnold Schumann, schumaw@ufl.edu, 863-956-1151

Additional Information:

- ☐ Provide additional information available (i.e. publications, websites, photographs) that is not applicable to any of the prior sections. Charts, graphs, photos, etc. should be attached as separate file(s) and referenced in the text of the report.

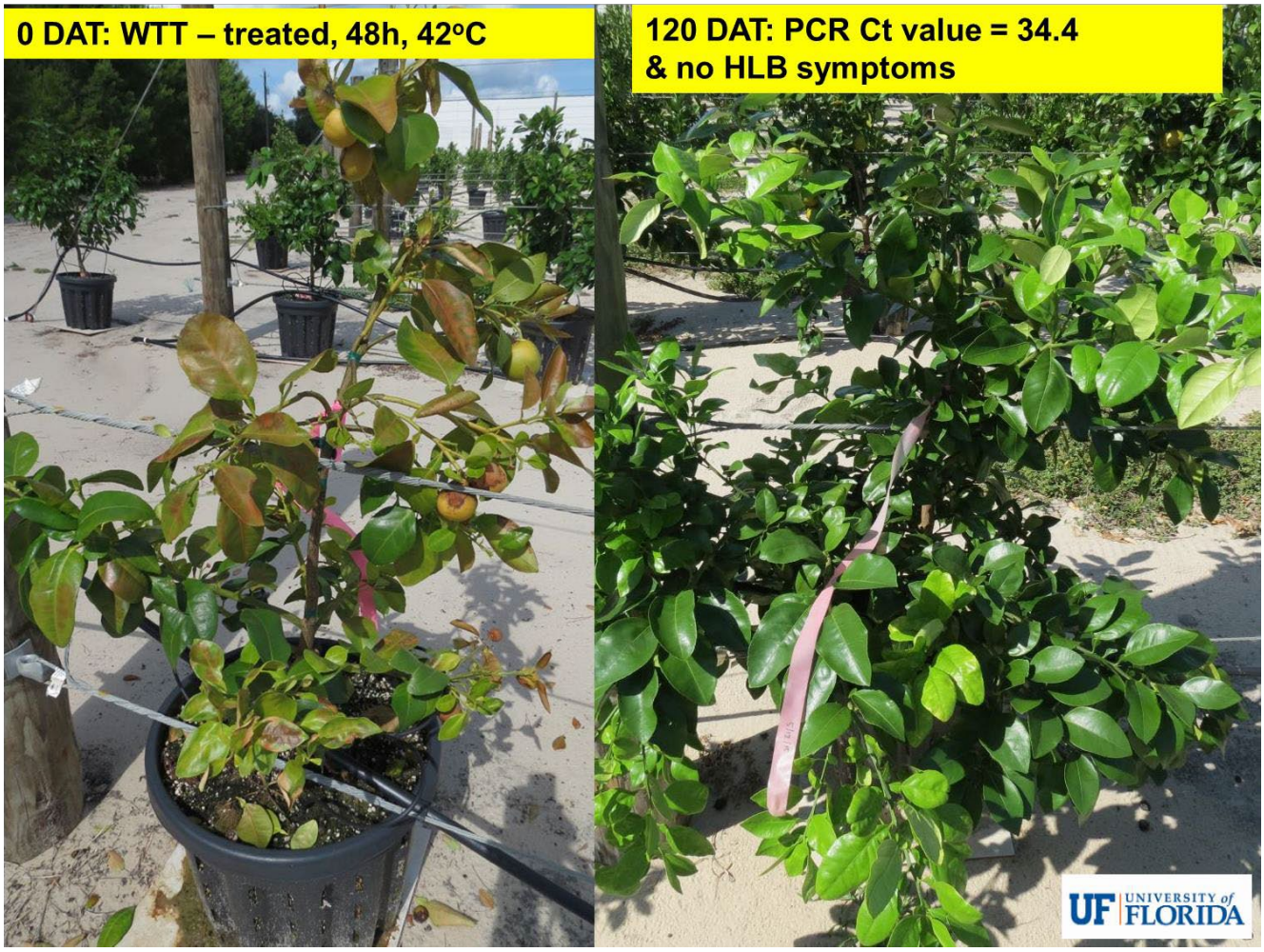
Please refer to the following photos, figures, data tables and field day evaluation sheet in support of the above report:

'Ray Ruby' grapefruit @ 2.5 years, 10-gal pots, 871 trees /acre: 346 boxes/acre (total 496 in 2 years)

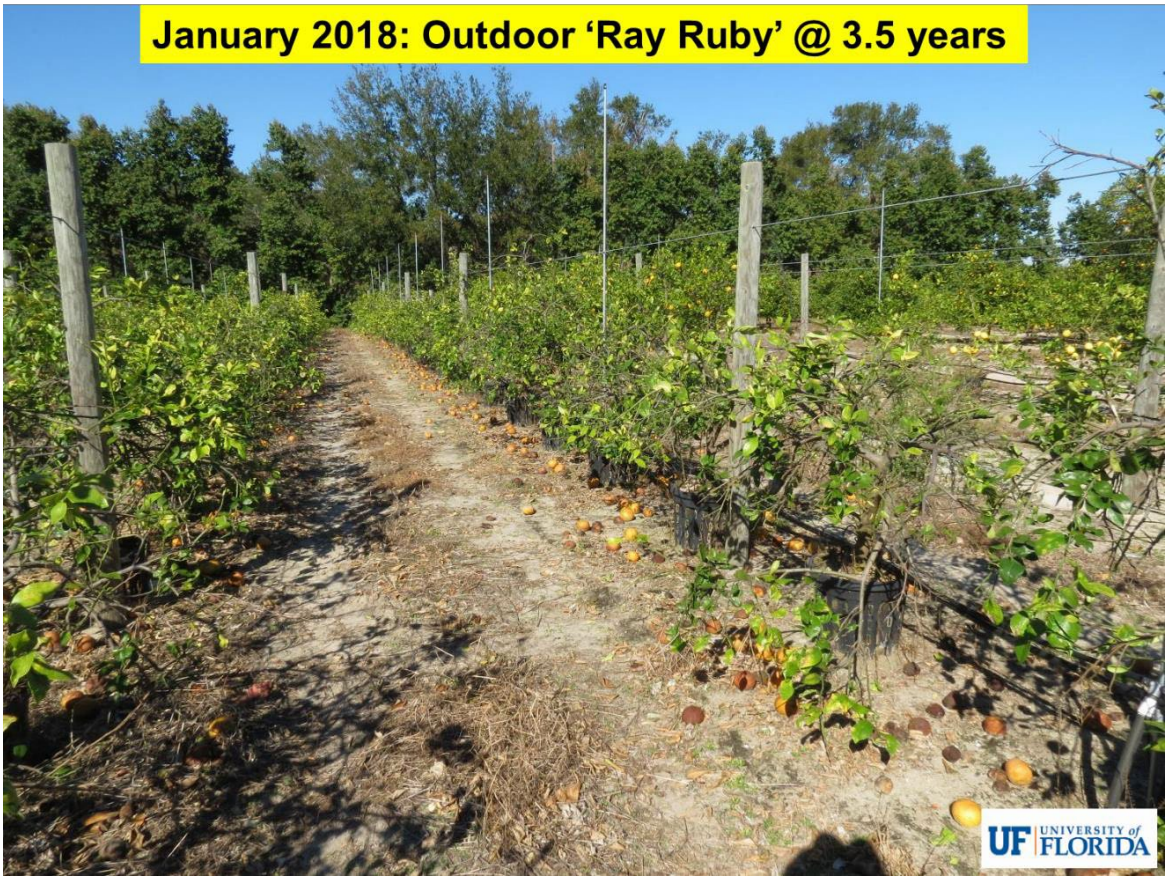
February 2017:
'Honey Murcott' @ 2.5 years, 7-gal pots, 1,361 trees /acre: 680 boxes/acre, 99% pack-out

Bumblebee colony (class A commercial hive) placed inside the south end of the CREC CUPS to study insect pollination, with murcott bloom shown below.

Thermotherapy example on HLB-positive grapefruit (above and below)



Thermotherapy example and recovery on HLB-positive grapefruit (above)


January 2018: Outdoor 'Ray Ruby' @ 3.5 years
UF UNIVERSITY of FLORIDA

Outdoor 'Honey' murcott @ 3.5 years
UF UNIVERSITY of FLORIDA

CREC Yield results at 3.5 years

- 'Ray Ruby' grapefruit trees grown in CUPS or Outdoors:

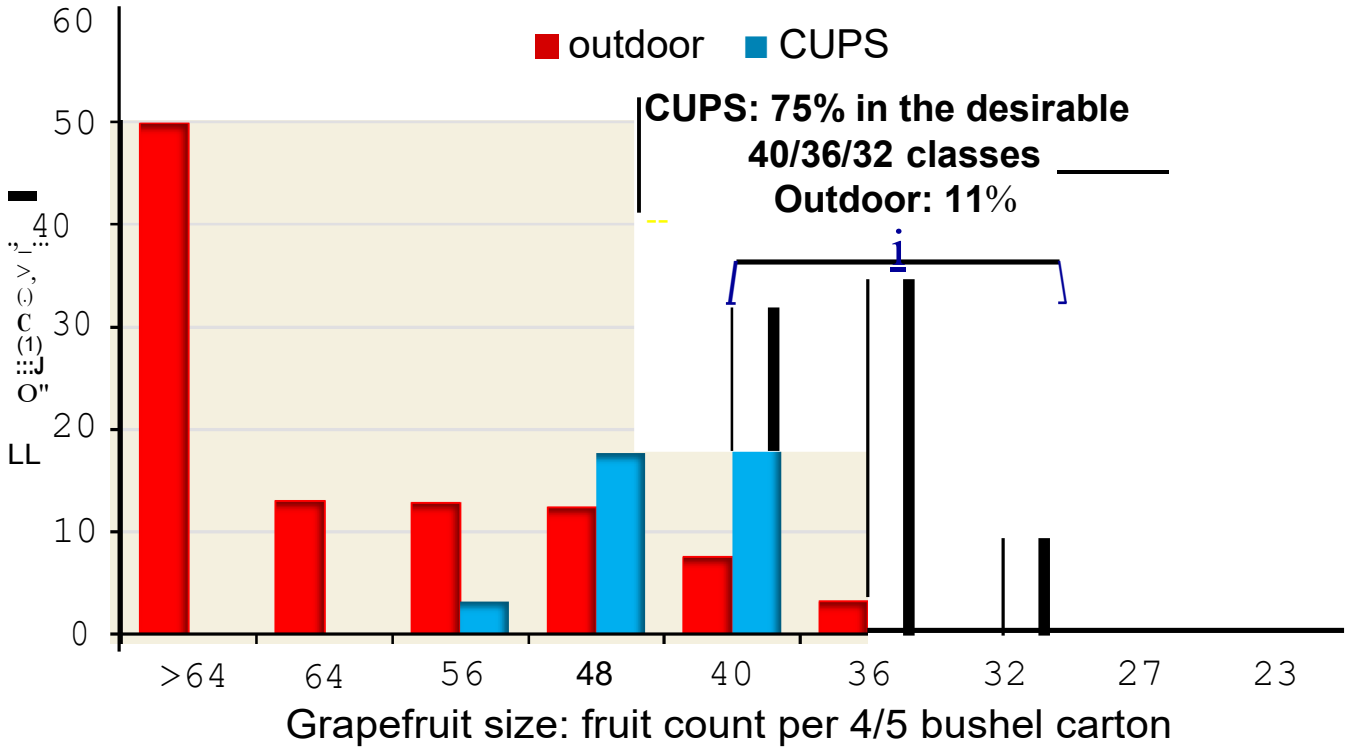
'Ray Ruby' grapefruit	Fruit yield boxes/ac	Fruit diameter (cm)	Acid (%)	Brix	Ratio	SS lb/box
CUPS	731	10.11	0.763	7.80	10.23	3.52
Outdoors	80	8.42	0.929	7.08	7.69	3.07
F-prob	*<0.001* ***	*<0.001* ***	*<0.001* ***	*<0.001* ***	*<0.001* ***	*<0.001* ***

juice yield: NS

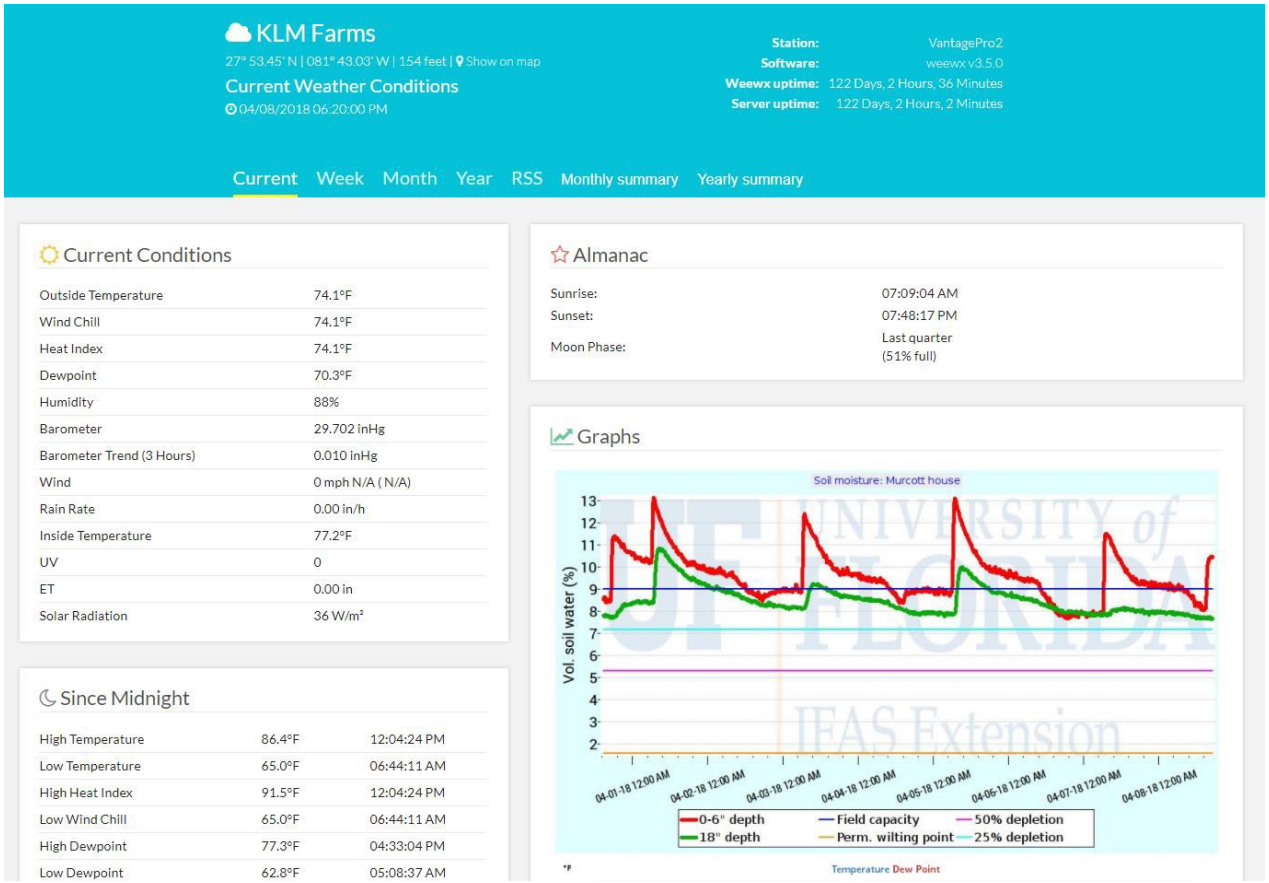
All significant effects were due to HLB disease affecting the trees outdoors

UF Floitio

CREC 'Ray Ruby' fruit size: 2018 plot harvest



http://166.130.8.210:4252/weewx/index.html



http://166.130.8.210:4252/weewx/index.html

CUPS Field Day Program Evaluation

Interim Sustainable Solutions for Fresh Florida Citrus Fruit Production in Florida 12-07-16

67 attendees

38 surveys

	Useful or Yes	%	Not useful or No	%	N/A	%
1. Evaluate the importance of the information presented.	38	100%	0	0	0	0
2. Have you gained any knowledge or new information from this program?	37	97.37%	1	2.63%	0	0
3. Will you share the gained information with others?	37	97.37%	0	0	1	2.63%
4. Will you change or improve your management practices because of the information gained from this program?	25	65.79%	0	0	13	34.21%
5. Would you like UF/IFAS to create a comprehensive guide to replace the CUPS quick start guide?	30	78.95%	1	2.63%	7	18.42%

Please feel free to write down any comments and/or suggestions

-Arnold Schumann is an excellent scientist. Promote him to eminent scientist and enhance his salary
-Repeat questions from audience
-We need to work on the state to have cost share programs for growers to invest in these systems and research all the variables on a larger scale. Extremely important.
-Keep the guide updated with new information. Is it published online at EDIS?
-Excellent seminar. Good mix of scientists and growers
-Well done!
-The CUPS quick start is an excellent go to guide.
-Need more info on which varieties will work in these structures
-Don't invite growers to speak who aren't willing to disclose info about their operation. ie. tree spacing
-More detailed info on actual irrigation and fertilization tried and comments on them. Look into canopy structural selection and maintenance cost
-Good program
-Good presentations and data-excellent financial analysis; EIP was old news-the historical part

Please write down suggestions for seminar topics in the future

-Great seminar, I look forward to seeing results in the coming year.
-Follow-up on reducing HLB infection on WTT production
-In-depth nutrient-fertigation and water quality calculations, calibration and system design for hydroponic irrigation/fertigation

Project (9): Management of laurel wilt of avocado with clonal and seedling rootstocks

Contact Person

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Email: bas56@ufl.edu

Project Partners

University of Florida, Institute of Food and Agricultural Sciences - Tropical Research and Education Center

Project Summary

The purpose of this project was to evaluate clonal and seedling rootstocks for avocado trees and determine whether any of the candidate rootstocks demonstrated decreased susceptability to laurel wilt. Options for treating laurel wilt at the time the project began were limited to time and labor-intensive treatments of single trees or small segments of groves.

Project Approach

Our working hypothesis was that vascular traits affect a cultivar's susceptibility to laurel wilt. For example, lumen dimensions are thought to be positively correlated with susceptibility (wider lumen = greater susceptibility). Work continued on the association of avocado race with vascular attributes. To date, replicates of different clones of pure Mexican (M), Guatemalan (G), and West Indian (WI) from Florida have been examined, and additional sources of the same clones have been sought. Arrangements are being made to obtain Duke (M), Reed (G) and Nabal (G) branches from collections held by the University of California at Riverside. Material from California would increase sample size and enable an examination of the G(enotype) x E(nvironment) interaction, which could influence results and conclusions from this work.

We evaluated the effects of LW on three clonal avocado rootstocks commonly used in California, 'Toro Canyon', 'Dusa', and 'Duke-7'. Two-year-old plants were inoculated with R. lauricola; control plants were inoculated with sterile water. Chlorophyll fluorescence [the ratio of variable to maximum fluorescence (Fv/Fm)], leaf chlorophyll index (LCI) net CO_2 assimilation, transpiration, and stomatal conductance were measured on inoculated and non-inoculated plants of each cultivar every 2-3 days. Xylem sap flow was monitored continuously in all plants. External symptoms (leaf wilting and stem dieback) were assessed daily using a visual scale (1 to 10). At the end of the experiment, the amount of vascular staining was assessed using the same scale and stem sections were assayed for the pathogen on potato dextrose agar.

Goals and Outcomes Achieved

External LW symptoms developed 22, 33, or 36 days after inoculation (dai) for 'Dusa', 'Duke-7', and 'Toro Canyon', respectively. The LCI was lower for inoculated than non-inoculated plants 45 dai in 'Toro Canyon' and 48 dai in 'Dusa'. The Fv/Fm was lower for inoculated than non-inoculated plants 27 dai in 'Toro canyon' and 31 dai in 'Dusa'. Inoculation did not affect the LCI or the Fv/Fm in 'Duke-7'. Net CO2 assimilation, transpiration, and stomatal conductance decreased as a result of inoculation 34, 41, and 48 dai for 'Dusa', 'Toro Canyon' and 'Duke 7', respectively. We did not observe significant differences in xylem sap flow between inoculated and non-inoculated plants of any cultivar. The amount of vascular staining was low in each cultivar, with inoculated plants exhibiting 20-30% internal stem discoloration.

Goal 1: Reduce the susceptibility of commercial WI scion cultivarsof avocado to laurel wilt.

Preliminary findings indicated that Mexican and Guatemalan rootstocks reduce susceptibility of the West Indian scions to which they were grafted due to decreased sapflow.

Goal 2: Identify combinations of clonal scions and clonal rootstocks of each of the botanical races of avocado that would result in consistently low laurel wilt development.

Progress toward goal: Duke-7 rootstock was preliminarily shown to have increased resistance to LW.

Goal 3: Disseminate research findings of the project to the avocado industry.

Progress toward goal: Findings have been presented at meetings of the Florida Avocado Administrative Committee.

Beneficiaries

Beneficiaries included all Florida avocado growers (numbered 1,088 according to the 2012 USDA Census of Agriculture). Results were presented at an American Phytopathological Society Division meeting, and made available through IFAS EDIS (Electronic data information source).

Lessons Learned

As mentioned in previous reports, grafted plants with clonal scions and rootstocks produced by Brokaw Nursery in California were received late due to initial problems with preparation of the material. The material was received later, grown in the greenhouse at our research center and then subjected to the planned experiments.

Total Funding Expended: $71,233.54

Percentage of Project Completed: 100

Project (10): Teaching Nutrition with Specialty Crops and School Gardens

Contact Person

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Project Partners

Florida Agriculture in the Classroom, Inc. (primary)

The partner organizations are Florida Farm Bureau, Florida Fruit and Vegetable Association, Wm. G. Roe & Sons, Florida Citrus Mutual, Florida Strawberry Growers Association and UF/IFAS Extension.

Project Summary

School garden grants have been hugely popular with Florida teachers for several years, and Florida Agriculture in the Classroom Inc. (FAITC) has been able to provide teachers with a steady source of school garden grant funding thanks to Specialty Crop Block Grants. The continued school garden grant funding allows teachers to either pay for materials to start a garden or refurbish an existing garden and use FAITC school garden curricula Gardening for Grades and/or Gardening for Nutrition and the school garden as a teaching tool.

The 100 $500 school garden mini grants it provided with a 2015 Specialty Crop Grant (Contract #022896) funded teachers' school garden efforts for the 2016-17 and 2017-18 school years. We requested to split the grant funding to two school years because we did not receive the 2016 Specialty Crop Block Grant and wanted to make sure we had funding every school year. These grants allowed teachers to teach students about the specialty crops grown in Florida. Many of the teachers were able to have 2 different gardens during the school year due to the Florida climate. These school gardens changed many of the students' outlook on food. They began asking their teachers for fruit and vegetable snacks versus going to the snack machine. A few of the teachers made fruit and vegetable "chips" and allowed the students to eat those during the year. We also had an 85% increase of knowledge using the school garden to teach lessons.

Florida Agriculture in the Classroom, Inc. began providing school garden grants during the 2011-12 school year with funding it received from a 2010 Specialty Crop Grant (Contract #106853). After that, it received a 2012 Specialty Crop Grant (Contract #019713) to distribute school garden grants during the the 2013-14 school year, a 2013 Specialty Crop Grant (Contract #020734) to distribute school garden grants during the 2014-2015 school year and a 2015 Specialty Crop Grant (Contract # 21768) to distribute school garden grants during the 2015-2016 school year.

According to the applications and final reports submitted from the school garden grants, these school garden projects collectively reached over 17,000 students. In addition, we asked that teachers keep food diaries of their students eating habits after being exposed to the specialty crop school garden, and overall their consumption of fruits and vegetables grew at least 10 percent, which is a goal set for the grant.

Project Approach

FAITC promised to increase the number of students exposed to school gardens, increase their consumption of specialty crops grown in their school gardens and increase their knowledge of the nutritional benefits of Florida specialty crops. All of these goals have been reached.

Food diary summaries from teachers who received the 50 $500 school garden grants indicated students consumption of specialty crops rose during the project. Students' views on foods also changed. Teachers stated in their final reports that students asked for fruits and vegetables as snacks versus going to the vending machines. The teachers got creative and starting making dried fruits and vegetables and some called them "chips" and allowed students to come to their room and get those snacks throughout the day. Almost all of the grant recipients had garden food tasting days where they created salads, wraps, salsa and more. Some of the schools even hosted parents and community tasting/sharing days after school to share what they were growing at their school.

Of the 100 garden grants, 32 were new projects. That means that 32% of our garden grants were new gardens. During these two school years we had hurricanes in the fall so we are happy with that number of new garden projects.

In addition, teachers were exposed lessons from 'Gardening for Nutrition' through teacher workshops. We held over 10 workshops around the state of Florida and saw more than 1,000 teachers over those workshops. By hosting workshops teachers felt more comfortable going back to their classroom and teaching about the nutritional benefits of Florida specialty crops. The student knowledge of 'Gardening for Nutrition' lessons were an average of 46% on the pre-tests and increased to 85% on the post-tests. Examples of lessons taught were "My Garden, My Plate", "What We Eat" and "In Search of Essential Nutrients" where students learned about the importance of fruits and vegetables in their diets and the parts of the plants they are eating and what the difference between fruits and vegetables are.

We passed out over 2,000 'Gardening for Nutrition' and hosted 20 workshops in 2016 and 13 in 2017. Our goal was to increase by 10 percent, teachers exposed to 'Gardening for Nutrition' and we did not meet this goal. I think we did a great job maintaining the number and did have two hurricane seasons that effected teachers ability to attend workshops, facilitate workshops and start new projects.

Goals and Outcomes Achieved

Goal 1 of the grant was to increase the consumption of fruits and vegetables by student by 10 percent. According to the mini grant final reports we exceeded this goal. Teachers used dehydrators to make fruit and vegetable chips that students has access to throughout the day. They made salsa using a blender, they made salads and brought in salad dressing. A few schools added a free vegetable to the cafeteria so all the students could try something new. And one school sent all the food home on the weekends through a backpack program. Overall a great success.

Goal 2 was to increase student knowledge of the nutritional benefits of specialty crops grown in school gardens. According to final reports teachers used lessons in 'Gardening for Nutrition' to teach their students there was an increase of 85% and therefore we exceeded our goal of 20%.

Goal 3 was to increase the number of Florida teachers and teachers exposed to 'Gardening for Nutrition' by 10 percent. As stated above we did not meet this goal but we did maintain with the year before. We were able to host 32 teacher workshops over the 2016 and 2017 school years and handed out over 2,000 copies of 'Gardening for Nutrition'.

Beneficiaries

FAITC's mission is to increase the number of agriculturally literate students in Florida. We do that through grants, workshops and teacher resources.

Two great ways we have found to increase student knowledge is through free teacher resources and school garden grants. Teachers do not have the money to do "extra" projects on their own. Through this grant we were able to help 100 schools start or continue with their gardens. Through our school garden curriculum we have been able to help teachers more effectively use their gardens as teaching tools.

Through this grant we have been able to reach more than 1,500 teachers and 45,000 students in kindergarten through 12th grade who participated in school garden workshops and received grants. Through workshops teachers learned how to cultivate and enjoy specialty crop fruits and vegetables.

We had 32 out of 100 new school gardens created and an increase of 85 percent in school garden and nutritional student knowledge.

In addition, Florida's specialty crop producers benefited from the project because it introduced their commodities to young consumers who will be more likely to purchase them in the future. Every year FAITC produces an annual report that is accessible by the public.

Lessons Learned

We did not have the delays with this grant like we have had in years past because we were not creating a new educational curriculum or activity newspaper. We did not receive the 2016 Specialty Crop Block Grant and therefore asked to distribute the 100 school garden grants over two school years. This allowed us to be able to offer these grants every year. If we did not have to do this the grant would have been finished much sooner but we will still complete the grant project before the due date.

I do believe that having the hurricanes in the fall of 2016 and 2017 really messed up teacher's schedules due to missed school days and make up days. This decreased our attendance at fall workshops and requests for additional materials. There is nothing we can do about natural disasters and hopefully for the next few years we will get a break and not have any that disturb the school year.

Total Funding Expended to Date: $34,998.00

Percentage of Project Completed: 100

Project (11): FNGLA Economic Impact Study

Contact Person

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Project Partners

Florida Nursery Growers and Landscape Assocation

Alan W. Hodges, PhD*, Hayk Khachatryan, PhD**
,Mohammad Rahmani, PhD*, Christa D. Court, PhD*
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Project Summary

Florida is a leading state, ranked second only to California in the US in terms of overall industry value. Nursery crops are one of the largest agricultural segments in Florida, along with fruits, vegetables and forest products. The fourth study in a series since 2000, FNGLA will capture this information in print, web-based communications and other means to position the information in front of the proper media channels and the end-consumers media sources to promote the size, scope and relevancy of Florida's nursery and landscape industry. In order to assess the market position and have a clear picture of the economic status of the industry, evaluation of nursery and landscape sales needs to be done on a consistent basis.

The Florida Nursery, Growers and Landscape Association (FNGLA) committed to providing timely statistics in 5-year increments. The relationship between academia and industry is critical. FNGLA and the University of Florida have enjoyed a strong partnership with this and many other programs. The project captures business data and produce statistics from the 2015 calendar year. Our industry needs to have timely science to back-up industry practices and procedures along with data to quantify and qualify the size of the industry and its impact to the State of Florida and its residents. This project is part of a series the industry plans to conduct every four to five years. Armed with this qualified and timely information, FNGLA now has the tools and resources to properly position Florida's nursery growers, landscape professionals and garden center retailers in Florida's marketplace.

Project Approach

Full study has been completed and is available through the following link.
http://www.fred.ifas.ufl.edu/pdf/extension/EcoContribEnvirHortIndFL2015.pdf
The goals established for the research and development have been completed on-time and through the funding the University of Florida has created an executive summary outline the results of the survey.

As the nation's largest state nursery and landscape association, FNGLA represents Florida's environmental horticulture industry which generated $21.08 billion total output sales in 2015 and directly employed 232,000 people. FNGLA continues to saturate the appropriate markets and reach key audiences with the results.

In January 2018, Fresh From Florida magazine ran information in the form of a 4- page marketing piece. It appeared as an insert and overruns will also be requested for additional outreach. The Fresh From Florida print magazine's distribution is all year long and has a strong following. Potential is to have the economic impact's direct mailing reach political, legislative and consumers.

Contract is with Journal Communications for 2500 printed overruns and 300 complimentary pieces were generated @ the cost of $13,500.00. A smaller run of 250 brochures were produced for distribution prior to the production of Fresh From Florida magazine. See the digital piece attached at the end of this report. Detail digital version will also be available.

34x78 Pull-up banner with economic impact information was developed and will be used in industry events and tradeshows to showcase Florida's Economic Impact.

FNGLA's Officers took the economic impact information to Washington D.C, and provided the marketing piece to congressional leaders during their visit.

REPORT IS POSTED ON UF Webpage: http://www.fred.ifas.ufl.edu/extension/economic-impact-analysis-program/.
Currently the project has distributed a total funds to date and request has been submitted for the following amount:

Goals and Outcomes Achieved

The goal of this project was to estimate and evaluate the total economic impact of the environmental horticulture industry in Florida, including wholesale nursery and greenhouse production, landscape services, and wholesale/retail trade in horticultural goods and the impact it has on the state, regions, and counties of Florida.

Our target was 400 completed surveys for each industry group (production, services, and trade) for a total of 1,200 responses targeted. Actual number of responses was 1,546, well beyond this target.

Survey questionnaires were sent to 10,440 firms, and usable responses were provided by 1,546 firms, representing a 14.8 percent response rate. Survey respondents reported sales of $1.195 billion in 2015. Total industry sales were estimated at $10.711 billion, including $2.753 billion for nursery producers, $4.431 billion for landscape service firms, $1.239 billion for horticultural retailers, $1.041 billion for wholesalers, and $1.247 billion for allied firms. Approximately 25 to 30 percent of nursery, wholesaler and allied firm sales were to out-of-state (national and international) markets, while landscape and retailer sales were predominantly to state and local markets. Respondents reported employment of 14,794

persons, including 11,245 full-time and 3,549 part-time or temporary employees, and total employment in the industry was estimated at 109,821 jobs, including 64,777 jobs in the landscape sector and 19,152 jobs for nurseries. In addition, a total of 552 unfilled positions were reported. Respondents reported capital investment of nearly $50 million in 2015, including $21 million for buildings/structures, $23 million for equipment and vehicles, and smaller amounts for information technology and other types of capital. For the next three years (2016-18), respondents expected to invest nearly $90 million.

Beneficiaries

Florida's entire nursery and landscape industry will benefit from this study. In 2010, total direct sales by Florida nursery, landscape service firms and horticulture retailers were $15.35 billion and total direct employment was 204,700 jobs! The largest plant product types for nursery growers were tropical foliage, representing 35 percent of total sales, followed by potted flowering/bedding plants (22%), and shrubs (10%). Florida native plants represented about 7.8 percent of nursery sales. With the results of the 2015 study, we now know that the industry has grown to $21.08 billion total output sales in 2015 and directly employs 232,000 people.

Our industry needs to have science to back-up industry practices and procedures along with data to quantify and qualify the size of the industry and its impact. The study provides real-time data presenting the size and strength of the industry in Florida's marketplace.

Overall number of beneficiaries is estimated to be 1,200.

Lessons Learned

The Fall of 2017 was an incredibly challenging time for so many in the state of Florida. Due to Hurricane Irma, our industry not only suffered damage but FNGLA cancel a large tradeshow only a few days before the storm, rescheduled the same size show within a month and then put on our normal Tropical Foliage International Expo(TPIE), all within a 4 month time frame. Our team has been very busy professionally to say the least. The remaining funds were to be used for video production and photography to help promote the results of the Economic Impact Study but even though our industry is resilient and will recover, it was not the time to be videotaping production nurseries or landscape projects showing the size and scope of our industry. Even with this delay, we were able to produce video and photography. which provided a well rounded presentation of our industry.

Total Funding Expended: $73,450.35

Percentage of Project Completed: 100

Integration of a biological control alternative to the management strategy against vectors of the laurel wilt disease of avocados

Project Summary

Laurel wilt (LW) is a deadly disease of trees in the family Lauraceae. The disease has caused widespread mortality of redbay (*Persea borbonia*) and other native Laureceae throughout the southeastern United States and is now threatening the avocado industry in Florida. The key vector of this disease in native lauraceous hammock is the redbay ambrosia beetle RAB (*Xyleborus glabratus*). However, RAB is seldom detected in avocado orchards and other closely related species of ambrosia beetles (i.e, *Xyleborus bispinatus* and *Xyleborus volvulus*) have become vectors of the pathogen in south Florida avocado orchards.

Until recently, management strategies for the vectors of the laurel wilt fungus relied solely on chemical control and sanitation practices. Beneficial entomopathogenic fungi (EF) may have a key role in controlling these pathogen vectors (Carrillo et al. 2015), but there are important knowledge gaps regarding the compatibility of EF with other management tactics used by avocado growers.

The goals of this project were to: 1) determine antagonisms and potential complimentary and/or synergistic relationships between EF and the pesticides used by avocado growers, 2) use this knowledge to improve efficacy of EF already in use by avocado growers, 3) reduce the costs associated with management of LW vectors using EF, and 4) engage more growers into using EF in an integrated management approach. Surveys, workshops, and participatory research were used to promote the integrated use of EF by avocado growers. Multiple surveys were used to determine the number of growers that started using EF in an integrated pest management approach and the reduction in use of chemical insecticides as a measure of the success of this project.

Project Approach

Objective 1. Determine the compatibility of commercial formulations of EF with fungicides, insecticides, and adjuvants currently registered for use in avocado production.

a) Screen selected registered commercial agrochemical formulations including copper-based fungicides, with the commercial EF to determine their compatibility when mixed and applied together over time and under different spray regimes

Each EF (*Isaria fumosorosea*, *Beauveria bassiana*, and *Metarhizium brunneum*) was assessed *in vitro* after being mixed together for 1 or 6 hours with a specific commercial agrochemical product (fungicides, insecticides, and adjuvants) currently registered for application in avocado production to determine their compatibility when applied together. The protocol followed a modification of that described in Avery et al. (2013). The EF suspension was adjusted to 10^3 spores/ml and the agrochemical diluted to the label rate. After 1 or 6 hours of exposure of each EF to the agrochemical product in a vial, a 100 µl aliquot of each suspension was removed and spread under sterile conditions on 10 potato dextrose agar (PDA) plates. Plates were sealed and then incubated at 25 °C under a 14-hour photophase for 7 days. The resulting EF

colony forming units (CFU) present on the plate were counted and compared with the CFUs of each EF without any agrochemical added to the suspension.

Compatibility of the three EF products tested after a 1- or 6-hour exposure to agrochemicals are presented in **Tables 1-3**. The criterion for determining non-compatibility of the EF products when mixed with the agrochemicals was a CFU count significantly (Tukey's HSD test, $P < 0.05$) less than that of the EF product mixed with water only. This was based on two experimental replicates with five plates / treatment / experiment. Based on CFU counts of the 6-hour exposure treatments, PFR-97 is not compatible with the following fungicides: Abound, Flopan 80, Ridomil Gold SL, Tilt; insecticides: Danitol, Hero, Malathion, Permethrin, Agrimek, VErtimek or Epimek, Talstar, Mustang; and adjuvants: Citrus Oil, L1700, Keyplex 350. BotaniGard is not compatible with the following fungicides: Abound, Flopan 80 WDG, Switch 62.5 WG, and Pro Phyt Phosphonate; insecticides: Danitol, Malathion, and Permethrin; adjuvants tested were all compatible. Met52 is not compatible with the following fungicides: Flopan 80 WDG, Pro Phyt Phosphonate; insecticides: Danitol, Malathion; and adjuvant Keyplex 350.

Table 1. Compatibility of three entomopathogenic fungal products exposed for 1 or 6 hours (h) to fungicides used in avocado pest management of ambrosia beetles.

	Met52		PFR-97		BotaniGard	
Fungicides	1 h	6 h	1 h	6 h	1 h	6 h
Abound	C	C	NC	NC	NC	NC
Cuprofix-Ultra 40 Disperss	C	C	C	C	C	C
Flopan 80	NC	NC	NC	NC	NC	NC
Kocide 2000	C	C	C	C	C	C
Ridomil Gold Copper	C	C	C	C	C	C
Ridomil Gold SL	C	C	C	NC	C	C
Switch 62.5WG	C	C	C	C	NC	NC
Tilt	C	NC	NC	NC	C	C
Pro Phyt Phosphonate	NC	NC	C	C	C	NC

C = compatible; NC = Not compatible

Table 2. Compatibility of three entomopathogenic fungal products exposed for 1 or 6 hours (h) to insecticides used in avocado pest management of ambrosia beetles.

	Met52		PFR-97		BotaniGard	
Insecticides	1 hour	6 hours	1 hour	6 hours	1 hour	6 hours
Danitol	C	NC	NC	NC	NC	NC
Hero	C	C	NC	NC	C	C
Admire Pro	C	C	C	C	C	C
Malathion	NC	NC	NC	NC	NC	NC
Permethrin	C	C	NC	NC	C	NC
Agrimek, Vertimek or Epimek	C	C	C	NC	C	C
Actara	C	C	C	C	C	C
Talstar	C	C	NC	NC	C	C
Mustang	C	C	C	NC	C	C

C = compatible; NC = Not compatible

Table 3. Compatibility of three entomopathogenic fungal products exposed for 1 or 6 hours (h) to adjuvants used in avocado pest management of ambrosia beetles.

	Met52		PFR-97		BotaniGard	
Adjuvants	1 hour	6 hours	1 hour	6 hours	1 hour	6 hours
Banole	C	C	C	C	C	C
Citrus oil	C	C	C	NC	C	C
Nu-Film-P	C	C	C	C	C	C
L1700	C	C	NC	NC	C	C
Pentrabark	C	C	C	C	C	C
Keyplex 350	NC	NC	C	NC	C	C

C = compatible; NC = Not compatible

b) Determine the pathogenicity of EF when mixed with different agrochemicals against the different ambrosia beetles vectoring laurel wilt

The effect of agrochemicals mixed with BotaniGard on the mean survival time of *X. bispinatus* was assessed using an *in vivo* bark plug bioassay technique described in Zhou et al. (2018). In brief, bark plugs (15 mm Ø) were removed from 'Lula' avocado bolts and placed inside the tube and then treated with one of the following treatments: 1) EF suspension; 2) EF suspension mixed with one of the agrochemicals; 3) agrochemical alone; or 4) water alone. Ambrosia beetles from stock colonies maintained at the UF/IFAS Tropical Research and Education Center (TREC) in Homestead, FL were transported to UF/IFAS Indian River Research and Education Center in Ft. Pierce, FL. Upon arrival, an individual beetle was gently placed near the top of each tube by using a moistened camelhair brush. The tube was slightly tilted so that the beetle could gently slide down the tube until it reached the bark plug. Once the beetle reached the bark surface of the plug and was observed freely moving on the surface, the tube's screw cap was put in place. Tubes were randomized in a styrofoam rack according to the treatments tested and placed into an environmentally controlled chamber set at 25°C in total

darkness for 24 hours. After this time, the caps were replaced with Parafilm and again placed upright in a styrofoam rack.

Beetles were checked daily post-exposure under a binocular dissecting scope to determine if they were alive or dead. If the beetle was not visible, the tube was tapped on a hard surface several times until movement was apparent. If no movement was visible after tapping, then the beetle was presumed dead. Using a permanent marking pen, a specific colored dot was marked on the Parafilm corresponding to the day's color code. Dead beetles remained for several days in the tube with high moisture until mycosis was apparent. Bioassay experiments were conducted twice, and there were 10 replicates per treatment.

The fungicides Cuprofix and Root Shield Plus were compatible with BotaniGard as the combination of these fungicides + BotaniGard resulted in ambrosia beetle survival time being similar to that of BotaniGard alone (**Figure 1**). The fungicides Prophyt, Folpan and Abound were not compatible with BotaniGard.

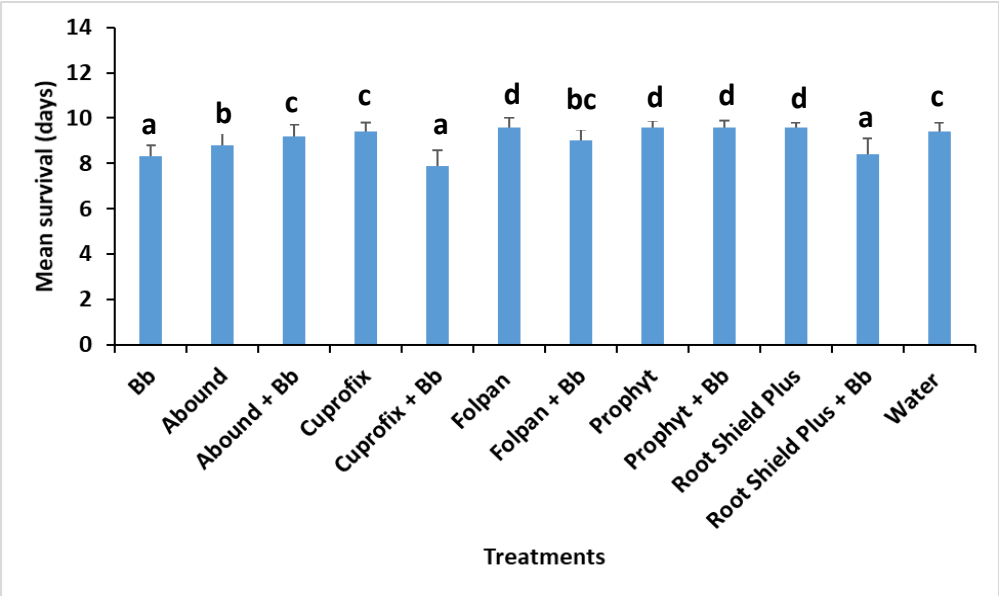

Figure 1. Comparison of mean survival (± SEM) of *Xyleborus bispinatus* adults after exposure to fungicide treatments alone or in combination with BotaniGard (Bb) in the bark plug bioassays after 10 days. Bars with different letters above them are significantly different (LSD test, $P < 0.05$).

The effect of agrochemicals mixed with BotaniGard on the development and reproduction of *X. bispinatus* was assessed using an avocado sawdust method designed by Menocal et al. (2017; 2018). Briefly, limbs (10-15 cm Ø) of healthy and non-sprayed 'Lula' avocado trees from an orchard at TREC were cut with a chainsaw and divided into smaller bolts (20 cm long). The bolts were sprayed with EF and pesticides alone or in combination depending on the experiment and left hanging in the field until dry. Ten *X. bispinatus* females were placed on the bolts and allowed to walk for 5 minutes. After this time, the beetles were placed individually in test tubes with avocado sawdust rearing media.

BotaniGard caused higher foundress mortality than the control and all fungicides when applied alone (**Figure 2**). None of the fungicides applied alone caused significantly higher mortality than the control. However, only the fungicides Cuprofix and Kocide combined with

BotaniGard had significantly higher mortality than the control. In general, mortality caused by BotaniGard alone or in combination with the different fungicides tested was similar, except for Folpan which was significantly lower.

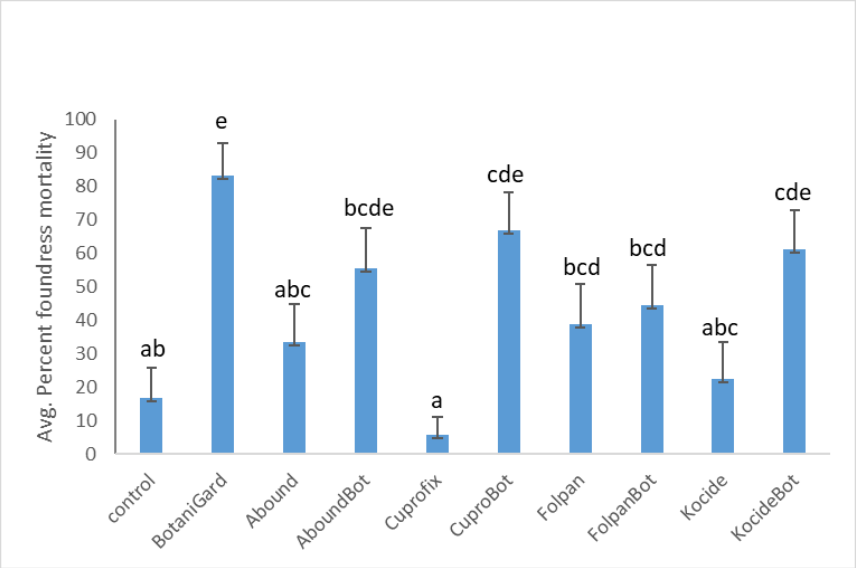

Figure 2. Mortality (mean ± SEM) of *X. bispinatus* foundress females exposed to BotaniGard (Bot) and fungicides alone or in combination. Letters above the bars that are not the same are significantly different (Tukey's test, $P < 0.05$).

The number of progeny from *X. bispinatus* foundresses exposed to BotaniGard was similar to the number of progeny in the control treatment (**Figure 3**). However, two treatments, Abound + BotaniGard and Cuprofix + BotaniGard, significantly reduced number of progeny compared to the control. In contrast, Cuprofix alone increased the longevity and reproductivity of *X. bispinatus*.

Among the insecticides tested using the *in vivo* bark plug bioassay (**Figure 4**), Hero (zeta-cypermethrin + bifenthrin), Talstar (bifenthrin), and permethrin did not inhibit infection by *B. bassiana* and allowed conidiogenesis from the beetles (**Figure 5**) more than 50% of the time (**Table 4**). Conidiogenesis from adult beetles is necessary to kill the brood inside the tree. These insecticides killed the beetles in less than three days after treatment and reduced the probability that a beetle would bore into or through the wood. Malathion and Danitol were less compatible with BotaniGard. Although they also killed the beetles in less than three days, the mycosis rate was only 20% and 25%, respectively. When Agrimek was mixed with BotaniGard, the survival time of the beetle was shorter compared to the fungal treatment alone, and beetles mycosed 70% of the time. However, this insecticide did not prevent the beetles from boring into or through the wood.

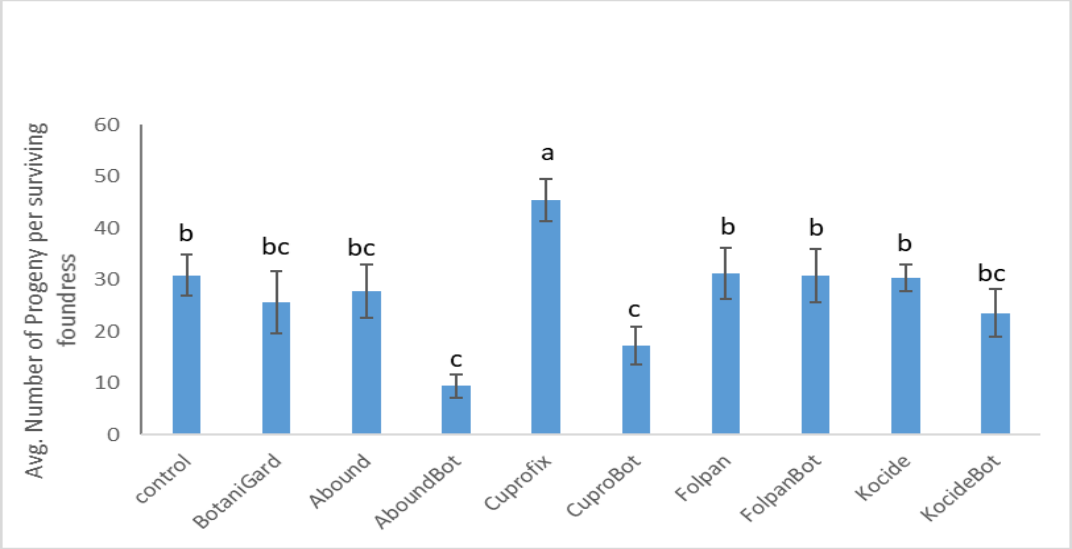

Figure 3. Average number of progeny (± SEM) per surviving *X. bispinatus* foundress exposed to BotaniGard (Bot) and fungicides alone or in combination. Letters above the bars that are not the same are significantly different (Tukey's test, $P < 0.05$).

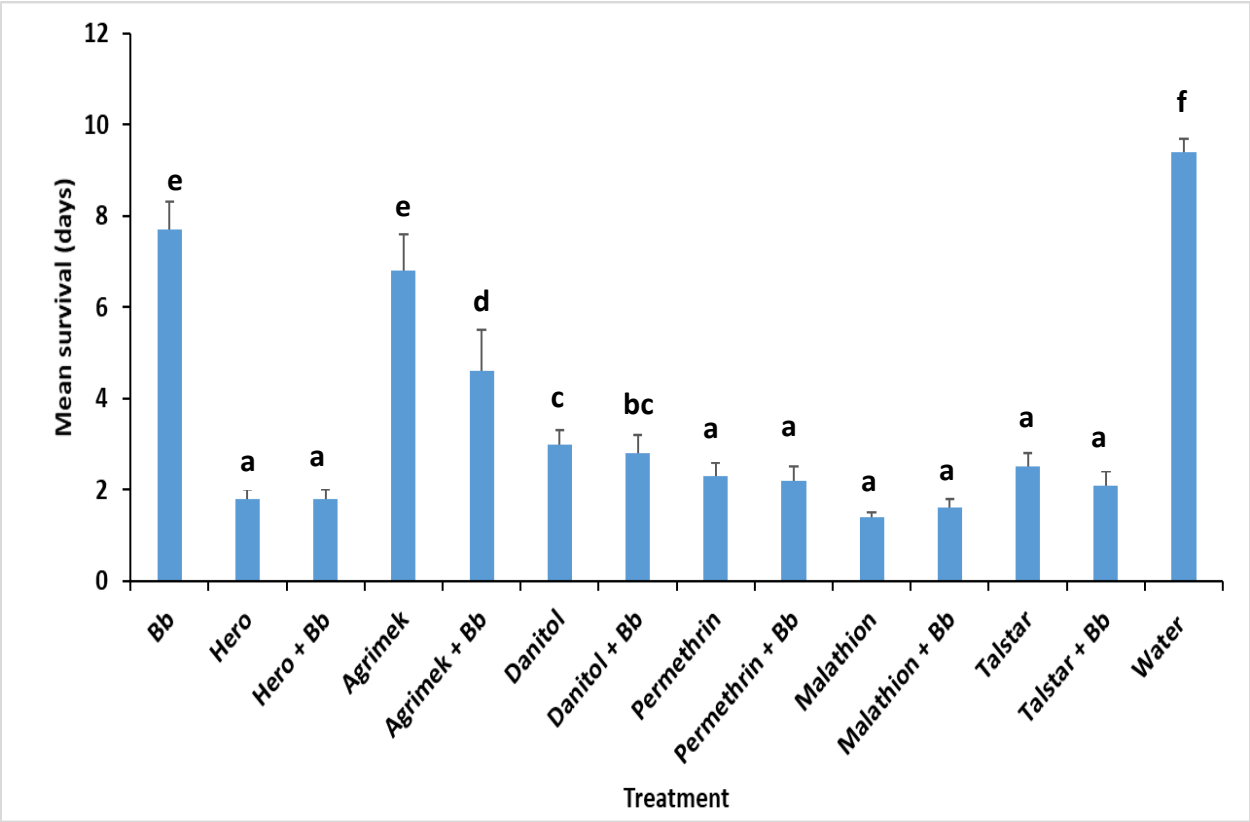

Figure 4. Comparison of mean survival (± SEM) of *X. bispinatus* after exposure to insecticide treatments alone and in combination with BotaniGard (Bb) in the bark plug bioassays after 10 days. Letters above the bars that are not the same are significantly different (LSD test, $P < 0.05$).

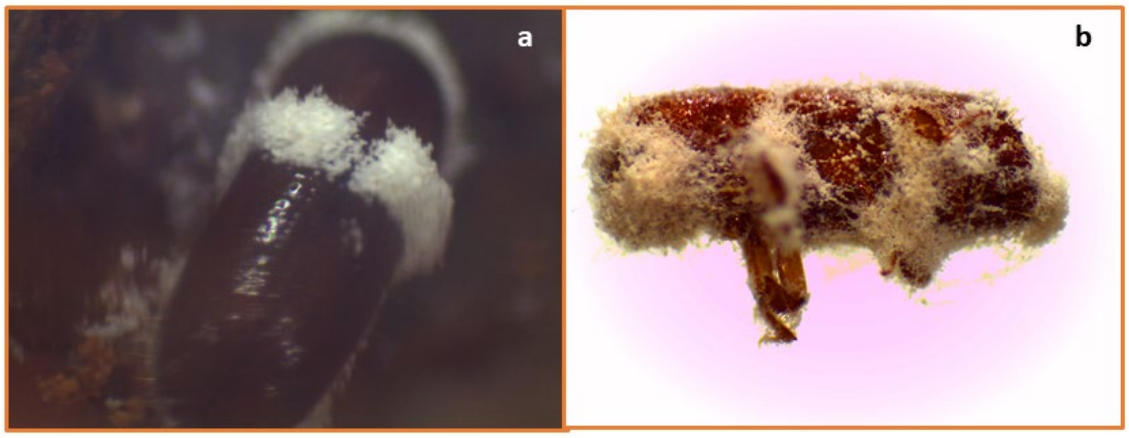

Figure 5. The ambrosia beetle *Xyleborus bispinatus* displaying mycosis after direct exposure to a) *Beauveria bassiana* alone and b) *B. bassiana* mixed with permethrin inside the avocado bark plug bioassay chamber.

Table 4. Comparison of mean percentage of *X. bispinatus* after exposure to insecticide treatments alone and in combination with BotaniGard (Bb) that mycosed or bored through the plug in the bark plug bioassays.

Treatment	% Mycosed	% bored through bark plug
Bb	95	25
Hero	0	0
Hero + Bb	50	0
Agrimek	0	30
Agrimek + Bb	70	10
Danitol	0	0
Danitol + Bb	25	0
Permethrin	0	0
Permethrin + Bb	55	0
Malathion	0	0
Malathion + Bb	20	0
Talstar	0	0
Talstar + Bb	55	0
Water	0	55

c) Determine if either the EF or compatible insecticide can be reduced from the label rate and still obtain similar efficacy against the beetles as at label rate and ultimately reduce the application costs to the grower.

The initial assessment was the same as described above for the bark plug assay except either the EF or insecticide concentration was reduced. At first, one product was reduced while the other product remained at label rate and vice versa. Then different combinations of concentrations of the insecticide reduced from 1.0X to 0.5X, that of the label rate and the EF concentration reduced from 10^6 to 10^5 was combined to determine the best mixture efficacy.

The mean survival for *X. bispinatus* adult beetles after exposure to selected insecticides (Hero, Permethrin) or fungal biopesticide and BotaniGard (Bb) alone at label rates or at a reduced rate were compared with different combination treatments (**Figure 6**). All insecticide treatments alone at either the label or reduced rate as well as the combination treatments with Bb killed the beetles significantly faster than either the Bb treatments alone or the controls (water, supernatant). The mean survival for beetles exposed to the different Hero insecticide rates as well as the combination treatments were similar except for the treatment with reduced rates of both the insecticide and Bb. Beetles exposed in the Permethrin treatments at the reduced rate had a significantly longer survival time compared to those at the label rate. In the combination treatments with the Bb concentrations, beetles exposed to Permethrin at the reduced label rate died significantly slower compared to the same insecticide combinations at the label rate. Percentage mycosis and beetles that bored through the avocado bark plug when exposed to Bb alone was 1.8 times higher at the label rate compared to those at the reduced rate (**Table 5**). The percentage of beetles that bored through the plug in the controls were slightly lower, compared to the Bb treatment alone at the lower concentration. The lowest mycosis rate (5%) occurred in the Hero combination treatments at the highest rate for both the insecticide and fungal treatments. However, the highest mycosis rate (55%) occurred with beetles exposed to the reduced Hero insecticide treatment plus Bb at the label rate. Only the combination treatment of Hero plus Bb at the lowest label rates allowed for beetles to bore through the bark plug. Permethrin insecticide at the reduced rate plus Bb at the label rate allowed for the highest percent beetle mycosis (60%) amongst the combination treatments. Amongst the combination treatments, only exposure to lower rates of the Permethrin insecticide plus either of the Bb rates allowed for the beetles to bore through the plug.

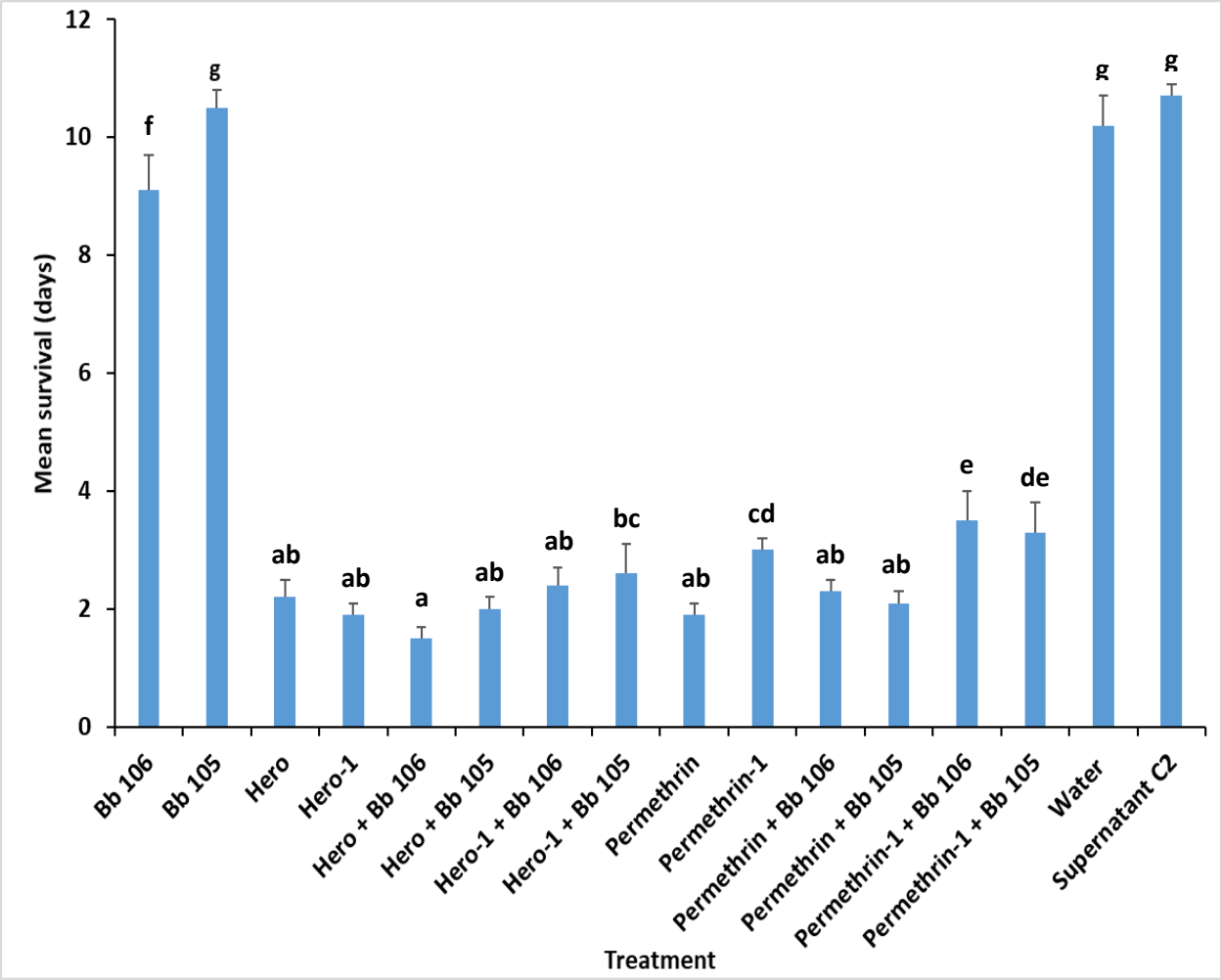

Figure 6. Comparison of mean survival (± SEM) of *X. bispinatus* after exposure to insecticide treatments alone and in combination with BotaniGard (Bb) at label (10^6) or reduced rates (10^5) in the bark plug bioassays after 10 days. Letters above the bars that are not the same are significantly different (LSD test, $P < 0.05$).

Table 5. Comparison of mean percent of *X. bispinatus* after exposure to insecticide treatments alone and in combination with BotaniGard (Bb) at label or reduced rates that mycosed or bored through the bark plug in the bark plug bioassays.

Treatment	% Mycosed	% bored through bark plug
Bb 10^6	70	65
Bb 10^5	40	95
Hero	0	0
Hero-1	0	0
Hero + Bb 10^6	5	0
Hero + Bb 10^5	15	0
Hero-1 + Bb 10^6	55	0
Hero-1 + Bb 10^5	10	10
Permethrin	0	0
Permethrin-1	0	0
Permethrin + Bb 10^6	35	0
Permethrin + Bb 10^5	15	0
Permethrin-1 + Bb 10^6	60	5
Permethrin-1 + Bb 10^5	25	5
Water	0	80
Supernatant C2	0	80

Overall the results of the *in vivo*, bark plug, and reproduction experiments suggest that the fungicides Abound, Flopan 80 WDG, Switch 62.5 WG, and Pro Phyt Phosphonate are not compatible with BotaniGard. By contrast Cuprofix-Ultra, Kocide 2000, Ridomil Gold Copper and Ridomil Gold SL were compatible with Botanigard. Among the insecicides tested only Danitol and Malathion were not compatible with Botanigard. These results are incorporated in the UF/IFAS Laurel Wilt Ambrosia beetle control recommendations (available at: https://trec.ifas.ufl.edu/RAB-LW-2/RAB_Recommendations.shtml).

Objective 2. Integrate EF into the management strategy currently being implemented in commercial avocado orchards for control of ambrosia beetles and laurel wilt.

The main strategy currently implemented by avocado growers is the use of the fungicide Tilt® (propiconazole) through trunk injections and infusions. Tilt is being used prophylactically to treat entire plantings or as a spot treatment in healthy trees adjacent to a LW affected tree. Groves treated prophylactically with Tilt are also being treated with BotaniGard. Therefore, it is important to determine compatibility and/or antagonisms between Tilt and BotaniGard. A modification of the avocado sawdust method described by Menocal et al. (2017, 2018) was used to incorporate Tilt into the rearing media at a concentration of 200 ppm. This fungicide concentration has been found in commercial trees treated with this fungicide (Crane JH, De la Torre C, Monterroso, A. - personal communications). Groups of 10 *X. bispinatus* females were placed on BotaniGard treated bolts and allowed to walk for 5 minutes on the bolt (as described above). Control beetles were exposed to water treated bolts. After this exposure period, beetles were placed individually in test tubes with avocado sawdust rearing media with or without Tilt and the rearing media dissected 27 days later to determine the effects of the different treatments

on the beetle reproduction. The experimental treatments were 1) BotaniGard 2) Tilt 3) BotaniGard + Tilt, and 4) control.

BotaniGard caused higher foundress mortality than the control, Tilt alone and Tilt + BotaniGard. Interestingly, the presence of Tilt inhibited the action of BotaniGard on the foundress female (**Figure 7**). In addition, the number of progeny from *X. bispinatus* foundresses exposed to BotaniGard was similar to the number of progeny in the Tilt alone and Tilt + BotaniGard treatments (**Figure 8**). These results suggest that the presence of Tilt alone reduces the number of *X. bispinatus* progeny to levels similar to those achieved with BotaniGard treatments alone. Moreover, Tilt showed an inhibitory effect on the foundress female mortality caused by BotaniGard. More research is needed to determine if applications of BotaniGard are justified in groves treated prophylactically with Tilt.

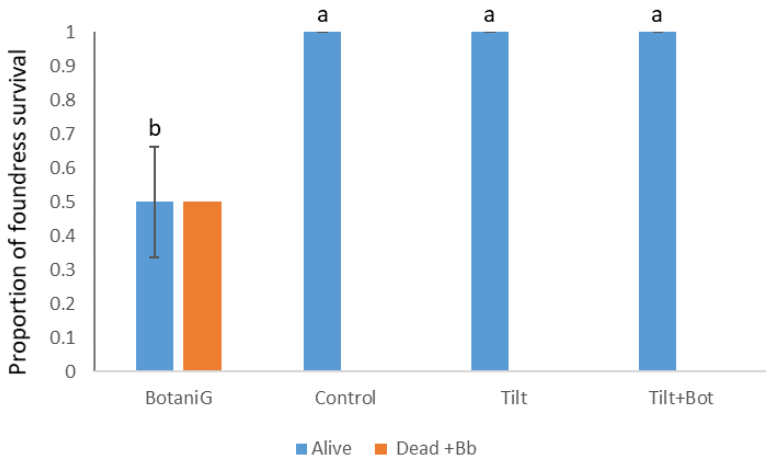

Figure 7. Mean proportional survival (± SEM) of *X. bispinatus* foundress females exposed to BotaniGard (BotaniGard and Bot) and avocado sawdust containing the injectable fungicide propiconazole (Tilt). Letters above the bars that are not the same are significantly different (Tukey's test, $P < 0.05$).

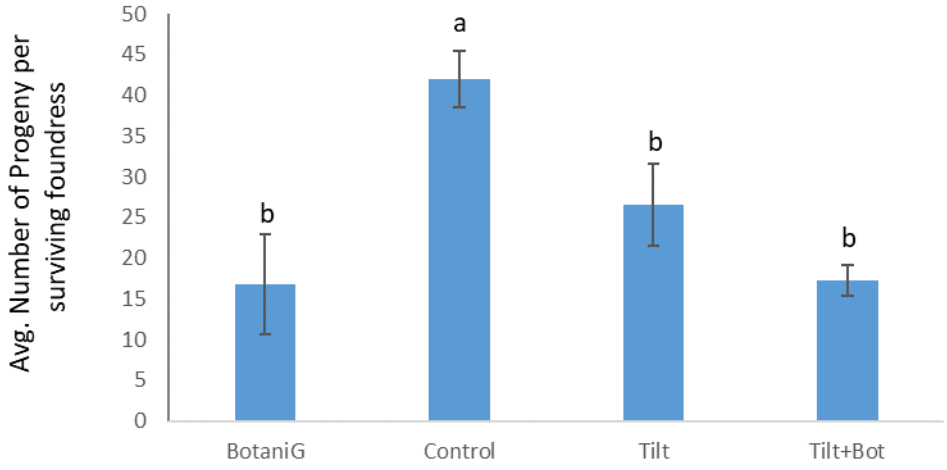

Figure 8. Average number (± SEM) of progeny per surviving *X. bispinatus* foundress exposed to BotaniGard (= BotaniG and Bot) and avocado sawdust containing the injectable fungicide propiconazole (Tilt). Letters above the bars that are not the same are significantly different (Tukey test, $P < 0.05$).

Beneficiaries

The beneficiaries of this project involve not only avocado growers and homeowners, but workers employed by growers and ancillary industries such as packinghouses, chemical companies, and irrigation companies. The Florida avocado industry consists of about 7,500 acres, which represents about 60% of the total tropical fruit crop acreage and is the state's second-largest fruit industry. There are over 400 growers that make part, most, or all of their income from avocado production.

The research findings of this project have been presented to the local growers during the Florida Avocado Administrative Committee meetings (the second Wednesday of each month) and through separate workshops on management of laurel wilt vectors that are conducted every 3-4 months. In addition, research findings from this project were presented at the Laurel Wilt Summit on Nov 3-4 (2016) to approximately 100 participants.

<u>Workshops and meetings where the research findings of this project have been presented</u>

Laurel Wilt Workshop as part of the FIU Veterans and Small Farmers Outreach Program, 22 attended, took place at FIU Veterans Building. 2016.

Laurel Wilt Seminar--Entomology, 45 attended, took place at UF/IFAS Extension Miami-Dade County. 2016.

Laurel Wilt Summit, 103 attended, took place at UF/IFAS Extension Miami-Dade County. 2016.

Laurel Wilt Field Day, 42 attended, took place at various locations within the industry. 2016.

Laurel Wilt Growers' Forum, 22 attended, took place at UF/IFAS Extension Miami-Dade County. 2016.

Tri-State Initiative Project Tour, 24 attended, took place at various locations, began at UF/IFAS TREC. 2017.

TREC Research, Extension Faculty Seminar Series to Inform the California Avocado Industry of Laurel Wilt, 110 attended across three locations, took place in Fallbrook, San Luis Obispo, and Ventura California. 2017.

Round Table Discussion of Potential Effects of Hurricane Irma on Laurel Wilt and Ambrosia Beetles, 38 attended, took place at UF/IFAS TREC. 2017.

Epidemiology and Management of Laurel Wilt, 44 attended, took place at UF/IFAS Extension Miami-Dade County. 2017.

Ambrosia beetles associated with laurel wilt in avocado. University of Georgia, Athens, GA, Feb 2017.

The research findings of this project were also summarized and made available at: https://trec.ifas.ufl.edu/RAB-LW-2/RAB_Recommendations.shtml. This website receives approximately 7K visits and 24K page views per year.

Goals and Outcomes Achieved

Goal 1
Established: Avocado growers increase their use of EF and do not rely solely on use of pesticides to manage LW vectors.

Accomplished: According to the Florida Avocado Administrative Committee records from the last 3 years 3,138.45 acres (~42% of the commercial acreage) and approximately 260 parcels incorporated entomopathogenic fungi in their pest management plan. Our target of a 30% increase in the number of avocado producers transitioning from complete reliance on chemical insecticides to adopting EF to manage LW vectors was accomplished.

Goal 2
Established: Decrease the amount of chemical pesticides currently being used to manage ambrosia beetles that vector LW.

Accomplished: Avocado growers that incorporated EF in their pest management plan against LW vectors replaced at least three applications of chemical insecticides (Malathion, Hero, Danitol, Talstar and EpiMek) with EF. This represents roughly a 25-30% reduction in the use of broad-spectrum chemical insecticides overall in the avocado producing area. We were aiming for 40% reduction; therefore, this goal was partially achieved.

Goal 3
Established: Avocado producers improve their management practices by combining EF with compatible pesticides within a comprehensive spray program.

Accomplished: Results of the EF compatibility tests were presented in multiple workshops and grower meetings (see Beneficiaries section) and incorporated in the UF/IFAS Laurel Wilt Ambrosia beetle control recommendations (available at: https://trec.ifas.ufl.edu/RAB-LW-2/RAB_Recommendations.shtml). All avocado producers using EF are avoiding incompatible mixtures and using compatible combinations of fungicides and insecticides with EF.

Goal 4 (established)
Established: Reduce pest management costs by combining chemical pesticides with reduced rates of EF while maintaining the same level of pest control of the full (costly) label rate of commercially available EF.

Accomplished: Avocado growers are currently using EF at the lowest recommended rate and combining it with compatible pesticides and adjuvants. Because of the high cost of EFs, the strategy is to attempt to suppress the ambrosia beetle population with 2-3 applications from February through May when beetle populations are increasing.

Goal 5 (established)
Established: Increase knowledge of avocado producers on biological control and IPM alternatives for management of ambrosia beetles that vector LW in avocados.

Accomplished: Participants of various workshops and seminars have reported high rates of knowledge gains (73-100%). Results of surveys show increased adoption of IFAS recommendations (50-75%) to manage vectors of laurel wilt by avocado producers.

Publications

Avery, P. B., Pick, D. A., Aristizábal, L., Kerrigan, J., Powell, C. A, Rogers, M. E. and Arthurs, S. P. 2013. Compatibility of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) blastospores with agricultural chemicals used for management of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae). *Insects* 4: 694-711.

Carrillo, D., Dunlap, C. A., Avery, P. B., Navarrete, J., Duncan, R. E., Jackson, M. A., Behle, R. W., Cave, R. D., Crane, J., Rooney, A. P., and Peña, J. E. 2015. Entomopathogenic fungi as biological control agents for the vector of the laurel wilt disease, the redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae). *Biological Control* 81: 44-50.

Menocal, O., Cruz, L. F., Kendra, P. E., Crane, J. H., Cooperband, M. F., Ploetz, R. C., and Carrillo, D. 2018. *Xyleborus bispinatus* reared on artificial media in the presence or absence of the laurel wilt pathogen (*Raffaelea lauricola*). *Insects* 9: 30; doi: 10.3390/insects9010030

Menocal, O., Cruz, L. F., Kendra, P. E., Crane, J. H., Ploetz, R. C., and Carrillo, D. 2017. Rearing *Xyleborus volvulus* (Coleoptera: Curculionidae) on media containing sawdust from avocado or silkbay, with or without *Raffaelea lauricola* (Ophiostomatales: Ophiostomataceae). Environmental Entomology 46: 1275-1283.

Zhou Y., Avery, P. B., Carrillo, D., Duncan, R. H., Lukowsky, A., Cave, R. D. and Keyhani, N. O. 2018. Identification of the Achilles heels of the laurel wilt pathogen and its beetle vector. *Applied Microbiology and Biotechnology* 102: 5673-5684. doi: org/10.1007/s00253-018-9037-y.

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Lessons Learned

The implementation of new pest management tactics by growers is influenced by multiple factors including economic considerations, side effects of the proposed tactic, and interactions with other components of the production system.

Total Funding Expended to Date: $55,323.23

Percentage of Project Completed: 100%

FINAL PERFORMANCE REPORT

FINAL PROJECT REPORT TEMPLATE

Final Performance Reports must illustrate the completion of each project within the grant agreement. Each project shall be outlined as separate project profiles. You will report on projects in the same order they were submitted in the approved State Plan and subsequent amendments.

FDACS CONTRACT NUMBER:

#22904

PROJECT TITLE:

Appropriate rootstock and fertilizer regimes for the efficient production of Finger Lime

PROJECT IMPACT AND FINDINGS

Include a summary of the project results of 250 words or less, suitable for dissemination to the public. A statement of results provides a brief description of your project, its success, and/or lessons learned. A statement of results should:

1. *Include the name of the organization that led and executed the project (State department of agriculture or subrecipient);*
2. *Capture the project's purpose and activities completed;*
3. *Outline the outcomes and indicators achieved, results/important findings and lessons learned; and*
4. *Highlight the target population reached and products developed (including intended use and audience).*

The University of Florida evaluated the Finger lime clone 'DPI50-36' budded onto 15 different commercial rootstocks and grown under three different fertilizer regimes. Results were also disseminated to educate stakeholders on improved cultivation practices. Trees were slower to establish when compared to conventional citrus and remained HLB negative for the duration of the study. In initial years, different fertilization treatments did not have a significant effect on tree growth. The major determinant for tree growth and development was the rootstock used. Trees on Volkamer Lemon has the best growth, while Cleopatra mandarin and C22 produced dwarf trees. Addition of supplemental micronutrients coated in a sulphur bentonite clay mix proportionately increased the leaf micronutrient content. Trees did not flower during the study period and the yield data could not be obtained. Several trees were uprooted following hurricane Irma, but 90% of the stressed trees recovered on being reset. Several stakeholders have shown interest in evaluating the Finger lime as a niche specialty crop for the fresh fruit market. Due to slow growth of trees, study could not be completed in the given time frame.

BENEFICIARIES

Number of project beneficiaries: 12

ACTIVITIES PERFORMED

OBJECTIVES

Provide the approved project's objectives.

#	Objective	Completed?	
		Yes	No*
1	Comparing Finger Lime-rootstock interactions for HLB tolerance and optimum tree growth.	X	

2	Develop a fertilization program that will allow profitable Finger Lime cultivation in the Florida HLB-endemic environment.		X
3	Increase in knowledge on Finger Limes and benefits to its cultivation	X	
4			

**If no is selected for any of the listed objectives, you must expand upon this in the challenges and lessons learned sections.*

ACCOMPLISHMENTS

List your accomplishments for the project's period of performance, including the impact they had on the project's beneficiaries, and indicate how these accomplishments assist in the fulfillment of your project's objective(s), outcome(s), and/or indicator(s).

Accomplishment/Impact	Relevance to Objective, Outcome, and/or Indicator
We have identified rootstocks that can result in vigorous growth of trees	Relevant to objective 1. Stakeholders have a better idea which commercial rootstocks are superior to produce Fingerlime.
Our pest management program has successfully prevented trees from getting infected with HLB after planting	Relevant to objective 1. Spray regime was sufficient to keep trees from getting infected with HLB. This is significant since HLB is a major issue in citrus and citrus like fruit production.
Fertilization programs have indicated optimum leaf nutrient status	Relevant to objective 2. Optimum nutrient availability can improve tree growth and productivity. Our results indicate that the fertilization program adopted in this study can provide optimum nutrients for sustained growth.
Several grower-oriented publications and presentations at professional meetings have been made	Relevant to objective 3. There has been an increase in interest on finger lime cultivation and several stakeholders have expressed interest in evaluating fingerlimes in their property. Additionally, dissemination of information has resulted in increased awareness of this crop to stakeholders who had no prior knowledge of its existence.
A superior commercially available finger lime clone has been imported from California	Relevant to objective 3. This was initiated through stakeholder discussions who expressed desire to import superior clones not available in Florida. A superior clone has been introduced by DPI and will be available soon after clearing quarantine procedures.

CHALLENGES

If you experienced any challenges during the project's period of performance, provide a listing of them below. Also, provide the corrective actions you took to address these issues. If you did not attain the approved outcome(s) and indicator(s), provide an explanation in the Corrective Actions column.

Challenge	Corrective Actions
Tree growth is slower than conventional citrus	Trees will have to be evaluated for a longer duration to obtain concrete results
Effects of fertilization treatments unclear	Trees will have to be evaluated for a longer duration till flowering and fruit set to obtain concrete results
Tree damage caused by hurricane and wild hogs	Wild hogs were trapped and removed. Hurricane damaged trees were reset.

LESSONS LEARNED

Provide recommendations or advice that others may use to improve their performance in implementing similar projects.

Woody plants are slower to establish than conventional row crops and require evaluation for longer duration than that permitted by the FDACS 2 year grant cycle. Complete evaluation of this crop would require at least 4 years to obtain fruit yield and other harvest data.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Describe your plans for continuing the project (sustainability; capacity building) and/or disseminating the project results.

We hope to complete this project in the next two years, beyond the current time frame of the current grant. We plan to request a renewal of this grant. A field day with interested stakeholders is also planned once the trees have flowered and fruited. We will continue to disseminate information on our field trial at appropriate intervals to interested stakeholders and to the scientific community.

OUTCOME(S) AND INDICATOR(S)/SUB-INDICATOR(S)

Provide the results of the project outcome(s) and indicator(s) as approved in your State Plan and project proposal. The results of the outcome(s) and indicator(s) will be used to evaluate the performance of the SCBGP on a national level.

OUTCOME MEASURE(S)

Select the Outcome Measure(s) that were approved for your project.

- ☐ **Outcome 1**: Enhance the competitiveness of specialty crops through increased sales
- ☐ **Outcome 2**: Enhance the competitiveness of specialty crops through increased consumption
- ☑ **Outcome 3**: Enhance the competitiveness of specialty crops through increased access
- ☐ **Outcome 4**: Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources
- ☐ **Outcome 5**: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems
- ☐ **Outcome 6**: Enhance the competitiveness of specialty crops through increasing the number of viable technologies to improve food safety
- ☐ **Outcome 7**: Enhance the competitiveness of specialty crops through increased understanding of the ecology of threats to food safety from microbial and chemical sources
- ☑ **Outcome 8**: Enhance the competitiveness of specialty crops through enhancing or improving the economy as a result of specialty crop development

OUTCOME INDICATOR(S)

Provide the indicator approved for your project and the related quantifiable result. If you have multiple outcomes and/or indicators, repeat this for each outcome/indicator.

Outcome indicator 1: Comparing Finger Lime-rootstock interactions for HLB tolerance and optimum tree growth.
Quantifiable result: We have identified several rootstocks that can be suitable for finger lime cultivation and tree growth has been optimum in several of them. Volkamer lemon rootstocks resulted in the most vigorous tree growth followed by the two commercially available rotostocks: Swingle and Carrizo. Rootstocks such as Cleopatra mandarin or C22 resulted in dwarf trees. All trees have been negative for HLB.
Outcome indicator 2: Develop a fertilization program that will allow profitable Finger Lime cultivation in the Florida HLB-endemic environment.
Quantifiable result: The fertilization treatments have provided valuable insight into tree growth. In the fist year there was no difference in any of the fertilizer treatments. In the second year, coated slow release treatments were superior to conventional quick release fertilizer. Addition of a sulphur bentonite clay mix did not substantially increase any of the tree growth parameters studied.
Outcome indicator 3: Increase in knowledge on Finger Limes and benefits to its cultivation

Quantifiable result: There has been an interest in Fingerlimes and its production among a section of the stakeholders. Primarily smaller growers have shown interest in evaluating Fingerlimes in their property as a niche specialty crop. Additionally, we have also had international visitors visit our test site and express interest in future collaboration.

DATA COLLECTION

Explain what data was collected, how it was collected, the evaluation methods used, and how the data was analyzed to derive the quantifiable indicator.

Trees have been supplemented with Fertilizers and Tigersul Micros as outlined in the project document (Activity 2). Trained scouts from the University of Florida have visually evaluated trees on a routine basis and suspect trees have been evaluated for the presence of HLB disease using qPCR. Our results indicate that HLB was not detected in any tree during 2017 – 2018. Tree growth in year 1 and 2 has remained slow when compared to conventional citrus cultivars. Similar results have also been obtained by CA growers. Trees were measured on a regular interval and trees visually rated on a scale of 0-4 based on the following parameters (0- dead tree, 1- unhealthy tree without significant growth, 2- unhealthy tree with limb dieback and significant chlorosis, 3 – healthy tree with mild chlorosis and 4, healthy trees with no chlorosis and limb dieback) (Activity 3). Data was analyzed using SAS. Leaves have also been sampled on an annual basis, acid washed and sent for analyses of all common nutrients. Leaf collection has been challenging due to small leaf size and large thorns on tree and we have had to use extra precautions during sampling. There has been no significant difference in NPK leaf nutrient content among the different treatments, but micronutrient levels were elevated in the treatment containing Tigersul micros. Soil sampling was conducted on a yearly basis. Tigersul micro amended soil has lower pH than the other treatments. By the end of this grant cycle, Chlorophyll fluorescence and quenching measurements have not resulted in significant differences among the treatments. An article in the Citrus industry magazine entitled "Finger Lime could be a new crop for Florida Growers" has been published and has been well received with it being shared on Facebook and other social media platforms. Additionally, an Electronic Data Information Source of UF/IFAS Extension (EDIS) document on finger limes has been published and another publication explaining finger limes is being submitted. An update from the field test was presented at the FSHS annual meeting in Tampa and the ASHS meeting in Hawaii. Additionally, an extension poster was presented and well received at the Florida Citrus Expo, held in Ft. Myers during 2017 (Activity 4). Several stakeholders have expressed interest in the red finger lime cultivar commercially grown in CA. We initiated paperwork to import in 2016 and budwood has been imported by DPI in fall 2017. Plants are in quarantine and budwood is expected to be released to the stakeholders in 2019. The lack of a good variety is the major issue with stakeholders and we hope the newly introduced variety will help increase popularity of the crop.

CONTACT PERSON

Contact Person for the Project Manjul Dutt

Telephone Number 8639568679
Email Address manjul@ufl.edu

FEDERAL PROJECT EXPENDITURES TO DATE

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	39026	35610.13
Fringe Benefits	4886	6793.25

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Travel	2400	1850.99
Equipment	0	
Supplies	15369.50	14564.61
Contractual	0	
Other	2000	1747.94
Direct Costs Sub-Total		
Indirect Costs		
Total Federal Costs	63681.50	60566.92

PROGRAM INCOME

Source/Nature (i.e., registration fees)	Amount Approved in Budget	Actual Amount Earned
Total Program Income Earned	None	None

ADDITIONAL INFORMATION

Provide additional information available (i.e., publications, websites, photographs) that is not applicable to any of the prior sections.

Be sure to include any documents, publications, or other attachments referenced throughout the report. If the attachments are large, the State Department of Agriculture should consider combining them as an appendix to the full report and submitting the appendix as a separate file.

1. Citrus industry grower magazine article on Finger Lime
2. UF/IFAS EDIS article on Finger Lime
3. Photographs of selected trees in the trial.

Project (14): Characterizing Fermentation Chemistry of Muscadine Grape Juice During Vinification

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Project Summary

In grape, the metabolites initially present in juice contribute to generation of flavor and aroma components during fermentation which are responsible for organoleptic properties of finished wines. The distinct chemical composition of muscadine berry yield wines with poor stability and shorter shelf life compared to Bunch grape. A comprehensive understanding of influence of various berry components on fermentation chemistry and organoleptic properties of finished wine is crucial for effectively improving muscadine wine quality, shelf life, market value and consumer acceptance.

Project Approach

This study was conducted to determine fermenting juice attributes to document the influence of grape berry juice components on organoleptic properties of muscadine wines. Juice of ripe Muscadine grape berry from cultivars Noble and Alachua (red), and Carlos and Doreen (white/bronze) were collected and

juice obtained using a screw press. The metabolites content and chemical properties of juice was determined using various biochemical and instrumental analysis. The Brix level of juice was brought to 22 with sucrose and fermentation initiated by adding (300 mg/L) rehydrated Red Star Montrachet yeast culture. Fermenting juice samples were collected at different time intervals and analyzed to determine changes in their metabolites content, composition and properties during vinification using appropriate biochemical assays and instrumental analysis.

The biochemical analysis showed major differences in chemical constituent's content and characteristics among the muscadine varieties studied. The juice pH varied among cultivars as follows: Carlos = 3.6, Alachua = 4.2, Noble = 3.3 while the Brix was 18.5 = Alachua, 17.5 = Noble, 14 = Carlos and 14.7 = Pam. The sugar analysis showed that muscadine grape juice contained significantly lower amount of sugar compared to European Bunch grape varieties. The titratable acidity of cultivars ranged between 2.27 to 2.81. Noble seed contained highest amount of phenolics followed by skin and pulp tissue. The juice of cv. Alachua contained highest amount of free amino acids while that of cv. Fry contained the lowest amount of free amino acids. Yeast assimilable nitrogen (YAN) and free amino acids content was 227 and 175mg/L (YAN mg/L), and 48.2 mg/L and 46mg/L in Noble and Carlos juices, respectively. Carlos contained lowest amount of phenolics while Noble and Alachua had highest content of phenolics. Thus, the total phenolics content of juice was 273 and 221 mg/L (mg/L Gallic acid equivalent) in Noble and Carlos juice, respectively. The flavonoids content of Carlos juice was 26.1 mg/L (mg/L Quercetin equivalent). HPLC analysis of juice showed that tartaric and malic acids were the major organic acids present in bronze cultivar Carlos whereas Tartaric acid was the only major organic acid found in red variety, Noble. Antioxidant activity of Noble and Carlos juice was 63.73 and 61.07 % radical scavenging activity, respectively.

Fermenting juice samples were collected at different time periods during vinification and subjected to biochemical analysis. The results revealed major changes in free amino acids, phenolics, flavonoids content and antioxidant activity of fermenting juice. Free amino acids content of fermenting juice increased from 48 mg/L to 58 mg/L during vinification in red cv. Noble while their concentration increased from 46 to 54 mg/L in white cv. Carlos. Total phenolics content increased from 273 mg/L to 505 mg/L (mg/L Gallic acid equivalent) in cv. Noble, and from 221 to 333 mg/L in white cultivar Carlos during juice vinification. Flavonoids content also increased from 26.1 to 71.8 mg/L (mg/L Quercetin equivalent) during vinification in cv. Carlos. Antioxidant activity (% radical scavenging activity) decreased from 68% to 48% during vinification in cv. Carlos while in cv. Noble the antioxidant activity changed from 63.73% to 54.13% during vinification. The major organic acids of muscadine juice were tartaric acid, succinic acid, malic acid, lactic acid and citric acid whose content changed during fermentation. The results also showed that in bronze cultivar (Carlos), succinic acid and citric acid concentrations increased during fermentation while in red grape (cv. Noble), tartaric acid was found to be the major organic acid at later stages of vinification. Analysis of samples collected at different periods of vinification also showed major changes in their phenolics content and composition. The total phenolics content of fermenting juice was found to increase during the initial stages (0 to 30 days) of vinification. For example, the phenolics content of fermenting juice doubled in varieties Carlos and Doreen while it remained similar in Noble, Alachua and Pam after 30 days of fermentation. The observed changes in

phenolics content may be attributable to their increased release from berry tissue into surrounding media and enhanced solubility during vinification.

Changes in stilbenes content and composition of fermenting juice was also monitored during vinification. The data showed that piceid content of both white and red cultivars decreased in varying amounts during fermentation. For example, in cv. Carlos piceid content decreased from 56.79 ng/ml to 9.68 ng/ml during fermentation while in cv. Doreen it decreased from 8.11 ng to 4.74 ng/ml. In contrast, in Alachua it increased from 56.48 to 342.23 ng/ml. Quercetin content of cv. Carlos remained similar (around 230 ng/ml) during the fermentation while that of cvs. Doreen, Noble and Alachua increased during the 18 days of fermentation. Resveratrol content of Carlos and Doreen remained similar (3.6 to 5.98 ng/ml) while that of Noble decreased (1799 to 776 ng/ml) and Alachua increased (17 to 134 ng/ml) during fermentation. The syringic acid content of Carlos, Noble and Doreen decreased (11 to 5.6 ng/ml) while that of Alachua increased (1.8 to 18 ng/ml) during fermentation. Scopoletin content of Carlos and Alachua increased (9 to 12.6 ng/ml) while that of Noble decreased (70.89 to 25.46 ng/ml) during fermentation. Caffeic acid content of Carlos and Noble decreased (34 and 226 to 12.7 and 97.34 ng/ml respectively) while that of Doreen and Alachua increased (12 and 3 to 21.97 and 39.48 ng/ml, respectively). P-Coumaric acid content ofCarlos and Noble decreased (19.89 and 219.8 to 1.29 and 35.53 ng/ml, respectively) while that of Doreen and Alachua increased (7 and 6.4 to 6.6 and 13 ng/ml, respectively). E-viniferin content of Carlos and Noble decreased (5.9 and 358 to 3.9 and 217 ng/ml, respectively) while that of Alachua increased (11.4 to 184 ng/ml). Epicatechin content of cvs. Carlos, Noble and Doreen decreased (3.08 and 8.5 to 2 and 0.3 ng/ml, respectively) while that of cv. Alachua increased from 0.5 to 19 ng/ml). Catechin content of juice of all cultivars decreased to varying levels during fermentation. These data suggest that chemical constituents of fermenting juice vary widely and undergo varying level of compositional changes irrespective of berry/juice color. Further, certain phenolics components of juice found to undergo major quantitative changes than others. Overall, the phenolics constituents' content of all juices appeared to decrease during fermentation, especially with increasing periods of vinification. The observed decreases in phenolics content can be attributed to their conversion to flavor/aroma volatiles that are generated during vinification.

Learning about flavor volatiles production in fermenting juice would help correlate changes in juice chemical content/composition with the flavor volatiles production during the same period. The fermenting juice samples collected at different vinification periods were subjected to GC/EI-MS analysis to determine changes in their flavor volatile composition. The analysis revealed presence of > 22 components in juice of cv. Noble. Of these components, 1-butanol, 3-methyl-isopentyl alcohol (40%), ethanol (24%), ethyl acetate (16.7%), isobutanol (7%), 2, 3-butanediol (2%), isobutanol (1.25%), 1-hexanol (0.7%), n-octanol (0.49%), and acetaldehyde (0.3%) were some of the notable compounds found in cv. Noble juice. These components endured varying level of changes during vinification. The isobutanol, acetone, n-octanol, 1-hexanol, and acetaldehyde content decreased while that of ethanol, ethyl acetate, octanoic acid and acetic acid content increased following 18 days of fermentation. The volatiles analysis revealed that before vinification, 1-butanol and 3-methyl-isopentyl alcohol were the major components of unfermented juice (Noble) which decreased from 40% to 30% during vinification. In contrast, ethanol content of juice increased from 24% to about 40% of the total volatiles amount. Overall, the results showed that flavor volatile components content of fermenting juice changed during

vinification where certain components content greatly increased or decreased while the others remained unchanged.

Hazing of wine during vinification, ageing and storage is a major issue that adversely affects muscadine wine stability, shelf life, market value and consumer acceptance. Hazing occurs because of formation of protein-phenolic-tannin insoluble complexes during vinification and ageing. Analysis of these insoluble complexes formed in fermenting juice of cv. Carlos after 30 days of ageing showed that it is mainly composed of low molecular weight proteins/polypeptides. Long storage/ageing periods beyond 60 days resulted in precipitation of high molecular weight protein-tannin complexes suggesting that increasing period of ageing enhanced polymerization of these components and caused formation of more complex high molecular weight products. The results also showed that 'lees' formation, precipitation and hazing grew with increasing alcohol concentration in fermenting juice suggesting that increasing levels of alcohol promotes formation of insoluble protein-phenolics complexes resulting in hazing. Muscadine grapes appears to be more prone to this problem than Bunch grape because they contain relatively high levels of nitrogenous compounds such as amino acids and protein.

Goals and Outcomes Achieved

*The unfermented juice of cultivar Noble was found to contain highest amount of total phenolics compared to other muscadine cultivars tested. Thus, the total phenolics content of juice was 273 and 221 mg/L (mg/L Gallic acid equivalent) in Noble and Carlos juice, respectively, and the flavonoids content of Carlos juice was 26.1 mg/L (mg/L Quercetin equivalent). Among berry tissue, seed contained the highest amount of phenolics followed by the skin and pulp tissue.

*The unfermented juice of cv. Alachua contained highest amount of free amino acids while that of cv. Fry contained lowest amount of free amino acids. Yeast assimilable nitrogen (YAN) and free amino acids content was 227 and 175mg/L (YAN mg/L), and 48.2 mg/L and 46mg/L in Noble and Carlos juices, respectively.

*Tartaric acid and malic acids were found to be the major organic acids in unfermented juice bronze cultivar Carlos whereas Tartaric acid was the only major organic acid found in red variety, Noble.

*Antioxidant activity of Noble and Carlos juices was 63.73 and 61.07% radical scavenging activity, respectively in unfermented juice.

*Muscadine grape varieties were found to contain relatively lower amount of sugar in unfermented juice compared to European Bunch grape varieties.

*Biochemical analysis of fermenting juice samples showed major changes in their amino acids, phenolics, flavonoids, organic acids content and antioxidant activity. These data showed that during vinification, pH of cv. Noble juice decreased gradually after initiation of fermentation while that of cv. Carlos juice decreased by Day 7 and then increased by day 21.

*Amino acids analysis of fermenting juice revealed that their concentration increased from 48 mg/L at the beginning of fermentation to 58 mg/L towards the end of fermentation in red cv. Noble while their concentration increased from 46 to 54 mg/L towards the end of fermentation in white cv. Carlos.

*Sugar analysis of fermenting juice showed that in cv. Carlos, sucrose, glucose and fructose levels remained unaffected until day 3 of fermentation, then degraded rapidly from day 4 onward and

disappeared completely by day 5 of fermentation. In contrast, in cv. Noble glucose breakdown began from day 1 onward and vanished by day 6 of fermentation while sucrose hydrolysis began on day 2 and completed by day 10. In contrast, fructose breakdown began on day 4 and finished by day 10 of fermentation. These data revealed that till day 3 of vinification alcohol production was limited and increased from day 4 onward peaking by day 10.

*Flavonoids content of fermenting juice increased from 26.1 to 71.8 mg/L (mg/L Quercetin equivalent) during fermentation in Carlos grape.

*Antioxidant activity (% radical scavenging activity) of fermenting juice decreased from 68% at the beginning to 48% towards the end of fermentation in cv. Carlos whereas in cv. Noble the antioxidant activity changed from 63.73% to 54.13% towards the end of fermentation.

*Muscadine berry juice found to contain varying amounts of organic acids such as tartaric acid, succinic acid, malic acid, lactic acid and citric acid. The results showed that in bronze grape (cv. Carlos), concentration of succinic and citric acid increased during fermentation. Unlike the white cv. Carlos, in red grape (cv. Noble), tartaric acid was the major organic acid present toward the end of fermentation.

*During vinification, pH of cv. Noble juice decreased gradually after initiation of fermentation. In contrast, the pH of cv. Carlos juice decreased by Day 7 and then increased by day 21.

*The phenolic profiling of muscadine grape juice revealed that juice composition varies widely and undergo varying level of structural changes irrespective of berry/juice color. Further, certain phenolics components of juice found to undergo more dramatic changes than the others. Quantification of total phenolics during fermentation revealed an increase in total phenolics content from 273 mg/L to 505 mg/L (mg/L Gallic acid equivalent) in cv. Noble. Likewise, phenolics content also increased in white variety, Carlos from 221 at the beginning to 333 mg/L towards the end of fermentation.

*Among the 42 muscadine varieties studied, piceid and resveratrol were the major stilbenes while Ɛ-viniferin and pterostilbene were present in trace amounts. The highest stilbenes concentration was found in 'Fry Seedless' (270.20 µg/g fresh weight) while lowest amount was in 'Doreen' (1.73 µg/g fresh weight).

*Resveratrol content of Carlos and Doreen remained similar (3.6 to 5.98 ng/ml) while that of Noble decreased (1799 to 776 ng/ml) and Alachua increased (17 to 134 ng/ml) during fermentation. Overall, the phenolics constituents' content of all juices appeared to decrease during vinification, especially with increasing periods of fermentation.

*The GC/EI-MS analysis of berry volatiles showed that isobutanol, acetone, n-octanol, 1-hexanol, and acetaldehyde content decreased while that of ethanol, ethyl acetate, octanoic acid and acetic acid content increased following 18 days of fermentation.

Beneficiaries

The main beneficiaries of this research will be the 26 wineries in the state of Florida as well as other wineries located across the southern States. It would also help hundreds of hobbyist wine makers and entrepreneurs who currently consume Muscadine wine. The outcome of this research will significantly increase muscadine berry and wine quality, number of wineries, consumers and entrepreneurs associated with Muscadine industry.

Lessons Learned

The information gained points to the fact that chemical constituents of fermenting juice differ widely and undergo varying level of structural and compositional changes irrespective of berry/juice color. Furthermore, some juice components appear to undergo more dramatic changes than others. Overall, the phenolics constituents' content of all juices appeared to decrease during fermentation, especially with increasing periods of vinification.

The grape berry composition is known to differ season to season due to varying environmental conditions which significantly impacts its finished wine characteristics. To better understand the interrelationship between season, berry composition and finished wine quality ripe berry from four red and bronze muscadine cultivars viz. Noble and Alachua (red), Carlos and Magnolia (white) will be collected, juice extracted and analyzed to determine its metabolite content and composition and subjected to fermentation studies. Aliquots of fermenting juice will be collected at different vinification points and aging periods, and analyzed to determine changes in their metabolites content, and identify flavor volatiles generated during vinification and aging of muscadine wines. Comparative analysis of biochemical data obtained from multiple seasons will be conducted to better understand the impact of season and environment on berry composition and its effect on finished wine characteristics to help develop vinification protocols to consistently produce superior muscadine wines. The outcome will be disseminated to grape growers, wineries and consumers to increase awareness about influence of berry composition on finished muscadine wine characteristics, stability, shelf life and market value.

The original Principal Investigator, Dr. Devaiah Kambiranda, who was initially involved in directing and managing the project research accepted a faculty position at a different institution and parted with Florida A&M University in May 2017. Fortunately, we were able to identify another faculty member Dr. Ramanjaneya V.R. Mula who has expertise to continue project activities assumed the PIship of this project and continued with various experiments and activities proposed in the project. Unfortunately, Dr. Mula passed away unexpectedly shortly after starting the project due to cardiac arrest. I, being a co-PI of the project took over PIship and had to recruit another researcher to complete project research which slightly affected project timeline. During the 2017 season hurricane Erma caused severe damage to grapevines and loss of ripe berry that were dedicated for use in this study. As a result, we had to rely on berry from second harvest for experimentations to meet project objectives.

These unexpected events slightly affected project time line by delaying sample collection, processing and analysis protocols. Although these unforeseen problems slightly affected originally planned activities they did not adversely impact overall project activities and progress. We were still able to collect necessary plant material, conducted planned research activities and achieve project goals.

Total Funding Expended: $71,019.40

Percentage of Project Completed: 100

Project 15: Breeding Lettuce for High Resistance to Lettuce Bacterial Leaf Spot

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Collaborators: Drs. Yunwen Wang and William Wadlington, postdoctoral researchers. EREC

Dr. Christian Miller. Commercial Vegetable Production and Tropical Fruits. Extension Agent Palm Beach County

Dr. Richard Raid. Plant Pathology Department. EREC

Industry Collaborators:

General Management Growers Inc

Duda Fresh

TKM-Bengard

Veg-Pro

Roth Farms

3 Star Lettuce

Other Collaborators:

Dr. Richard Michelmore. The Genome Center and Plant Sciences Department. University of California, Davis

Project Summary

Lettuce (*Lactuca sativa* L.) is grown in the winter months in the Everglades Agricultural Area (EAA) of South Florida; lettuce is an economically important vegetable crop to the state, with a market value of $60 to $70 million. In Florida, climate can be conducive to the development of bacterial leaf spot (BLS) disease caused by the bacterium *Xanthomonas campestris* pv. *vitians* (*Xcv*). This disease is sporadic, and outbreaks may occur whenever the climate is cool and humid and when occasional rains occur during the season. Because any leaf spotting in lettuce if highly undesirable to the consumer, even low disease severity can affect market value.

BLS outbreaks with varying severity have occurred in Florida for the last 25 years. The first widespread outbreak of BLS in Florida occurred in the winter of 1992-1993, causing approximately $4 million in losses (Pernezny et al, 1995). The worst incident was in 2010 when a widespread outbreak devastated the regional lettuce industry. BLS was responsible for causing significant damage resulting in near 100% crop losses, which translated to millions of dollars of losses. Since 2010, sporadic outbreaks have occurred at the EAA; the last one during the season of 2017-2018. This outbreak did not cause economic crop losses, because it was detected early and crop residuals were immediately destroyed. No chemical controls are consistently effective against this bacterium, and because a BLS outbreak cannot be predicted by forecast, it's unlikely any preventive chemical control for BLS would ever be cost effective for growers.

The best solution to the persistent BLS threat continues to be breeding BLS resistance lettuce varieties adapted to Florida. The initial work to breed BLS resistant lettuce in Florida was funded by a previous 2013 Florida Specialty Crop Block Grant Program "*Enhancing Bacterial Leaf Spot Resistance in Lettuce for Sustainability*". In the previous project, a novel source of resistance was discovered among 30 lettuce cultivars. The USDA Plant Introduction (PI) 358001-1 was immune to three representative Florida strains of *Xcv,* L44, JF196 and L7, one of the most virulent strains collected in the state to date (Wang et al, 2015).

After identifying PI 358001-1, the genetics of resistance was studied in a $F_{2:3}$ mapping population made between PI 358001-1 (resistant) and cv. Tall Guzmaine (susceptible) (**Figure 1.A.**). The population was genotyped using molecular markers such as Simple Sequence Repeats (SSRs), Augmented Fragment Length Polymorphisms (AFLPs), Resistance Gene Candidates (RGCs), Cleaved Amplified Polymorphisms Sequences (CAPS) and phenotyped in two greenhouse experiments. A single locus was mapped to the end of linkage group 2, which is a genomic region known for a high density of resistance gene candidates (RGCs) (Wang et al. 2016).. The resistance locus from PI 385001-1 was named *Xcvr* (*Xanthomonas campestris vitians resistance*) (**Figure 1.B.**) (Wang et al, 2016) The finding was significant because a single resistance locus trait is more efficiently transferred to adapted susceptible varieties using conventional breeding approaches.

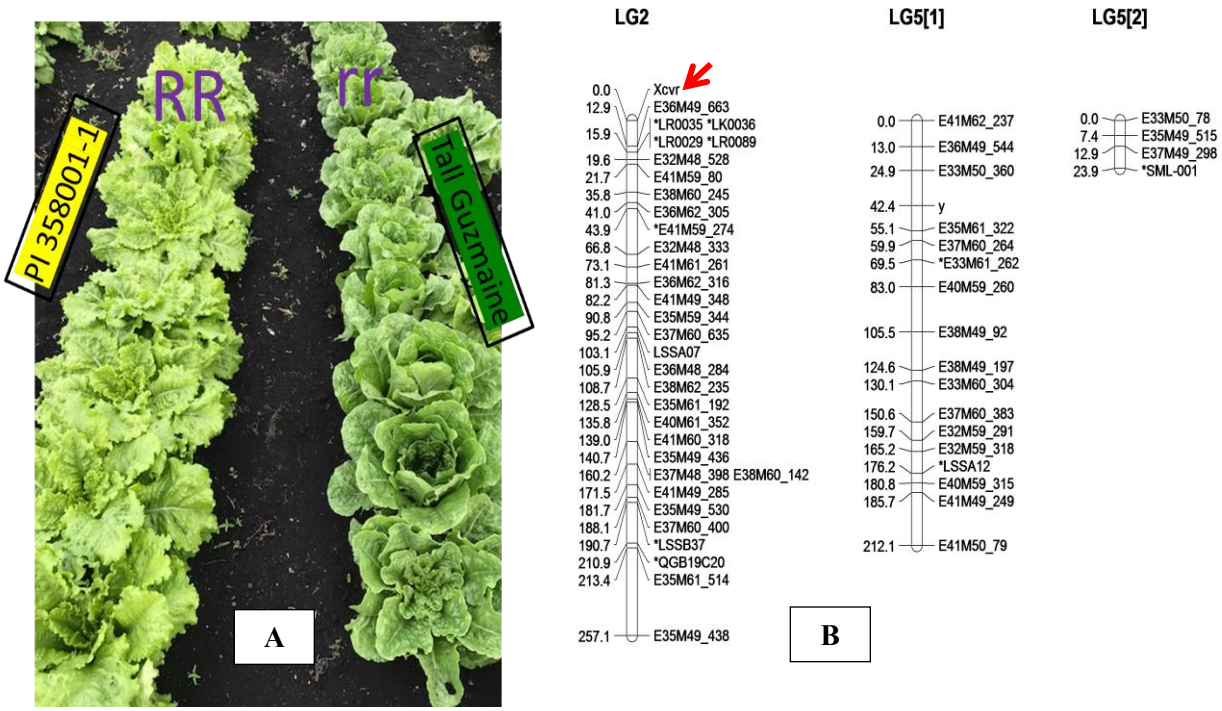

Figure 1. The resistant, 'PI 358001-1' on the left is a light green, leaf type lettuce with savoy and undulated leaves. The susceptible, Florida adapted cultivar 'Tall Guzmaine,' on the right, is a darker green, romaine type lettuce with flatter leaves **(A)**. The genetic map of population 'PI 358001-1 × Tall Guzmaine' with resistance locus named *Xanthomonas campestris vitians resistance* (*Xcvr*) mapped in Linkage Group (LG) 2. The position of *Xcvr* is indicated with a red arrow **(B)**.

This project is a continuation of findings in the grant "*Enhancing Bacterial Leaf Spot Resistance in Lettuce for Sustainability*" which paved the way for a straightforward breeding project to develop marketable Florida adapted cultivars of lettuce with BLS resistance from PI 358001-1. This work involves creating and maintaining multiple breeding populations that segregate for resistance and marketable traits. Families from those populations that are both BLS resistant and marketable can be selected and continued for further breeding.

Traditional breeding approaches (crossing, selecting, trait testing, selecting again) are time consuming. Molecular Markers have been successfully used to transfer several single dominant loci and widely adopted by the seed industry. We aimed to develop a molecular marker for Marker Assisted Selection (MAS) to accelerate the breeding process.

Descendants from those horticulturally acceptable resistant lines must be further tested in detail for desirable horticultural traits, such as postharvest quality, because lettuce growers in the EAA ship their lettuce from Florida all the way up north to the east coast of the USA and Canada. A desirable breeding line/cultivar must possess good horticultural traits to be commercialized.

Results of this project were constantly shared with the lettuce growers, stakeholders and extension agents to understand breeding targets for the lettuce industry. It is imperative to get feedback especially for growers to design and decide breeding strategies. This project was a collaboration between university scientists (researchers and extensionists) and lettuce growers. To structure this project, work was clustered into four major objectives:

1. to continue to transfer the resistance to adapted iceberg cultivars and additional romaine cultivars,
2. to conduct large scale variety trials for evaluation of yield, horticultural characteristics, and post-harvest qualities of the newly selected resistant varieties at multiple locations, and,
3. to develop molecular markers that are associated with the resistant gene and can be used in marker-assisted breeding to accelerate resistant cultivar development, and,
4. to increase grower knowledge about the benefit of using resistant cultivars for control of BLS.

Project Approach

Objective 1. to continue to transfer the resistance to adapted iceberg cultivars and additional romaine cultivars

In 2015, 12 initial populations were developed to accomplish this objective between the resistant PI 358001-1 and the romaine cvs. Terrapin, Okeechobee; EREC breeding lines 70096, 10178, 10179, 10198, 10207, 10221, 10380, 50100, and 60182; and the crisphead breeding lines 60167 and H1078. At this point populations were in the F_2 stage. In 2016, 8500 F_2 plants (≈500 plants per population) were grown in the greenhouse. Approximately 200 F_2 plants from each cross combination were selected using as selection criteria "romaine or crisphead appearance" at seedling stage and continued to the F_3 family generation.

Temperatures in winter of 2015 to 2016 were warmer than usual and seed production was poor; therefore, only seed of 271 F_3 families were harvested. DNA was extracted from seedlings of each of the 271 F_3 families and an additional selection of 60 potentially resistant F_3 families was made using the preliminary molecular marker LR0035. In the 2016-2017 season, the 60 F_3 families were planted in the field at the Everglades Research and Education Center (EREC) for selection based on horticultural traits (**Table 1**).

Table 1. Breeding selection of resistant families crossed with PI 358001-1

Crossed to PI 358001-1	Type	Number of Selected F_2	Selection of F_3 using molecular Marker	Resistant F_3 Families [1]	F_4 Families	F_5 Families
10168	Romaine	200	3	1	0	0
10178	Romaine	200	0	0	0	0
10179	Romaine	200	10	1	2	5
10198	Romaine	200	5	2	1 [2]	0
10207	Romaine	200	7	2	3	0
10221	Romaine	200	10	0	0	0
10380	Romaine	200	5	1	10 [2]	9
50100	Romaine	200	1	1	5	4
60167	Iceberg	200	0	0	0	0
60182	Romaine	200	9	0	0	0
70096	Romaine	200	5	1	0	0
H1078	Iceberg	200	1	0	0	0
Terrapin	Romaine	200	0	0	0	0
Okeechobee	Romaine	200	4	1	4 [2]	5
Total		**2,800**	**60**	**10**	**24**	**23**

[1] After testing the 60 F_3 families in two greenhouse experiments with *Xcv* isolate L7

[2] These lines or part of these lines are considered partially resistant to *Xcv*

Separately, seed from the 60 F_3 families were also planted in a controlled greenhouse environment for BLS testing by inoculation with the L7 isolate of *Xcv*. Ten F_3 families were found to be resistant to partially resistant to BLS (**Figure 2**).

No resistant families were selected from the field as they did not have good horticultural traits; a predominant inherited leaf type was observed in field conditions (**Figure 3**). Nonetheless, the partially resistant families identified in greenhouse experiments were planted in the field and a total of 24 selections were made from 13 of the16 resistant families. Those 24 selections were continued to the F_4 and F_5 generation using Single Seed Descend (SSD) selection method.

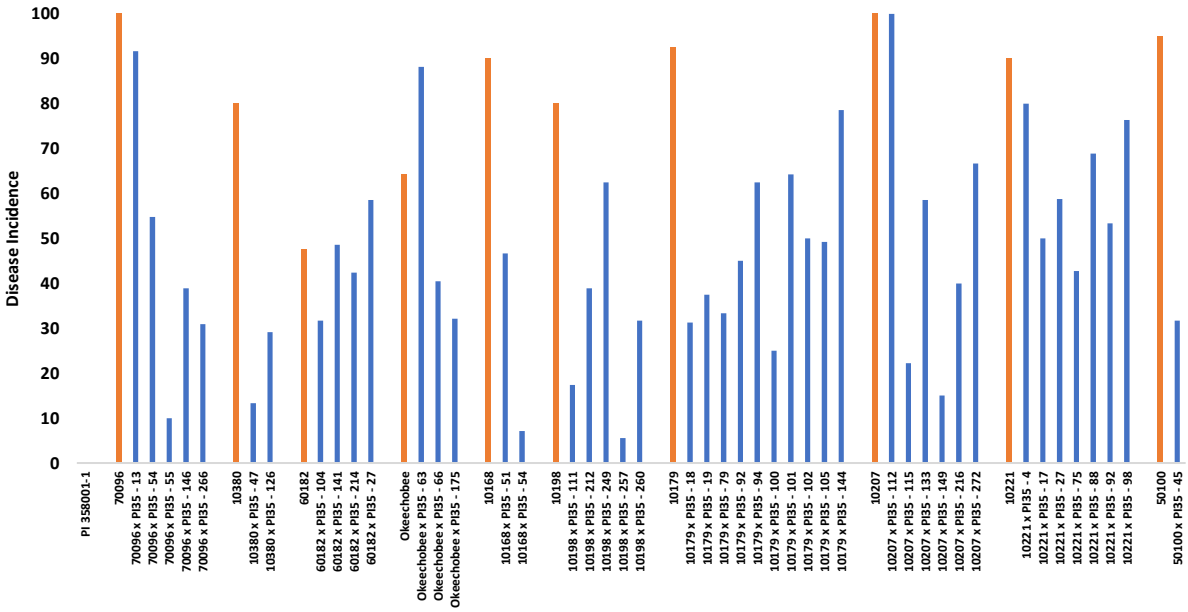

Figure 2. F_3 Families of the crosses of PI 358001-1 with EREC breeding lines 700096, 10380, 60182, 10178, 10198, 10179, 10207, 10221, 50110 and cv. Okeechobee. Lines and parents were tested using isolate L7 in controlled conditions. The graph represents the average of two experiments.

Figure 3. F_3 families planted in field conditions during 2016-2017 season. Families presented a predominant leaf type.

Because many F_3 families were lost due to heat during the 2015- 2016 season and a high frequency of those remaining were of the undesirable leafy type, the F_2 seed from populations listed in **Table 1** were planted in the 2016-2017 field season to select plants with horticultural traits resembling iceberg and romaine types.

The difference between the first selection described and the current selection was plant age; instead of making selection at seedlings, the new strategy adopted was selection at market maturity.

A total of 136 F_3 families potentially resistant to BLS were harvested. In the Fall of 2017, those 136 families were tested in a glasshouse that was equipped especially for testing BLS resistance (**Figure 4**).

In this experiment 12 susceptible lines and the resistant parent PI 358001-1 were included as checks. No disease was observed in PI 358001-1, the resistant donor of these populations. The rest of the parents were confirmed to be susceptible to isolate L7 of *Xcv* (**Figure 5A**).

No F_3 family was found to be as immune to *Xcv* as their resistant parent (disease severity of 0). One line had a media of 0.5 and 13 lines had a media of 1.0 indicating partial resistance in the rating scale of 0 (no disease) to 5 (completely colonized) (**Figure 5B**). Therefore, we identified 14 F_3 families for breeding partial resistance against BLS with acceptable horticultural traits.

Figure 4. Glasshouse specially equipped for BLS testing with two misters and plastic liners to hold the warm air and humidity.

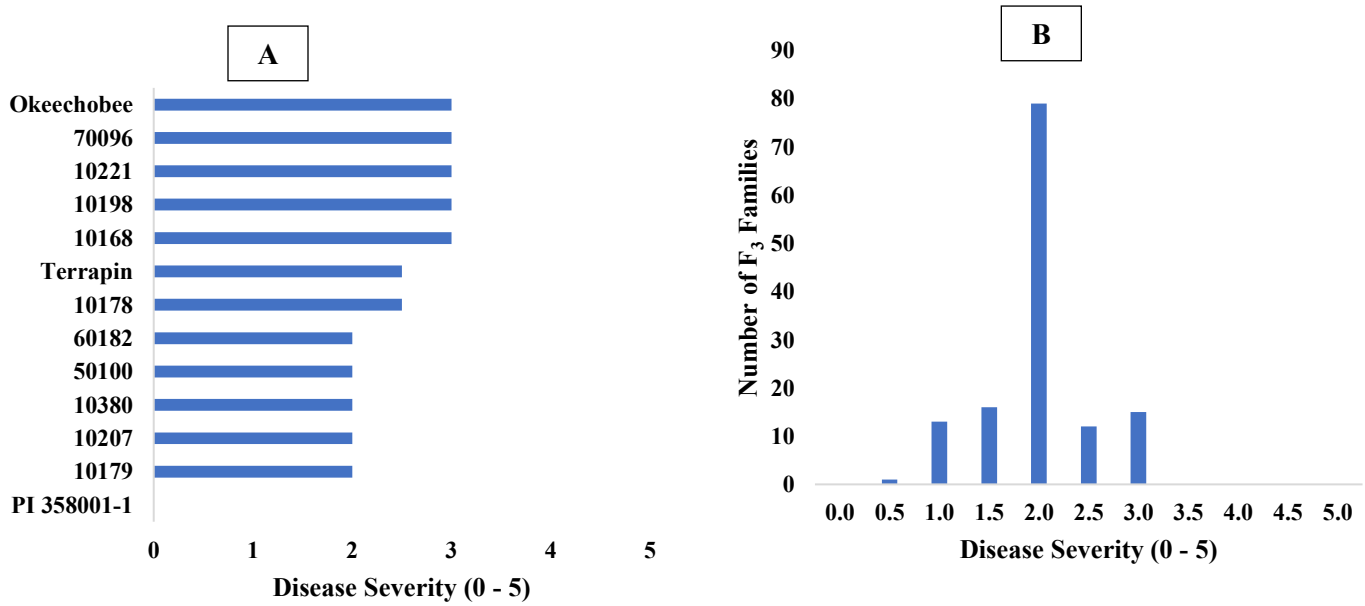

Figure 5. Disease Severity (0 to 5) of the resistant parent (PI 358001-1) and 12 susceptible lines tested in greenhouse conditions with isolate L7 of *Xcv* (A). Number of F_3 families on each category of the Disease Severity (0 to 5) with a total of 136 tested families; families were the result of crossing the resistant parent and the susceptible lines in figure a (B).

Those lines were planted for further selection and tested a second time for BLS resistance with two isolates of *Xcv*; L7 and a newly (2017-2018 season) collected isolate called Sc8B. Four families (BG17-0052, BG17-0111, BG17-0136 and BG17-0141) were resistant to both isolates tested (**Figure 6**). A few other lines showed resistance against isolate L7, but no others showed resistance above level 1 against isolate Sc8B.

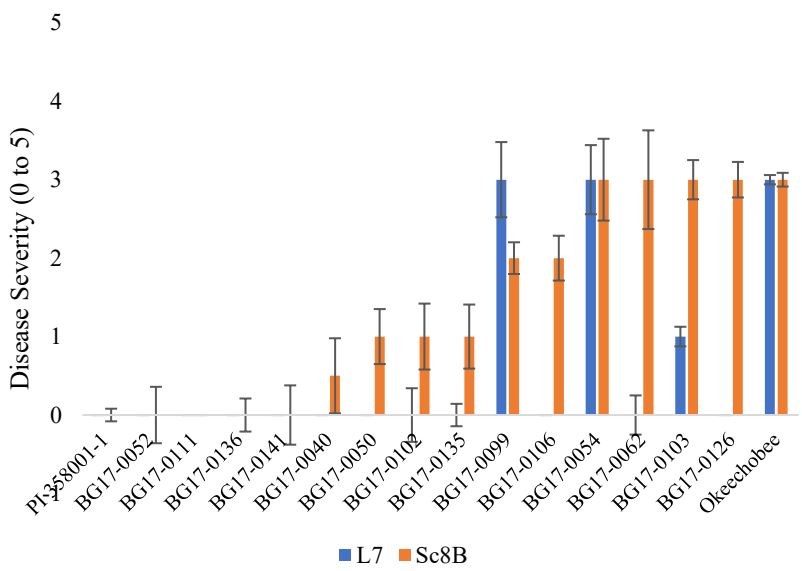

Figure 6. Disease Severity (0 to 5) of PI 358001-1 the resistant parent Okeechobee susceptible control and 14 breeding lines tested in greenhouse conditions with isolates L7 and Sc8B of *Xcv*.

There are yet more experiments to be conducted with the new collected isolate Sc8B, but it seems that this isolate may be more aggressive than L7. Regardless, PI 358001-1 is also resistant to this new isolate. These lines are currently in the F4 stage, but they do not present a horticulturally acceptable type.

The results of the previous season found a predominance of undesirable leafy type characteristics in resistant selections. In the fall of 2017, a backcrossing strategy was implemented to increase the probability of obtaining resistant material with romaine or iceberg character in selections. The F_1 from crosses between PI 358001-1 and Florida adapted varieties (**Table 2**) were backcrossed to their respective susceptible parent to produce BC_1F_1 or "Backcross generation 1" (noted as BC1). The BC_1F_1 seed was sent to the company 3 Star Lettuce in California to generate the F_2 of 10 BC_1F_2 in the spring of 2017 and produce the BC_2F_1 or "Backcross generation 2" (noted as BC2) populations which had a much higher proportion of romaine and iceberg characteristics (**Table 2**).

The 10 BC_1F_2 populations were planted at General Management Inc (GMI) for selection in the season of 2017-2018; a total of 112 BC_1F_2 plants were selected and continued to the BC_1F_3 families for BLS testing. When selecting, a high proportion of plants in these 10 populations had a romaine-like type except in the backcross of (Terrapin × PI 358001-1) ×Terrapin; this indicate the increase of favorable alleles controlling lettuce type. In addition, BC_2F_1 individuals were planted at the EREC in greenhouse conditions to develop seed of 10 BC_2F_2 populations for further field selections.

Table 2. Breeding selection of resistant families backcrossed to the susceptible parents. The first backcross was made in Florida (206-2017 season) and the second backcross was made in California (spring 2017)

Original cross	2016- 2017	Spring 2017 California		2017- 2018 Florida	
	EREC [1]	F_2 seed	Backcross [1]	Selected BC_1F_2	F_2 seed
10168 × PI 358001-1	× 10168	BC_1F_2	× 10168	20	BC_2F_2
10178 × PI 358001-1	× 10178	BC_1F_2	× 10178	14	BC_2F_2
10179 × PI 358001-1	× 10179	BC_1F_2	× 10179	9	BC_2F_2
10221 × PI 358001-1	× 10221	BC_1F_2	× 10221	0	BC_2F_2
10380 × PI 358001-1	× 10380	BC_1F_2	× 10380	9	BC_2F_2
50100 × PI 358001-1	× 50100	BC_1F_2	× 50100	24	BC_2F_2
60167 × PI 358001-1	× 60167	BC_1F_2	× 60167	19	BC_2F_2
70096 × PI 358001-1	× 70096	BC_1F_2	× 70096	8	BC_2F_2
Terrapin × PI 358001-1	× Terrapin	BC_1F_2	× Terrapin	4	BC_2F_2
Total				**112**	

[1] The backcross or cross was produced, the hybrid or F1 was crossed with the susceptible donor for example ((10168 × PI 358001-1) × 10168) × 10168.

We transferred BLS resistance from PI 358001-1 into a diverse array of lettuce varieties. During the lifespan of this project, PI 358001-1 has been crossed into a total of 84 varieties of lettuce, including 43 iceberg, 20 romaine, 12 bib, 5 leaf, 2 Latin types.

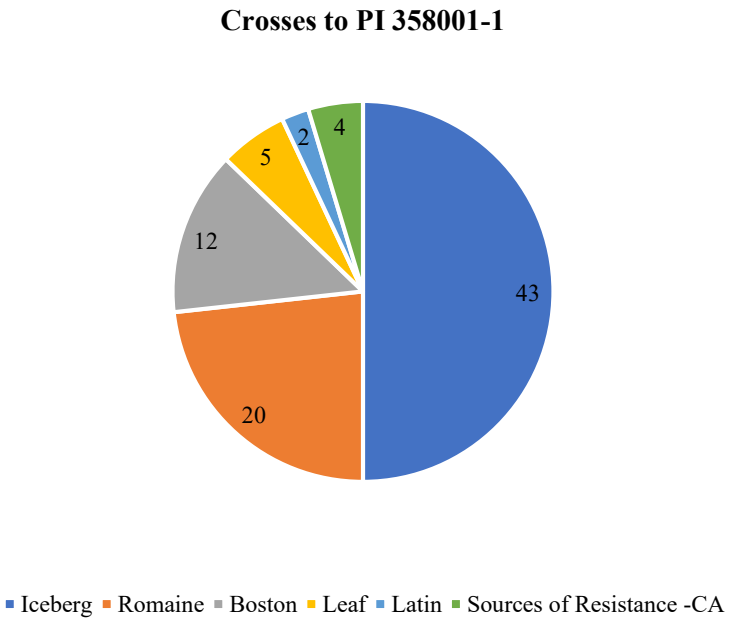

Figure 7. Number of crossed cultivars, plant introductions and other germplasm to PI 358001-1 the resistant donor of lettuce BLS.

In addition, four crosses have been made with well-known sources of resistance to the California *Xcv* strains, cvs. La Brillante, Little Gem, Pavane, and Batavia Reine des Glaces (**Figure 7**).

Sixty-four of these crosses are in the F_2 stage and further breeding could be accomplished in the immediate future.

The new crosses have expanded the UF lettuce breeding pool and transferred resistance into other types of lettuce, like Bib and Latin, which have potential to be commercialized to the Florida market in the future.

In conclusion, crosses of the existent germplasm of the University of Florida to the resistant PI 358001-1 had significantly increased in numbers. There is a substantial and additional amount of F_2 seed for field selection purposes. However, advanced breeding lines (F_4, F_5) do not present both good agronomic traits (e.g., lettuce type) and complete resistance. It seems that genes controlling both traits, resistance and type, may be linked, but this linkage remains yet to be elucidated.

Objective 2. to conduct large scale variety trials for evaluation of yield, horticultural characteristics, and post-harvest qualities of the newly selected resistant varieties at multiple locations

In the 2015-2016, thirty-five F_4 breeding lines from crosses between PI 385001-1 and susceptible romaine lettuce varieties Okeechobee and Tall Guzmaine were planted in General Management Growers Inc (GMI) field for further selection and evaluation of horticultural traits along with cultivars Manatee and Okeechobee for controls (**Figure 8**).

The 2015-2016 season was particularly warm which may have caused plants to bolt earlier as a consequence of the heat. Lettuce is a susceptible crop to heat and respond in several ways when temperatures are warmer than usual, including bolting early, producing unmarketable heads, increased susceptibility to other anomalies such as tipburn, and greatly reduced seed production.

Figure 8. Field experiment planted with 35 F_4 breeding lines of the crosses 'PI358001-1 × Tall Guzmaine' and 'Okeechobee × Tall Guzmaine' during the lettuce season of 2015 – 2016 at General Management Growers Inc (GMI) Belle Glade, FL.

A new strategy was adopted to accomplish this objective. In May 2017, 33 F_4 breeding lines were planted and were inoculated with the isolate L7 of *Xcv* in a controlled environment. The controls in this experiment were the resistant parent 'PI 358001-1' and the susceptible romaine cultivars Tall Guzmaine and Okeechobee. Evaluations for BLS resistance were made 10 days after inoculation using a subjective rating scale 0 to 5, where 0 indicates no spots and 5 is a plant with necrotic collapsed spots. Three lines from the cross of 'PI 358001-1 × Tall Guzmaine' showed little to no disease and were significantly less diseased than the susceptible parents and the other breeding lines. The remaining lines were discarded as they were susceptible to *Xcv* (**Figure 9**).

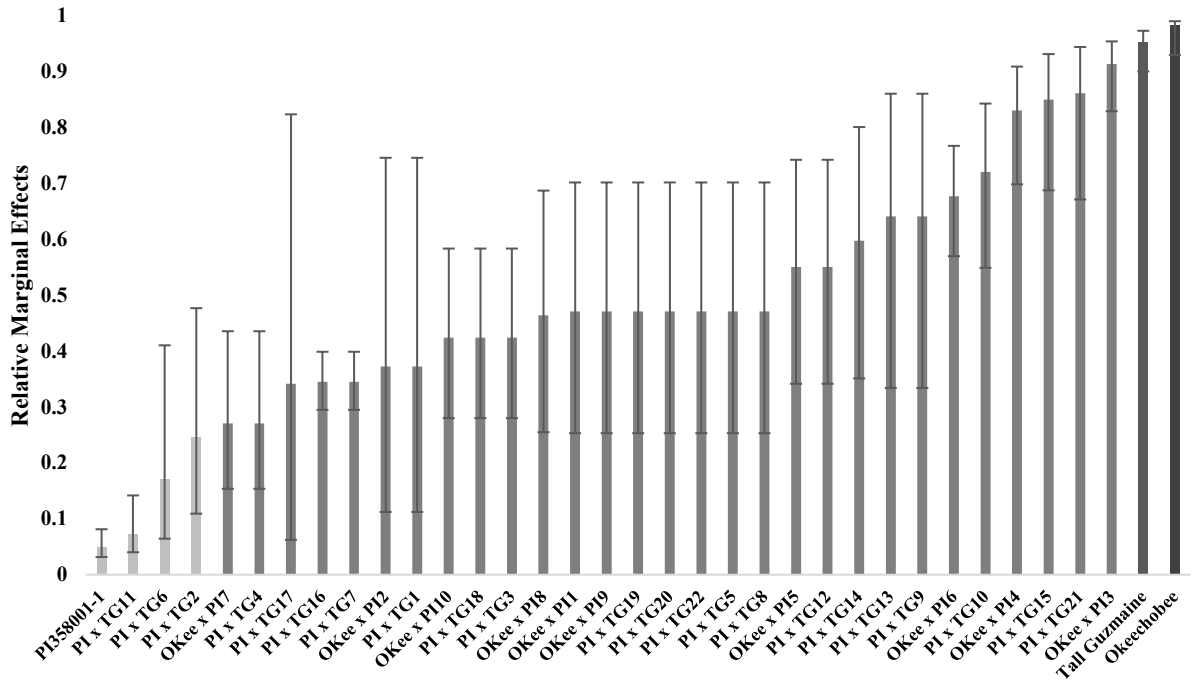

Figure 9. Relative Marginal Effects of the Disease Severity of 31 F_4 breeding lines of the crosses PI 358001-1 × Tall Guzmaine (PI × TG) and Okeechobee × PI 358001-1 (Okee × PI) with isolate L7 of *Xanthomonas campestris* pv. *vitians* (*Xcv*) in a greenhouse experiment in Belle Glade, FL 2017.

These lines were subsequently renamed as BG15-0203 (PI×TG11), BG15-0206 (PI×TG6), and BG15-0210 (PI×TG2) and planted in the field in the 2017-2018 season for postharvest quality evaluations, further selection, and continued to the F_5 generation. The three F_4 lines, the resistant parent PI 358001-1, a highly related to 'Tall Guzmaine' EREC line '70096' and control cultivar 'Okeechobee' were harvested from Belle Glade, FL on December 15, 2017.

Immediately following harvest, lettuce was rinsed to remove excess organic matter. Samples were then hydrocooled with chlorinated water (100mg/L) at pH 7 to 7/8ths cooling (roughly 4-6°C). Excess water was removed, and heads were placed in wax-corrugated boxes within ice coolers. Lettuce was transported to Gainesville, FL for a storage study. Upon arrival, lettuce was stored overnight in 1°C; the following day, noted as 'day 0', evaluations began. Lettuce samples remained in 1°C with 95-98% relative humidity (RH) in breathable packaging film until their evaluation period. The following parameters were measured at days 0, 7, 21, and 28 days after harvesting. For purposes of this report, results are presented only at day 0 and day 21 for moisture content and off flavor and day 7 and day 21 for midrib crispness.

Moisture content. - Samples were weighed immediately after removal from storage, weighed, cut in 1" segments, and whole leaf was dried at 65°C for 24 hours. Upon retrieving dry weight, moisture content was calculated as: $Moisture\ content\ \% = \frac{Fresh\ weight\ (g) - Dry\ weight\ (g)}{Fresh\ weight\ (g)} * 100$

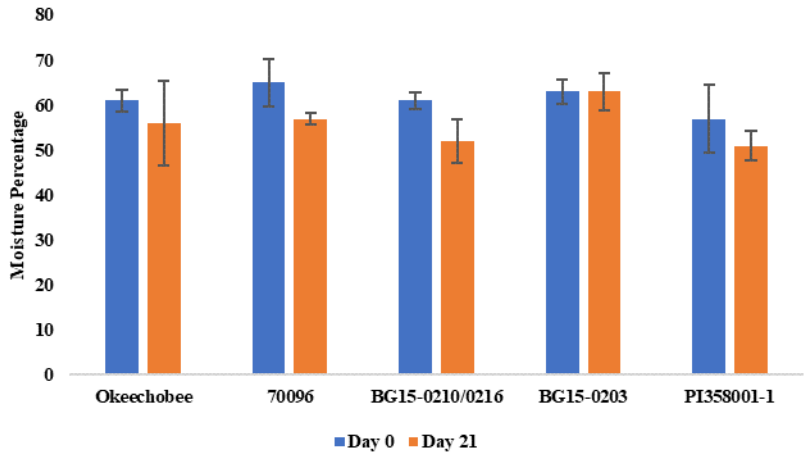

Figure 10. Moisture content at day 0 and day 21 after harvesting of PI 358001-1, the resistant parent, three F_4 breeding lines, noted as 'BG15', a highly related line (70096) to the susceptible parent and cv. Okeechobee.

Results indicated no differences in the percentage of moisture across the tested lines at day 0.

At day 21, the resistant parent PI 358001-1 had the lowest moisture content, as well the mix of two of the F_4 lines, BG15-0210 and BG15-0216. The breeding line BG15-0203 had the highest percentage moisture content 21 days after harvesting (**Figure 10**).

Crispness and Off flavor. - Ratings for crispness, and off flavors were assessed during each storage interval. Samples were rated on a 9-point hedonic scale: 9 = excellent or free from defects; 7 = good only minor defects, no objectionable defects; 5 = fair, slightly to moderately objectionable defects, lower limits of sales appeal; 3 = poor, excessive defects, limit of salability; and 1 = extremely poor, not useable (Kader et al. 1973).

Subjective ratings for midrib crispness indicated differences among the breeding lines and the cultivars tested. The control cv. Okeechobee presented the highest value for midrib crispness followed by the breeding line BG15-0203. Breeding line 70096 and PI 358001-1 had lower values of midrib crispness 7 days after harvesting (**Figure 11**).

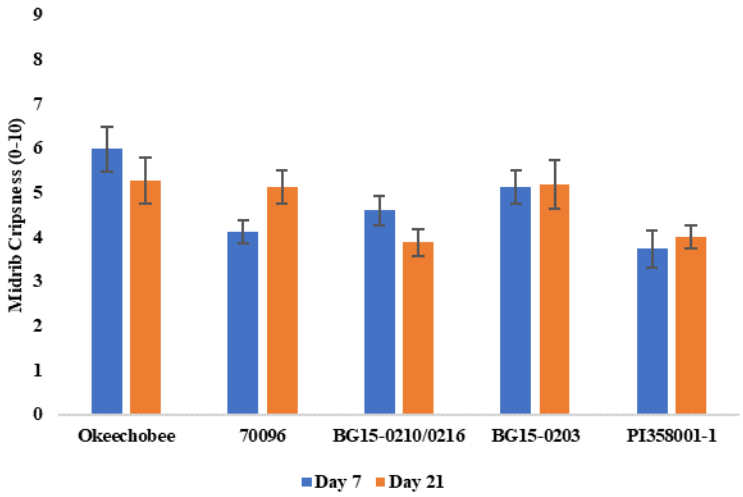

Figure 11. Midrib crispness at day 7 and day 21 after harvesting of PI 358001-1, the resistant parent, three F_4 breeding lines, noted as 'BG15', a highly related line (70096) to the susceptible parent and cv. Okeechobee.

Ratings for midrib crispness 21 days after harvesting followed the same pattern as in day 7. In this experiment, the breeding line BG15-0203 had an acceptable level of midrib crispness when compared to Okeechobee, PI 358001-1, and 70096 (**Figure 11**).

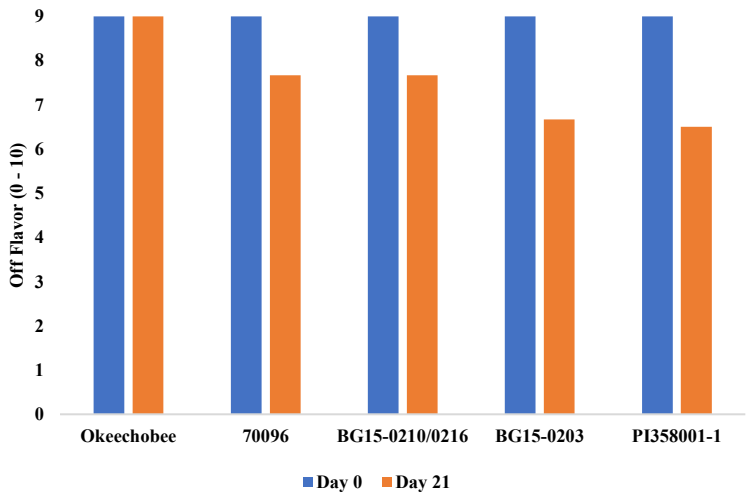

Figure 12. Off Flavor at day 0 and day 21 after harvesting of PI 358001-1, the resistant parent, three F_4 breeding lines, noted as 'BG15', a highly related line (70096) to the susceptible parent and cv. Okeechobee.

The flavor of controls, parents and breeding lines were good, and no differences were found among the breeding lines at day 0 in this experiment (**Figure 12**).

The differences were notable 21 days after harvesting. Okeechobee kept a good flavor, meanwhile PI 358001-1, 70096 and the breeding lines used in this study. The resistant PI 358001-1 had the worst flavor in this group of tested cultivars and breeding lines (**Figure 12**).

In addition to testing these advanced lines, postharvest in modified atmosphere packing was initiated on a set of 10 EREC breeding lines that are part of populations developed for objective 1. Breeding lines 10168, 10179, 10198, 10207, 10221, 10380, 50100, 60182, 70096, H1078, plus the commercial cultivar Okeechobee were harvested, packed in wax-corrugated boxes and passed through the vacuum cooling facility at TKM-Bengard facility, then the samples were sent to Canada for processing by the company VegPro. Briefly, the samples were cut and washed following VegPro protocols; lettuce heads were cut in 1 3/8" x 2" pieces and washed with peracetic acid (65 – 85 ppm) and packed in bags with 454 grams of sample in modified atmosphere (5 to 7% O2 and 0% CO2). Evaluations were done at 6, 10, 13 and 15 days after packing considering appearance (6 = good to 1= poor), color (3=typical to 1=discolored), odor (6 = fresh 1= foul smell), texture (3=crisp to 1=soft) and moisture (3=normal to 1=wet). These samples were processed conjunctly with a control cultivar (not disclosed to us).

Results indicated that our breeding lines had a poor shelf-life compared to the control used by our partner (VegPro) in modified atmosphere packages (**Figure 13**). However, at harvest time in this experiment our material suffered wind damage prior to being harvested in addition to not being completely mature. They also showed greener hearts than the commercial cultivars which might have contributed to these results (**Figure 14**).

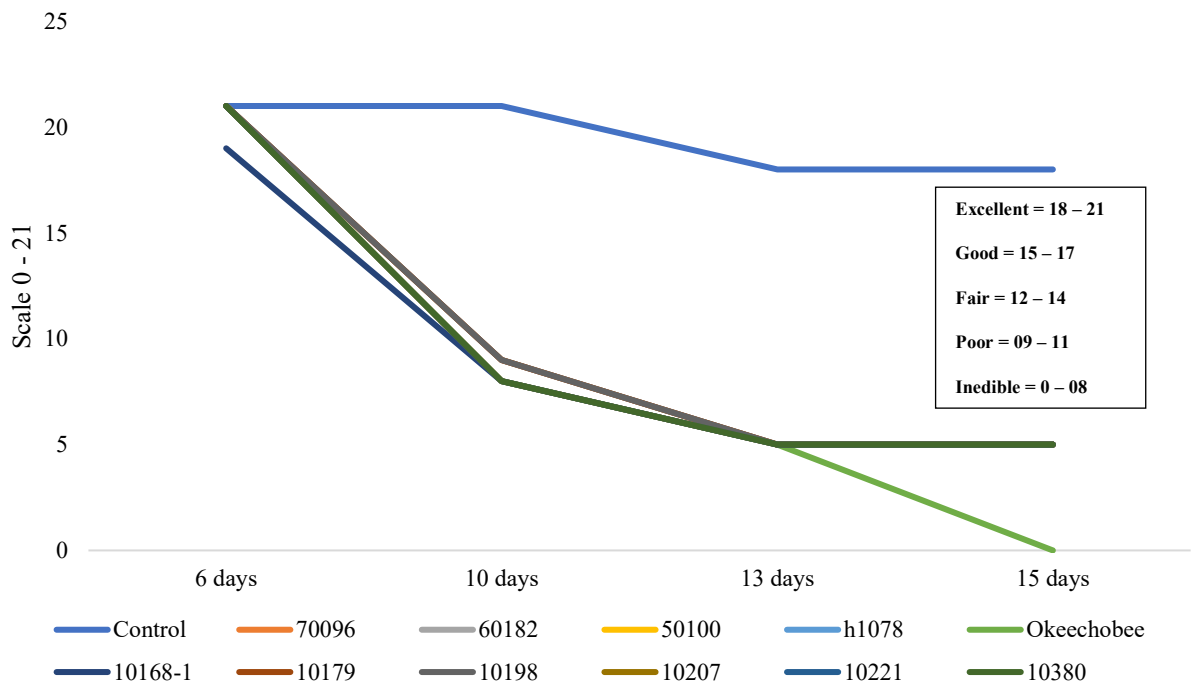

Figure 13. Control and EREC breeding lines processed in modified atmosphere packaging by VegPro. Samples were harvested, vacuum cooled and transported to Canada for processing. Days in the figure indicate days after initial sample processing. The data is a sum of the following parameters: appearance (6 = good to 1= poor), color (3=typical to 1=discolored), odor (6 = fresh 1= foul smell), texture (3=crisp to 1=soft) and moisture (3=normal to 1=wet).

Both tests indicate that our lines have poor postharvest characteristics suggesting that breeding lettuce for BLS resistance should consider additional traits such as horticultural traits and postharvest. Our University of Florida breeding germplasm remains to be characterized for postharvest characteristics (decay, pinking, browning, shelf-life, etc.).

In conclusion, three romaine breeding lines currently in the F_5 stage are part of the lettuce breeding program. These lines have poor postharvest quality and poor agronomic characteristics. There is a need to cross these lines with other romaine germplasm to improve postharvest and other important traits to lettuce grower. Test of these lines for Corky Root Rot (CRR) remain to be conducted, but it is expected that these lines may have tolerance to this disease as 'Tall Guzmaine' is a tolerant cultivar to CRR.

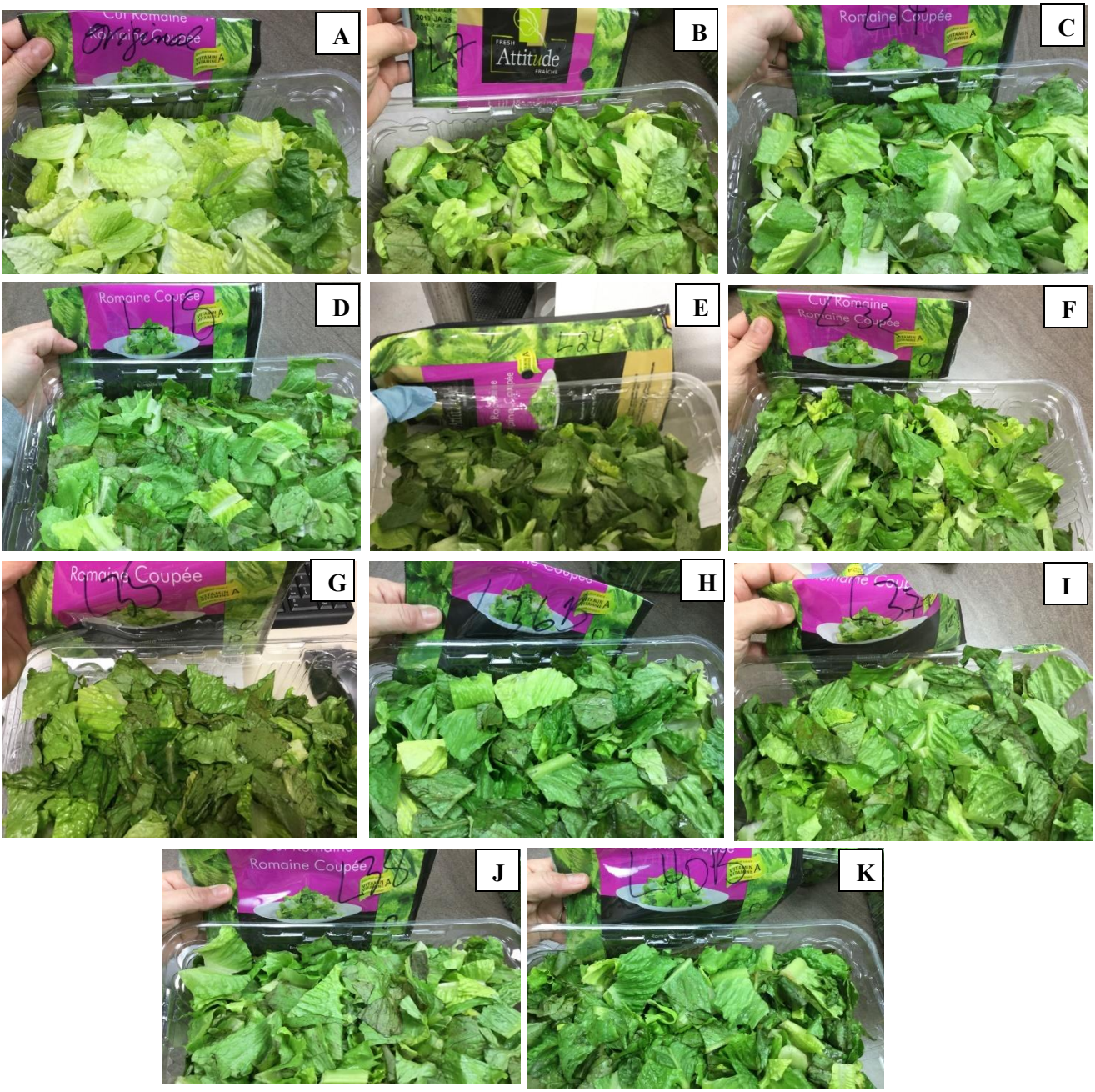

Figure 14. Control (A) and EREC lines 70096 (B), 60182(C), 50100(D), H1078(E), 10168(F), 10179(G), 10198(H), 10207(I), 10221(J), and 10380(K) in a modified atmosphere package test conducted by VegPro International. These lines were used as a susceptible parent to transfer resistance from PI 358001-1.

Objective 3. To develop molecular markers that are associated with the resistant gene and can be used in marker-assisted breeding to accelerate resistant cultivar development

Previously, a mapping population between the resistant PI 385001-1 and the susceptible FL adapted romaine cultivar Tall Guzmaine was used to identify the locus controlling resistance named *Xcvr* (*Xanthomonas campestris vitians resistance*). A genetic map was made using 149 AFLPs, 14 SSRs, 5 RGCs, and 2 CAP markers. These markers were distributed along 6 Linkage Groups (LG) out of the 9 chromosomes in the lettuce genome. The PCR marker closest to the locus of resistance, LR0035 (RGC, Resistance Gene Candidate) was used for selecting resistant F_2 individuals.

In this project, offspring of populations listed in **Table 1** were used to screen F_2 plants for Marker-Assisted Selection (MAS). DNA was extracted from 2,304 F_2 plants and screened with the *RCG* marker (LR0035) (**Table 3**). Sixty potentially resistant F_3 families (**Table 1**) were identified with presence of the resistance allele from PI 3580001-1. Based on these results 60 F_3 families were planted in field conditions as previously described and tested for lettuce BLS in two greenhouse experiments (**Figure 2**). Only 10 families showed partial resistance to *Xcv* isolate L7 indicating that the marker developed from LR0035 was not efficient at selecting resistant families, because the marker was 17 cM (centimorgan) from the resistant locus, making it suboptimal for marker-assisted selection.

A new genetic map was made using genotyping-by-sequencing (GBS) with more complete representation of the lettuce genome and to provide variants that could be used to make PCR-base markers. Genomic DNA of the 162 F_2 individuals used to develop the segregating mapping population was sent to the Genome Center at the Michelmore lab at UC Davis to construct GBS libraries using the *AvaII* enzyme. Libraries were sequenced at the DNA Technologies Sequencing Core of the Genome Center at UC Davis. Reads were aligned to version 5 of the lettuce genome assembly using the TASSEL3 pipeline. Construction of the genetic linkage map was redone with the GBS data using MapMaker. QTL analysis of BLS resistance was computed with QTL Cartographer software using composite interval mapping method at 1,000 permutations.

A new improved genetic map contained a higher marker density (457 Single Nucleotide Polymorphisms) distributed along all 9 Linkage Groups (LG). Previous findings were confirmed as a major and single resistance QTL located on LG2 (**Figure 15**) for Disease Incidence (DI) in two experiments (LOD scores=17.8 and 28.6 for first and second experiment, respectively). One additional small QTL was identified in LG3 and two in LG8 which most likely are “ghost QTLs” with no significant effects. Unlike the first map, the new map constructed using GBS has the physical location of each marker anchored to the chromosomal sequence.

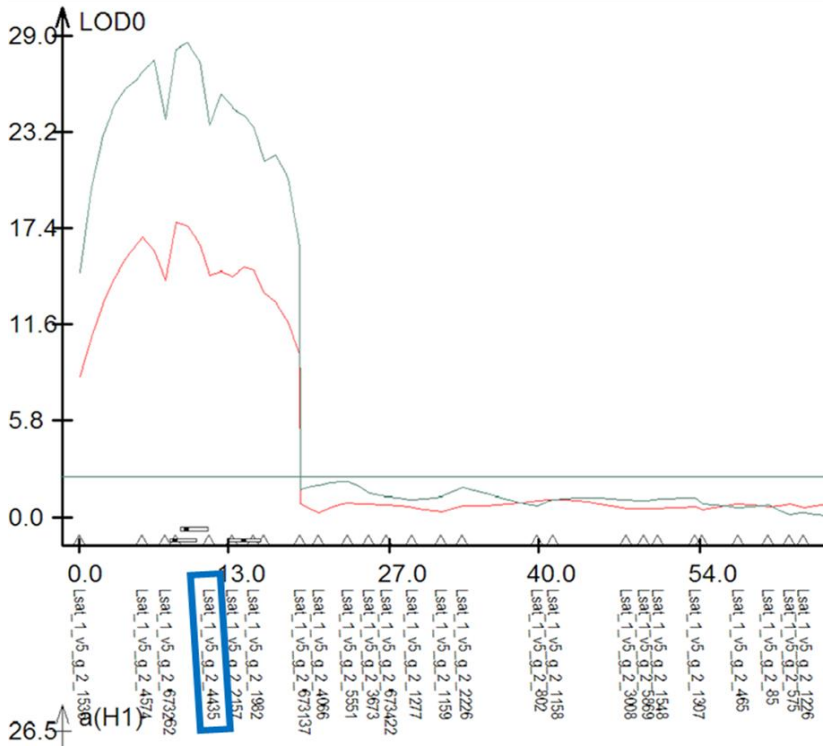

Figure 15. QTLs for Disease Incidence in two experiments with lettuce BLS. Both QTLs are in LG 2. The first experiment is indicated with an orange line (LOD=17.8) and the second indicated with a green line (LOD=28.6). Contigs in version 5 of the lettuce reference genome where GBS tags mapped to was noted along the X-axis. The contig Lsat1_v5_2_4435 is indicated with a blue box which contains the target sequence

This physical map allowed for the design of novel PCR markers based on the variants detected by the GBS data. Six new PCR-based markers were designed to detect sequence variants within ~5 cM of the estimated genomic position of *Xcvr* (**Table 3**). The markers were tested on genomic DNA of the parents used to construct the mapping population PI 358001-1 and Tall Guzmaine (RR, rr). Markers that detected a variant between parents were further validated on genomic DNA of 8 F_2 individuals known to be either homozygous resistant, susceptible, and heterozygous.

Markers were validated on F3 lines from population 'PI 358001-1 × Tall Guzmaine' using three homozygous resistant (RR) lines, heterozygous (Rr) lines, and homozygous susceptible (rr) lines to confirm a genotype-specific banding pattern (**Figure 16**).

Table 3. Primer sequences of markers near BLS resistance

Marker	Forward primer sequence	Reverse primer sequence	Distance from R
LR0035	GTGGGGAAGACGACAATGAT	CCACAAACTGCCATACATCG	17.1 cM
BLS_ww_15	GGGAAGAAGATCGCAGGAAG	CGGTAGCAAGTTTTTAAAACATGAATC	~4 cM

One marker designed for contig 44235 (**Figure 15**) on the lettuce genome (BLS_ww_15) amplified a 600 bp band from Tall Guzmaine (rr), three heterozygous F_2 (Rr), and two

homozygous susceptible families (rr). The marker does not amplify the 600 bp band from PI 358001-1 or three homozygous resistant families (RR) (**Figure 16**).

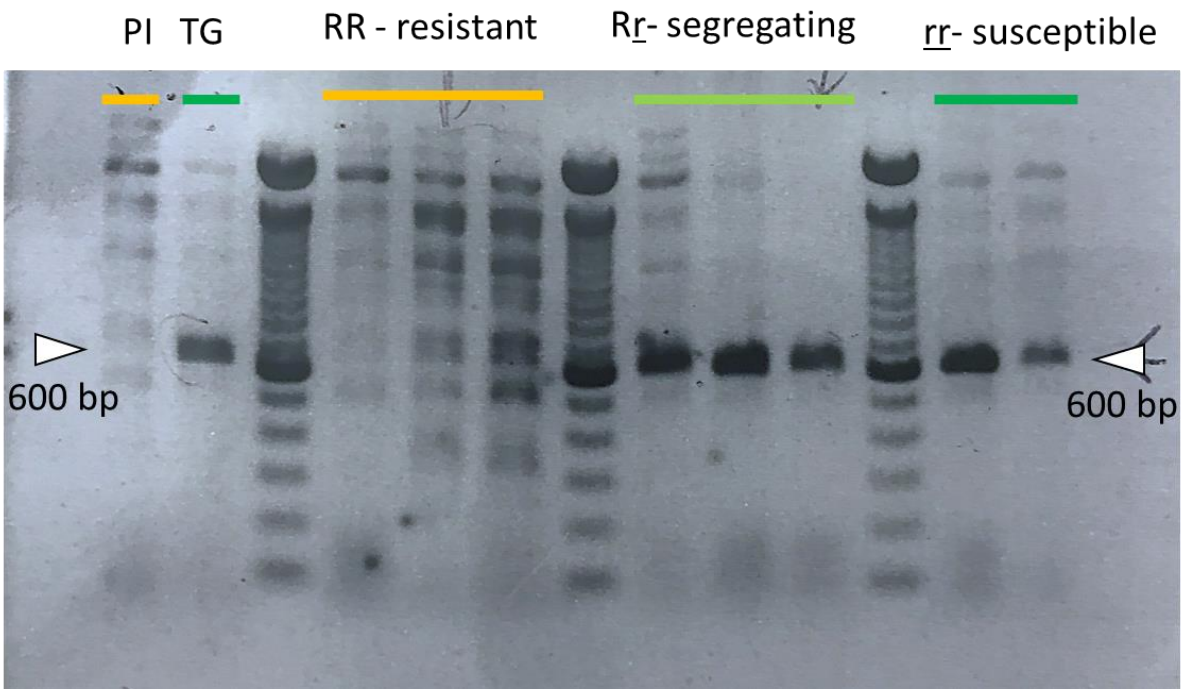

Figure 16. Validation of BLS_ww_15. BLS_ww_15 tested on 10 genomic DNA templates of known resistance genotype. The parents of the mapping population were used: PI 358001-1, PI (RR) and Tall Guzmaine, TG (rr). DNA from three homozygous resistant (RR), three heterozygous (Rr), and two homozygous susceptible F_2 individuals were tested. BLS_ww_15 amplifies a genotype specific 600 bp band, and a regular non-specific banding pattern.

This banding pattern, where the dominant marker detects the susceptible haplotype is ideal for selecting homozygous resistant (RR) lines developed in objective 1. The proximity to *Xcvr* is 3-5 cM which makes it close enough for MAS. BLS_ww_15 amplifies the 600 bp band from all 17 EREC breeding lines and cultivars tested with the marker to date (i.e., EREC breeding lines: 10168, 10179, 10198, 10380, 50011, 50100, 50111, 60167, 60182, 70096 and named cultivars: Belle Glade, Cooper, Hialeah, Manatee, Okeechobee, Sawgrass, Terrapin**)**. Thus, the marker should be useful to transfer resistance into each of those 17 genetic backgrounds and likely many more if PI 358001-1 is used as the resistant donor. BLS_ww_15 is currently being applied to screen 300 selections (BC_1F_3s and F_3s) made in the 2017-2018 season. Furthermore, the marker may be applied to F_4 selections from previous seasons and future seasons to come.

BLS_ww_15 may be effective for screening F_3 families in selections for crosses of PI 358001-1 and the 17 susceptible lines currently used for breeding, but it has room for improvement. Specifically, a marker with a codominant banding pattern and closer to the *Xcvr* locus is more desirable. The whole genome of PI 385001-1 was sequenced to obtain an unabridged data set for

marker development. Four DNA samples were sent for library preparation and sequencing to the Beijing Genome Institute at 100X coverage paired-end 100. The resulting reads were mapped to the 'Salinas' and 'Valmaine' assemblies detecting 1839 insertion/deletion polymorphs between PI 358001-1 and Salinas (in the 9 Mbp region around resistance). These polymorphisms will be used to develop an improved codominant marker. Design and validation of novel markers will be continued beyond the scope of this grant initially with 17 positions preliminarily detected in chromosome 2 using *Integrated Genome Viewer* and *Variant Calling Format*. Ten positions are suspected to be closer to the *Xcvr* locus compared to the dominant marker BLS_ww_15 (**Table 4**).

Table 4. Candidate positions detected in chromosome of the lettuce genome near the *Xcvr* (*Xanthomonas campestris vitians resistance*) locus

Initial position on chromosome 2 base pairs (bp)	Closer or farther from resistance than BLS_ww_15	Susceptible band bp	Resistant band bp
8505800	Closer	400	200
8503900	Closer	800	600
9961800	Closer	800	600
9985040	Closer	660	460
9941000	Closer	800	600
8537631	Closer	85	60
8542708	Closer	85	60
8545194	Closer	85	60
8514197	Closer	85	60
8461449	Closer	85	60
12687600	Further	800	600
12694300	Further	400	200
12620600	Further	800	600
11613398	Further	65	60
11698123	Further	85	60
11246745	Further	85	60
14226338	Further	85	60

Initially, the marker LR0035 which is related to a Resistance Gene Candidate (RCG) was not effective for MAS, indicating that this gene or genes around the sequence connected to LR0035 is not the gene controlling resistance against *Xcv*. During this project, a new genetic map was developed to overcome the difficulty of designing markers for MAS; the new map had a better

representation of the lettuce genome. A dominant marker (BLS_ww_15) is the most effective marker to date to likely select resistant families against *Xcv*; however, the marker has its limitations to one specific genetic background (PI 358001-1). Ideally, it is more efficient for MAS to develop a codominant marker with potential use for other genetic backgrounds; currently, four other cultivars are resistant to California strains of *Xcv*.

Objective 4. To increase grower knowledge about the benefit of using resistant cultivars for control of BLS

An initial survey using pre- and post-quizzes was given to 31 attendees at the 2015-2016 Florida Lettuce Advisory Committee (FLAC) meeting on 6 May 2016 by Dr. Christian Miller (Extension Agent for Commercial Vegetable and Tropical Fruit Production in Palm Beach County) (**see PDF file "Initial Survey"**). These surveys were designed to measure the knowledge of the audience on lettuce bacterial leaf spot (BLS disease). As part of these surveys, two presentations: "*Management of Lettuce Bacterial Leaf Spot through Resistance Breeding*" and "*Lettuce Disease and Management Update*" were given by Dr. Huangjun Lu (previous lettuce breeder at UF) and Dr. Richard Raid (Plant Pathologist at EREC).

The impact of the event was measured using 11 questions. The test asked the participants about their knowledge on BLS resistant breeding material as a method to control the disease. On average, 68/100 was the knowledge when attendees were examined prior to presentations. After presentation, the attendees were again tested; on average they scored 87/100 about their knowledge on using resistant cultivars against BLS. Initially, results seem to indicate that the participants at the FLAC meeting understood the importance of using resistant cultivars against BLS in lettuce (**Table 5**).

Table 5. Initial Survey conducted with attendees to the Lettuce Advisory Committee Meeting in May 2016 at initiation of the project "*Breeding Lettuce for High Resistance to Bacterial Leaf Spot*".

Question	Pre-survey	Post-survey
What is the correct name of the pathogen that causes lettuce bacterial leaf spot (BLS)?	36	0
Which type of lettuce is most susceptible to lettuce BLS?	72	100
Which types of lettuces show lettuce BLS symptoms?	82	100
Which description below best describes the symptoms of lettuce BLS?	82	100
The environmental conditions best suited for developing a lettuce BLS outbreak?	55	100
How effective are bactericides at preventing and curing lettuce BLS?	55	82
How might the initial infections of a lettuce BLS outbreak occur?	91	91
Which weed(s) can be infected by the pathogen that causes lettuce BLS?	72	100
What cultural options can NOT be utilized for preventing a lettuce BLS outbreak?	91	100
Which lettuce cultivar has been determined to have moderate resistance to lettuce BLS?	55	100
How can cultivars with undesirable commercial characteristics with diseases resistance be utilized?	55	82
Average	**67.8**	**86.8**

During the lifespan of the project, the University of Florida Lettuce Team shared advances and results of this grant with growers and stakeholders of the leafy vegetable community. These results were mainly presented at the two public meetings held by the Florida Lettuce Advisory Committee (FLAC) every season. Other venues included the Florida Foundation for Seed Producers (FFSP) and the Florida Seed Association (FSA) (**Table 6**).

Table 6. Presentations to growers and other stakeholders of the lettuce industry in Florida during the lifespan of the project "*Breeding Lettuce for High Resistance to Bacterial Leaf Spot*"

Meeting	Presentation	Speaker
FLAC 15-16 – 2	*Management of Lettuce Bacterial Leaf Spot through Resistance Breeding*	H. Lu[1]
FLAC 16-17 – 1	*Future to breed lettuce for Florida, including resistance in lettuce against BLS*	G. Sandoya[2]
FLAC 16-17 – 2	*Update on the Grant: Objective 3. Development of a molecular marker for high resistance to Bacterial Leaf Spot in lettuce*	Y. Wang[3]
FLAC 17-18 – 2	*Breeding Lettuce for High Resistance to Bacterial Leaf Spot*	W. Wadlington[4]
FLAC 18-19 – 1	*Breeding Lettuce for High Resistance to Bacterial Leaf Spot* *Molecular Marker Development for Bacterial Leaf Spot*	G. Sandoya W. Wadlington
FFSP	*Improving Lettuce for the Challenges of the EAA. Lettuce BLS*	G. Sandoya
33th FSA	*Improving Lettuce for the Challenges of the EAA. Lettuce BLS*	G. Sandoya
34th FSA	*Advances in Breeding for Lettuce Bacterial Leaf Spot (BLS)*	G. Sandoya
SCRI – Annual Meeting	*Improving Lettuce for the Challenges of the EAA. Lettuce BLS*	G. Sandoya

[1] Previous lettuce breeder at the University of Florida,
[2] New lettuce breeder at the University of Florida,
[3] First postdoctoral associate working in this grant,
[4] Second postdoctoral associate working in this grant

At the end of the project, a pre- and post-presentation survey was conducted among the attendees of the FLAC 2018-2019 first annual meeting. The survey asked questions related to the objectives of the project, future needs, and the general knowledge about BLS as disease (**see PDF File "Final Survey"**).

The survey asked questions about this project on whether the attendees (growers and stakeholders) were aware of the objectives of the grant. Satisfaction on accomplishment of the objectives of the grant and how important is to keep breeding resistance against Bacterial Leaf Spot (BLS) in lettuce and continue the work initiated by the University of Florida Lettuce Breeding Program.

Overall attendees were aware of the objectives of the grant by listing them correctly. They also expressed an 80% satisfaction with the accomplishment of the objectives and believe it is important to keep searching sources of resistance against BLS and keep breeding lettuce cultivars against this devastating disease (100%). More importantly, since 2010 growers and other stakeholders have seen the BLS disease 3 times (**Table 7**).

Table 7. Final Survey conducted with attendees to the Lettuce Advisory Committee Meeting in October 2018 at termination of the project "*Breeding Lettuce for High Resistance to Bacterial Leaf Spot*". General knowledge about objectives of this project

Questions	**Score**
Which of the following objectives were included in the Breeding against Bacterial Leaf Spot (BLS) in Adapted Lettuce cultivars for Use in the EAA grant awarded to Dr. Sandoya? (objectives of the grant listed)	100%
How do you rate your satisfaction with the progress made on the objectives of the BLS Resistance grant?	80%
How do you rate the importance of continuing the development of locally adapted BLS resistant breeding lines of lettuce?	100%
How do you rate the importance of continuing the search for new sources of resistance to develop locally adapted BLS resistant breeding lines of lettuce?	100%
Please indicate the number of BLS outbreaks that have occurred in EAA lettuce since 2015:	3

One additional test was conducted in the same survey. The test asked the participants about their knowledge on BLS resistant breeding material, management, symptoms and other important issues related to this disease. On average, 67/100 was the knowledge when attendees were examined prior to the presentation. After presentation, the attendees were again tested; on average they scored 80/100 about their knowledge BLS (**Table 8**).

Table 8. Final Survey conducted with attendees to the Lettuce Advisory Committee Meeting in October 2018 at termination of the project "*Breeding Lettuce for High Resistance to Bacterial Leaf Spot*". Pre and post presentation questions.

Pre and post presentation questions	**Pre-survey**	**Post-survey**
Process of developing BLS Resistant cultivars	60%	80%
Pathogen Races	60%	80%
BLS Management	60%	80%
BLS Symptoms	80%	80%
Best weather for BLS	80%	80%
Weeds as a BLS source	60%	80%
Average	**66.7%**	**80%**

Goals and Outcomes Achieved.

Objective 1. To continue to transfer the resistance to adapted iceberg cultivars and additional romaine cultivars

Initially the target of this project was to develop twenty lettuce populations per year. These populations refer to crosses made to produce a first generation, called F_1 which are then self-pollinated to produce enough seeds for field selections using Single-Seed-Descent Method in the F_2.

A total of 84 crosses were made in the lifespan of this project which are currently in F_1 stage. These crosses were made between the resistant PI 358001-1 and romaine, iceberg, butterhead, leaf, and Latin types. A total of 45 of these crosses are in the F_2 stage and are ready for further selection in the lettuce breeding program.

During the lifespan of the project, 698 indivual plant selections were made in the F_2 stage to later produce F_3 families. Currently, 25 families were advanced into the F_4 stage and 27 are in the F_5 stage.

The University of Florida Lettuce germplasm has approximately 120 breeding lines . In this project, 80% of the UF germplasm was crossed to the resistant PI 358001-1 indicating that the grant has surpassed the initial target of the projected work by 60% more crosses produced.

However, these lines present undesirable characteristics such as leafy types. An additional Backcross Selection Method was initiated to reintroduce the romaine type characteristic in 10 of these populations. Already we selected approximately 100 BC_1F_2 plants with romaine type appearance and it is expected to identify at least one resistant line.

Objective 2. To conduct large scale variety trials for evaluation of yield, horticultural characteristics, and post-harvest qualities of the newly selected resistant varieties at multiple locations

Unfortunately, 32 out of the initial 35 breeding lines selected for this study were discarded, because these lines had no resistance against *Xcv*. Data in postharvest qualities in these 3 lines was gathered and concluded that postharvest quality needs improvement. The lines were the result of the cross between 'Tall Guzmaine' and 'PI 3580001-1' and currently in the F_5 stage.

Although these 3 lines need additional improvement, they may be sources of resistance against Corky Root Rot, another disease problem important to the lettuce growers in the Everglades Agricultural Area (EAA). Additional experiments have yet to be conducted to determine the additional value of these lines and will continue to play an important role for lettuce growers.

Initially, another 11 University of Florida lettuce lines were studied in modified atmosphere packaging as a measure of their postharvest quality. Results indicated that postharvest quality should also be a breeding target in the lettuce breeding program.

Objective 3. To develop molecular markers that are associated with the resistant gene and can be used in marker-assisted breeding to accelerate resistant cultivar development

The initial work in this project was to design one molecular marker; however, it proved to be ineffective at selecting resistant families. A better genetic map of population 'PI 358001-1 × Tall Guzmaine' was generated using a different technology which allowed the design of new molecular markers.

The new marker designated BLS_ww_15 may select resistant individuals, but validation is still in the process. This new marker is dominant for susceptibility. The marker has been applied to screen 300 F_3 and BC_1F_3 families that were already selected for horticultural traits. By applying the marker to F_3 families after selection, the need to screen thousands of field plant selections is eliminated, as was originally described as a target objective. In addition to the time savings, the cost per sample of the entire marker protocol (seed to data) is less than 25 cents.

There is still the need to develop a codominant molecular marker. For this, the whole genome of PI 358001-1 was sequenced to have an appropiate dataset for marker design. At the final stage of this project, 17 putative codominant markers were designed; testing and validation of these markers will be done beyond the scope of the project.

Objective 4. To increase grower knowledge about the benefit of using resistant cultivars for control of BLS

Two surveys at the beginning and the end of the grant indicated that growers understand the need to use resistant cultivars as the most effective control method against lettuce BLS. Postsurvey indicated an 80 to 87% gain of knowledge. In fact, growers at the EAA have closely collaborated with university scientists to plant and conduct field selection trials during the execution of this project.

Dissemination of results of this project have been shared with growers and other stakeholders at the local Florida Lettuce Advisory Committee. Additional dissemination has been made throughout the state in meetings with the Florida Seed Association and Florida Foundation of Seed Producers. A total of 8 presentations were shared with growers and stakeholders.

The grant also was effective at increasing knowledge of two postdoctoral associates trained in lettuce breeding and genetics of resistance against BLS. The former postdoctoral associate Dr. Yunwen Wang is currently employed in the seed industry, and the current postdoctoral researcher Dr. William Wadlington, is at the University of Florida. Three local high school students also participated in this research.

Beneficiaries

Florida's lettuce production is primarily in the Everglades Agricultural Area (EAA) of South Florida, which is home to 5 large lettuce growing companies. They account for approximately 80% of the s production on 15,000 acres of planted operations. Throughout the state there are other small growers of lettuce in non-traditional settings; such operations include organic farmers, hydroponics, aquaponics, and protected structures producers (greenhouses). To the best of our knowledge, these small lettuce growers number as high as 150 in the state.

Any lettuce planting affected by Bacterial Leaf Spot (BLS) can reach 100% damage. The biggest operations in the state have a revenue of 70 million dollars per season. If a severe outbreak of the disease such as BLS occurs, lettuce growers in the state would lose up to 70 million dollars per season. Official acreage until the 2012 census was 12,000 acres, currently it is estimated that 15,000 acres are planted just at the EAA. This 15,000-acre data does not account for all the small operations that may get affected if this disease expands everywhere else in the state.

The lettuce growers in the state will soon have resistant breeding lines that can be released as a finished cultivar (licensed and distributed by the Florida Foundation of Seed Producers) or advanced breeding lines that can be commercialized by seed companies. At this point only one seed company provides lettuce seed to the 5 main growers with bigger operations in South Florida; at least three other companies are in the process of testing material for Florida environment. By releasing breeding lines resistant to BLS seed companies can continue the breeding process by incorporating this resistance into their elite cultivars.

Growers with small operations or with non-traditional settings typically purchase seed from small seed companies specialized in their type of operation. There are several seed companies that offer lettuce seed and other seed dealers which may be interested in commercializing our finished varieties resistant to BLS. This disease is believed to be seed transmitted therefore having BLS-free seed will be beneficial to any grower. In fact, organic growers are going to be the biggest beneficiaries of resistant material to diseases as their operations do not allow chemical pesticides. Although the resistance of PI 358001-1 to other lettuce diseases has not been tested yet, the likelihood of this cultivar being resistant to other pathogens is not discarded as the major resistance locus *Xcvr* is in a region in the lettuce genome well-known to host resistance candidate genes.

Advances of the grant were shared at the two annual Florida Lettuce Advisory Committee that take place at the EAA every year. Results of this project were shared with the Florida Foundation for Seed Producers and the Florida Seed Association in two occasions. The PI of the grant also shared results of this project in face-to-face interactions with growers and stakeholders throughout the state.

Lessons Learned:

The project main's delay was the change of Principal Investigators; the initial grant was awarded to a former faculty at the Everglades Research and Education Center (EREC). Another unexpected change occurred when the first postdoctoral researcher moved to the private sector with a permanent job. Despite these changes, the objectives of the grant were significantly advanced.

Hurricane Irma delayed both our ability to test lettuce for disease resistance and our ability to evaluate lettuce in the field. The extent of the damage and difficulty getting speedy repairs post-Irma delayed our ability to start testing the lettuce F_3 families for resistance by two months. This set back will delay our tests but did not negate our ability to complete testing this season. Also delayed by the hurricane was our field season 2017 - 2018. After Irma, we rescheduled plantings the breeding lines part of objectives 1 and 2 for several weeks. This delayed the date when we selected the lettuce which resemble to cultivated varieties which was done later in the season. This delay in selection pushed seed production into spring, which diminished the seed because of warmer temperatures.

Objective 1. Continuing to transfer the resistance to adapted iceberg cultivars and additional romaine cultivars.

No unexpected expected delays on transferring the resistance from PI 358001-1 into adapted romaine cultivars were experienced. More crosses than planned were produced between the resistant PI and the University of Florida breeding lines. Nearly all breeding lines belonging to the University of Florida lettuce breeding program were crossed to the resistant donor.

Initially significant amounts of F_3 families of crosses between romaine and the resistant PI 3580001-1 were lost (Table 1). This fact was resolved by planting more F_2 seed of these populations and making new field selections resulting in additional F_3 families. Twenty-four lines of this first group of populations were advanced into F_4 and F_5 but these families do not show acceptable horticultural traits.

It has also been established that the leafy type may predominate in the segregation pattern at the F_2 stage. Because of this complication, the genetics of transferring resistance seems to be more difficult than expected and required backcrossing to the recurrent commercial-like parent. As we learned that resistance is conditioned by a single dominant locus (*Xcvr*) located on LG2. We initiated a backcross selection method, which was repeated for two generations. We sought collaborations with a lettuce breeding seed company in California to help us accelerate the breeding process. The BC1 (Backcross 1) was produced at EREC, and the BC2 was produced in California reaching two generations of breeding in one calendar year. More than 100 new F_3 families from the BC_1 generation were selected, the new selected families present a higher proportion of romaine aspects.

In addition, we produced new cross combinations (populations) between PI 358001-1 and Florida adapted cultivars such as Belle Glade, Cooper, Chosen, Flagler, Gator, Hialeah, Homestead, Lantana, Manatee, Okeechobee, Sawgrass and Terrapin. These cultivars are either

romaine or crisphead lettuce commonly used in the Everglades Agricultural Area and are planted by commercial growers in different planting slots (fall, winter, spring). An extra accomplishment was to cross the resistant PI 358001-1 to well-known sources of resistance to different races of *Xcv* which are found in California environments.

At close of this project no breeding line with both, resistance and good horticultural traits are available. However, our resistant lines will soon be crossed again with romaine and/or iceberg lines to increase the "type". These crosses will be made with parents that offset specific undesirable traits of PI 358001-1, such as early bolting and susceptibility to tip burn. There are hopes to find horticulturally acceptable lettuce lines with resistance against BLS in the recent backcross families selected during last season. Hence, it is important for a breeding program to adopt more than one selection scheme, parallel to the main breeding scheme; in this project the single seed descend was initially used and a backcrossing selection scheme was initiated in parallel.

Objective 2. Variety trials for evaluation of yield, horticultural characteristics, and post-harvest qualities of the newly selected resistant varieties/lines

We currently have no advanced breeding lines (F_5 and beyond) for semi-commercial trials, because previous selections performed poorly for horticultural traits. The F5 lines bolted prematurely and most of these lines resulted not to be resistant against *Xanthomonas campestris* pv. *vitians* isolate L7. However, three lines had an acceptable level of partial resistance, which means the families had some disease but were consistently less diseased than other breeding lines in the same genetic background and susceptible parents.

We established collaborations with the vegetable postharvest specialist of the University of Florida. As part of a specialized research area in the lettuce breeding program, a graduate student was hired to study the postharvest of lettuce. The graduate student conducted preliminary trials with the three lines listed above. Results indicated that these lines as so as the resistant parent had poor postharvest quality.

Other lettuce cultivars and some breeding lines that are part of other breeding populations were planted in field conditions and with the help of VegPro International. When the post-harvest quality was studied, our lines performed poorly indicating that this trait should be taken into consideration in the lettuce breeding program.

These lines are missing the typical "type" needed by lettuce growers and have poor postharvest qualities. The lines need additional crossing to improve the quality of their romaine character. Lines should be first evaluated for corky root rot as they have as parent Tall Guzmaine a well-known source of resistance to this disease that is also a problem to lettuce growers worldwide. These lines should be crossed to parents that present romaine hearts, open heads, short cores and acceptable weights.

Objective 3. Development of molecular markers for marker-assisted selection for BLS resistance

The genetic map in population PI 358001-1 × Tall Guzmaine (Wang et al., 2016) had markers in LG 2, 4, 5, 6, 8 and 9, but none were associated with LG1, 3 and 7. We selected markers for four Resistance Candidate Genes (RCG, LR0089, LR0035, LK0036 and LR0029) that we suspected were near the *Xcvr* locus. RCG LR0035 was the closest to the locus of interest. However, LG2 is a large chromosome with a size larger than 250 Mbp; on average 1 cM in our current map is equal to 1 Mbp. The RCG marker, LR0035 was not as useful as was first thought, because it is 17.1 cM (centimorgans) from the locus *Xcvr*.

Instead of committing money and time to trying to develop a marker using available data. We adopted a better strategy (genotyping by sequencing) that allowed us to saturate all the Linkage Groups (a total of 9) in the lettuce genome and thus defining the region of interest (LG2) with more markers. Once we saturated the region near the *Xcvr* locus with GBS markers, novel PCR-based markers were designed resulting in one dominant marker (BLS_WW_15) that may target susceptibility. This makes the marker able to detect resistant individuals with a negative result (no banding pattern present). The position of the marker is close enough the estimated genetic position of resistance that if should be ~85% effective at selecting resistance families. The marker may be only effective at selecting resistant individuals when the donor is PI 358001-1.

However, the GBS data used to design marker BLS_WW_15 is only a sub-sample of the genomic sequence, and whole genome sequencing data was needed to develop marker with tighter linkage to resistance and a codominant banding pattern. Six putative markers were designed but testing was not accomplished in the grant lifespan

We experienced a delay to accomplish this goal as the postdoc (Dr. Yunwen Wang) took a position with the industry. We hired a new postdoc (Dr. William Wadlington) to continue the work initiated by Dr. Wang.

Objective 4. Increasing grower knowledge about the benefit of using resistant cultivars for control of BLS

No unexpected delays were experienced during the execution of two surveys with growers at the EAA. We shared information of this project beyond this specific location (EAA) with the idea to reach to other growers who do not participate in the Florida Lettuce Advisory Committee but also grow lettuce in other parts of the state.

Overall lessons learned

Resistance in lettuce against Florida isolates of *Xcv* was identified in PI 358001-1; no other sources of resistance to Florida isolates are currently available. The resistant PI has poor agronomic performance such as fast bolter, poor postharvest quality, susceptible to tipburn (identified in this research) and it seems that the “leafy type” is linked to the resistance; therefore, it is important to identify more than one source of resistance. Preliminarily 65 PIs were tested for resistance against three California isolates and three Florida isolates of *Xcv* to establish a better relationship between races of the pathogen and available resistance.

It is recommended to have more than one genetic background to do such as type on evaluations in advanced breeding lines. In the current project, two different romaines were crossed to PI 358001-1 but failing at testing for *Xcv* resistance in early stages reduced the amount of lines available for the breeding program. As results of this objective, it is recommended a characterization of the lettuce breeding program lines at UF for postharvest quality in both, whole heads and modified atmosphere packaging, this would give breeders a better and solid information on what parents to use for breeding and what other traits need to be considered in the breeding process at the same time as resistance.

Nowadays better sequencing technologies are available, and it should be easier and faster the development of molecular markers. Early in this project, available molecular markers were used for mapping; later during the execution of this project a mewer and better technology (GBS, genotyping by sequencing) was implemented. Results were better but not enough to develop a 100% efficient marker. In the current project there is not perfect molecular marker to be used for Marker Assisted Selection (MAS) in the University of Florida lettuce breeding program indefinitely. However, the marker named BLS_ww_15 should be effective at discriminating resistant and susceptible families from field selected plants.

We mostly shared the info of this project with the bigger growers at the EAA. We initially spoke to other stakeholders in statewide meetings with the idea to promote the overall goals of the lettuce breeding program of the University of Florida. In future research we are aiming to include other types of growers to cover more needs in the breeding industry of this specialty crop.

Literature

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Wang, Y., Lu, H., Raid, R. N., Nuessly, G. S. & Faroutine, G. (2015) Diverse Responses of Lettuce Cultivars and Germplasm Lines to Infections of Three Isolates of Xanthomonas campestris pv. vitians. *HortScience*, 50(5), 650-655.

Breeding Lettuce for Resistance to BLS

This survey's goal is to determine the baseline knowledge about bacterial leaf spot. Please circle the letter(s) of the most correct answer(s) to the questions below and return when completed.

1. What is the correct name of the pathogen that causes lettuce bacterial leaf spot?
    a. *Rhizomonas suberfaciens*
    b. *Xanthomonas campestris* pv. *vitians*
    c. *Erwinia carotovora* pv. *carotovora*
    d. *Pseudomonas marginalis* pv.*marginalis*

2. Which type of lettuce is most susceptible to lettuce bacterial leaf spot?
    a. Iceberg
    b. Romaine
    c. Boston
    d. Endive

3. Which types of lettuces show lettuce bacterial leaf spot symptoms?
    a. **Iceberg**
    b. **Romaine**
    c. **Boston**
    d. Endive

4. Which description below best describes the symptoms of lettuce bacterial leaf spot?
    a. Roots of infected plants are reduced in size and below brittle
    b. Leaf margins of infected plants turn yellow
    c. Black, angular, water-soaked lesions on mature leaves
    d. Affected plants decompose rapidly in a loose, wet mass

5. The environmental conditions best suited for developing a lettuce bacterial leaf spot outbreak?
    a. Cool and dry
    b. Cool and wet
    c. Warm and wet
    d. Warm and dry

6. How effective are bactericides at preventing and curing lettuce bacterial leaf spot?
    a. Not effective
    b. Slightly effective
    c. Effective
    d. Very effective

7. How might the initial infections of a lettuce bacterial leaf spot outbreak occur?
    a. Infected seed
    b. Infected crop residue from the previous lettuce crop
    c. Infected weeds
    d. Using contaminated tools, equipment, or personnel

8. Which weed(s) can be infected by the pathogen that causes lettuce bacterial leaf spot?
    a. Lambsquarters
    b. Amaranth
    c. Nutsedge
    d. Common groundsel

9. What cultural options can NOT be utilized for preventing a lettuce bacterial leaf spot outbreak?
    a. Planting disease-free seed
    b. Destroying infected crop residues before planting a 2nd lettuce crop
    c. Crop rotation
    d. Planting resistant cultivars

10. Which lettuce cultivar has been determined to have moderate resistance to lettuce bacterial leaf spot?
    a. 'Little Gem'
    b. 'Baby Gator'
    c. 'Tiny Dancer'
    d. 'Bitsy Belle'

11. How can cultivars with undesirable commercial characteristics with diseases resistance be utilized?
    a. They are totally useless
    b. They are planted, harvested, and sold to the public regardless
    c. Their resistant genes are cross bred into cultivars with good commercial characteristics
    d. They are planted as a border as protection around susceptible cultivars with good commercial characteristics

Breeding Lettuce for Resistance to BLS

1. Which of the following objectives were included in the *Breeding against Bacterial Leaf Spot (BLS) in Adapted Lettuce cultivars for Use in the EAA grant* awarded to Dr. Sandoya?
   a. Variety trials for evaluation of yield, horticultural characteristics, and post-harvest qualities of newly selected resistant varieties/lines.
   b. Continuing to transfer resistance to adapted iceberg and romaine cultivars.
   c. Developing molecular markers for marker-assisted selection of BLS resistance.
   d. Increasing grower knowledge about the benefit of using resistant cultivars for control of BLS.
   e. All of the above.

2. On a scale of 0-10 where 10 indicates the highest level, how do you rate your satisfaction with the progress made on the objectives of the *BLS Resistance* grant?

10	9	8	7	6	5	4	3	2	1	0
Very Satisfied		Moderately Satisfied			Satisfied			Slightly Satisfied		Dissatisfied

3. On a scale of 0-10 where 10 indicates the highest level, how do you rate the importance of continuing the development of locally adapted BLS resistant breeding lines of lettuce?

10	9	8	7	6	5	4	3	2	1	0
Very Important		Moderately Important			Important			Slightly Important		Unimportant

4. On a scale of 0-10 where 10 indicates the highest level, how do you rate the importance of continuing the search for new sources of resistance to develop locally adapted BLS resistant breeding lines of lettuce?

10	9	8	7	6	5	4	3	2	1	0
Very Important		Moderately Important			Important			Slightly Important		Unimportant

5. Please indicate the number of BLS outbreaks that have occurred in EAA lettuce since 2015: _____

6.

How do you rate your knowledge about:	BEFORE WORKSHOP					AFTER WORKSHOP				
	Very Low (1)	Low (2)	Moderate (3)	High (4)	Very High (5)	Very Low (1)	Low (2)	Moderate (3)	High (4)	Very High (5)
Process of developing BLS Resistant cultivars										
Pathogen Races										
BLS Management										
BLS Symptoms										
Best weather for BLS										
Weeds as a BLS source										

7. Please indicate any suggestions for improvement or topics of interest:

_____

_____

_____

Project (16): Adopting Integrated Pest Management Strategies Against Major Pests of Squash

Contact Person:
Name the Contact Person for the Project, including telephone number and email address.

Oscar E. Liburd, oeliburd@ufl.edu, (352) 273-3918

Project Partners:
List the primary organization implementing the project, as well as any partner organizations.

University of Florida, Florida Cucurbit Growers Association, Florida Fruit Vegetable and Growers Association

Project Summary:
Please refer to the "Project Purpose" section of your proposal when writing this section.

Provide a background for initial purpose of the project, which includes the specific issue, problem, or need that was addressed by this project.

Describe the importance and timeliness of the project.

If the project built on a previously funded project with the SCPGB or SCBGP-FB describe how this project complemented and enhanced, but is not duplicate, previous work.

Squash production in Florida is under serious threat due to attack by major above-ground insect pests, such as the melon and cowpea aphid (*Aphis gossypii* and *A. craccivora*, Hemiptera: Aphididae), the silverleaf whitefly (*Bemisia tabaci* Gennadius, B biotype; Hemiptera: Aleyrodidae), and major below-ground nematode pests such as root-knot nematodes (*Meloidogyne* spp.) (Nyoike and Liburd 2010). Aphids and silverleaf whiteflies injure squash directly and vector various damaging viruses; whereas root-knot nematodes significantly reduce squash production by damaging the roots and interfering with the absorption of mineral salts and water (Hooks et al. 2010).

Aphids transmit most of the viruses with great economic impact that infect squash including papaya ringspot virus (PRSV), watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV), and cucumber mosaic virus (CMV) (Nyoike and Liburd 2010). The incidence of whiteflies-transmitted viruses in cucurbit crops including cucurbit leaf crumple virus (CuLCrV), squash vein yellowing virus (SVYV), and cucurbit yellow stunting disorder virus (CYSDV) has increased substantially in the US eastern states (e.g. Florida, Georgia) during the last decade causing massive yield losses (McAvoy 2016). In addition, the squash silverleaf disorder caused by silverleaf whiteflies results in a decrease in photosynthesis that ultimately causes reductions in yield (Nyoike and Liburd 2010).

The use of pesticides is the common management strategy used by squash growers against both above- and below-ground pests; however, the sole use of chemicals is not sustainable as a pest management strategy and pest resistance to pesticides is always a major concern. The use of systemic or contact insecticides has little influence on the incidence of virus-transmitted diseases because viruses are transmitted before aphids acquire the lethal dose and immature whiteflies occur on the lower side of the leaves where they are hardly reached by the insecticides (Nyoike and Liburd 2010). Moreover, spraying chemical pesticides on a calendar basis (every 7–10 days) for pest control can be expensive, have negative effects on non-target organisms, especially pollinating agents and natural biological control organisms, and tend to negatively affect human health and the environment (Hooks et al. 2010).

The root-knot nematode, *Meloidogyne javanica* (Tylenchida: Heteroderidae), is one of the major nematode pests of vegetables in Florida. In squash, symptoms of *M. javanica* injury include stunting, premature wilting, leaf chlorosis, and other symptoms characteristic of nutrient deficiency. Because above-ground symptoms can be easily confused with diseases or nutrient deficiencies, root galling confirms nematode presence, infection severity, and potential for crop damage (Noling 1999). Currently nematode management techniques used in cucurbit crops in Florida include crop rotation of less susceptible crops or resistant varieties, cultural and tillage practices, use of transplants, and pre-plant nematicide treatments (Noling 1999). Increasing restrictions on chemical soil fumigants due to their high risk to human health and the environment have increase the need of new options for plant parasitic nematode management in vegetable crop production (Kokalis-Burelle et al. 2014).

Pasteuria species are gram positive, endospore-forming bacterial endoparasites of several important plant-parasitic nematodes (Dabire et al. 2005). Several studies have shown that parasitism of Pasteuria on root-knot nematodes reduces the infectivity of juveniles and prevents nematode reproduction (Dabire et al. 2005, Chaudhary and Kaul 2013, Swarnakumari and Sivakumar 2013). Pasteuria seed coating treatments have been used successfully to incorporate the bacteria into the soil without increasing the amount of bacterial endospores needed. A species of Pasteuria, (*Pasteuria nishizawae* Sayre) has demonstrated to be effective for managing *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), the soybean cyst nematode (SCN), in laboratory and large-scale field studies when soybean seeds undergo a Pasteuria seed coating treatment (Kokalis-Burelle et al. 2014). The seed coating treatment demonstrated no deleterious effect on plant health and plant height and showed high percentages of attachment to SCN. In 2014, this soybean treatment has been used in more than three million acres and the Pasteuria-coated soybean seeds are available in the market (Kokalis-Burelle et al. 2014). Despite the potential of *Pasteuria penetrans* Thorne (Sayre and Starr) for management of *M. javanica*, there is little information about *P. penetrans* seed coating evaluations in vegetable production.

Various cultural practices (e.g., cover crops and companion planting) and biological control techniques have been evaluated as sustainable management strategies. These include the use of plants with allelopathic potential against nematodes such as African marigolds (*Tagetes erecta* L., Asteraceae) (Hooks et al. 2010), root-knot nematode resistant cultivars including cowpeas (*Vigna unguiculata* (L.) Walp., 'Mississippi Silver', Fabaceae) (Wang and McSorley 2004), and the use of biological control agents such as *P. penetrans*, for *M. javanica* control (Hewlett et al. 2014). Despite the fact that there are several studies demonstrating the separate effects of cover crops, companion planting, and biological control on pest populations (e. g., Frank and Liburd 2005, Silveira et al. 2009, Youssef and Eissa 2014); there is little research evaluating holistic approaches for management of insect and root-knot nematodes attacking squash. These cultural practices in combination with biological control agents for management of both above- and below-ground pests can lead to the development of an integrated pest management (IPM) program for squash production.

We used these complimentary techniques including intercropping squash with African Marigold and cowpea, as companion plants and cover crops, and the application of a biological control agent (*P. penetrans*) for management of insect and nematode pests in squash production.

Project Approach:
Please refer to the "Work Plan" section of the proposal and the "Activities Performed" sections of your annual reports when writing this section.

Briefly summarize activities performed and tasks performed during the grant period.

Whenever possible, describe the work accomplished in both quantitative and qualitative terms. Specifically, discuss the tasks provided in the "Work Plan" section of the proposal. Include the significant results, accomplishments, conclusions and recommendations. Include favorable or unusual developments.

If the overall scope of the project benefitted commodities other than specialty crops, indicate how project staff ensure that funds were used to solely enhance the competitiveness of specialty crops.

Present the significant contributions and role of project partners in the project.

Activities performed

Objective 1. Evaluate the combined effect of cover crops and companion planting for management of aphids, silverleaf whiteflies, and thrips in squash production (spring and fall season).

• Browntop millet (Urochloa ramosa (L.) Nguyen) was planted as a winter cover crop from January to February (for the spring season). African marigolds 'Crackerjack' (Stokes Seeds, NY, USA) and cowpeas 'Mississippi Silver' (Urban Farmer, IN, USA) were planted as summer cover crops from August to September (for the fall season). Plants were mechanically rotated and the field was left fallow for one week before planting companion plants.

• Companion plants (African marigolds and cowpeas) and zucchini squash 'Cash Flow' were sown on raised beds with black plastic mulch and drip irrigated from March to June (spring season) and October to December (fall season). African marigolds and cowpeas were planted approximately three weeks before planting the squash to synchronize flowering times with the crop.

• DiPel® or Entrust® was applied to the entire field once or twice during the spring and fall season to suppress lepidopteran pests.

• The M-Pede® treatment was applied once per week for three weeks both seasons to suppress aphids and whiteflies, starting three weeks after planting the squash.

• Height and width of the squash plants were measured once after the squash flowering and once before squash termination.

• *In situ* counts were conducted weekly for five weeks, on squash plants to record densities of whiteflies and aphids, natural enemies, and parasitoids.

• Immature whiteflies and aphids were recorded weekly by destructive sampling for five weeks. For each treatment plot, four squash leaves were cut and taken back to the University of Florida's Small Fruit and Vegetable IPM Laboratory. Leaf discs were analyzed using a cork borer of 3.4 cm diameter.

• The silver leaf index was recorded weekly for the five-week period. Leaves were measured by randomly scoring six squash plants per plot with an arbitrary index from 0-5, modified from Yokomi et al. 1990, with 0 being no silvering, to 5 being a completely silvered plant.

• Densities of alate aphids and natural enemies were monitored for five weeks using two clear pan traps per plot filled with 5% soapy water. Traps were removed after 48 hours in the field.

• Densities of whiteflies, aphids, and natural enemies were recorded using two yellow sticky traps per plot. Traps were removed after 48 hours in the field, and data collection carried on over a five-week period.

• Randomly selected squash plants were screened for four aphid-transmitted cucurbit viruses (PRSV-W, WMV, ZYMV, and CMV) and the whitefly-transmitted CuLCrV using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and PCR, respectively (Nyoike et al. 2008).

• Squash plants were harvested two to three times a week by weighting the marketable and non-marketable yield (oversized fruit or fruit injured by pickleworms) per plot.

Objective 2. Determine the combined effect of squash seeds coated with P. penetrans and marigolds and cowpeas as cover crops and companion plants on the population density of root-knot nematodes (fall season).

• Zucchini squash seeds coated with *P. penetrans* endospores (Syngenta, USA) were incorporated into each treatment block. Uncoated 'Cash Flow,' seeds will be used as the second treatment in each block.

• Soil samples were collected prior to planting over crop, before planting companion plants, and at squash termination. The reproductive ability of root-knot nematodes in the soil was measured to determine if marigolds were an efficient host, and whether there was nematode resistant to any of the treatment combinations (marigold, marigold/cowpea, and cowpea) with *P. penetrans*. The Oostenbrink's reproduction factor (R=final nematode population/initial nematode population) was taken from five 100cc samples in each of the twenty plots and compared to treated with *P. penetrans* and untreated beds.

• The severity of *M. javanica* on squash roots was assessed with a root gall index from a scale of 1 (no galls) to 10 (severe galling).

Objective 3. Estimate the economic advantages of using a multi-tactic approach for management of insect and nematode pests in organic squash production.

• Data analysis was conducted using total marketable yield per season to identify differences with the pesticide treatment.

Objective 4. Develop and disseminate educational materials on sustainable management of insect and nematode pests in squash farming as part of an extension program.

• One field day was conducted during the Cucurbit Growers Workshop in Marianna, FL (January 18, 2017). Approximately 40 growers attended.

• Results were presented during the 2017 Florida Entomological Society in San Juan, Puerto Rico (July 2017) and during the 2017 Entomological Society of America (ESA) meeting in Denver, CO (November 2017).

Significant results

Objective 1. Evaluate the combined effect of cover crops and companion planting for management of aphids, silverleaf whiteflies, and thrips in squash production.

A total of 124 insect morphospecies from 64 families in the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Odonata, Orthoptera, and Thysanoptera, were collected from the experimental area. Parasitoids accounted for 34% (40) of the species, predators 20% (25), and plant feeders accounted for 35% (43) of the species, but only six were pests of concern in zucchini squash crops including cowpea aphids (*A. craccivora*), silverleaf whiteflies (*B. tabaci*), and thrips (Florida flower thrips, *Frankliniella bispinosa* Morgan; common blossom thrips, *F. schultzei* Tribom; tobacco thrips, *F. fusca* Hinds, Thysanoptera: Thripidae) and melonworms (*D. hyalinata*).

The applications of M-Pede® reduced the numbers of whiteflies and aphids on positive control treatments. Cowpeas were attractive to aphids in previous experiments during the spring season reason why Sweet Alyssum was used instead

Insect pest species. No significant differences in the numbers of silverleaf whiteflies and cowpea aphids were observed among treatments during both spring and fall seasons. Higher numbers of silverleaf whiteflies were observed during the fall season compared to the spring season. The same numbers of aphids were recorded during spring and fall seasons.

During the spring season, significant differences in the number of thrips were found in the squash and in

the flowers of the African marigolds (P= 0.013). The highest numbers of thrips (8 per leaf) were found on the squash planted next to the cowpeas. However, when scouting the companion plants, higher numbers of thrips (8 per flower) were observed in the flowers of the marigolds compared to the rest of the treatments. Few thrips were observed during the fall season.

Significant differences in the numbers of melonworms were observed among treatments during the fall season (P= <0.0003). The highest numbers (3 per plant) were observed in the treatment with cowpeas used as companion plants and the lowest numbers (2 per plant) in treatments with African marigolds used as companion plants. Melonworms established two weeks after planting and two spaced applications of Dipel® (Valent BioSciences LLC, Libertyville, IL) helped to minimize their damage. Low numbers of melonworms were recorded only one week before termination of the spring season. Moreover, cowpeas were attractive to aphids during the spring season as well hosting high numbers per plant (>25 per plant).

Predator and parasitoid species. Cowpeas harbored predatory species, especially adult and immature stages of coccinellids and carabid beetles. Mean numbers of immature lady beetles (4.5 per plant) and adult lady beetles (3.25 per plant) recorded in the companion plants were significantly higher in the cowpeas (P= 0.021 and P= 0.014. respectively) compared with marigolds and the mixed treatments (African marigolds mixed with cowpeas).

Contrary to cowpeas, marigolds hosted mainly adults and immature stages of *Orius* species commonly known as minute pirate bugs. Significant differences in the numbers of *Orius* spp. recorded in the companion plants were identified among treatments (P= 0.0005), being highest on the mixed treatments (9 per plant), followed by treatments with African marigolds only (6.5 per plant).

Despite finding high numbers of coccinellids in the companion plants, only *Orius* spp. adults were observed active in the squash plants with higher numbers recorded during the spring season (3.6 *Orius* per bed) compared to the fall season (0.5 *Orius* per bed). No significant differences in the numbers of *Orius* spp. were identified among treatments for both seasons, but numerical differences were found with the highest *Orius* spp. numbers (5 *Orius* per bed) in the mixed treatment, followed by treatments with marigolds only (3.5 *Orius* per bed).

Parasitoids were present in all treatments, throughout both seasons. There were changes in the species complex between seasons. Species from the family Scelionidae were more abundant (16 wasps per yellow trap) during the spring season, whereas wasps from the family Trichogrammatidae were more abundant (34 wasps per yellow trap) during the fall season.

Viral incidence. DAS-ELISA showed positive results only during spring seasons (higher aphid numbers compared to the fall season) with low incidence of all four aphid-transmitted viruses (less than 20% positive results). CuLCrV symptoms were only observed in a few plants during fall seasons (higher numbers of whiteflies compared to the spring season). However, PCR showed positive results in 70 to 85% of the samples collected.

Objective 2. Determine the combined effect of squash seeds coated with P. penetrans and marigolds and cowpeas as cover crops and companion plants on the population density of root-knot nematodes.

Nematode management and Pasteuria. Results showed no significant differences in the Reproductive Factor (RF) of *M. javanica* between treated and untreated squash seeds. Nevertheless, there was a significantly lower nematode RF in the treatments containing cowpea and the mixed treatments compared to the treatment with marigolds used as cover crop and companion plant (P< 0.05). While the positive and negative control treatments showed a 2-fold increment in the numbers of *M. javanica*, the RF was less than 1 for all treatments. This indicates that overall companion plant treatment combinations were not effective hosts for *M. javanica*.

The resistance to *Meloidogyne* nematodes of the cowpea variety 'Mississippi Silver' used during the experiments may explain the lower numbers of *M. javanica* in treatments containing cowpeas compared to the controls. The allellophatic effect of the marigold root exudates onto the nematodes seemed to affect only their RF and not *M. javanica* numbers.

The effectiveness of *P. penetrans* endospores had a high variability among treatments. This is because there was a natural population of the bacteria scattered in random patches throughout the experimental field. Additionally, the developmental stages of Pasteuria seemed to be in reproduction phase I (attachment and

germination) or II (exponential growth) that explain an increased infestation capacity in these random patches.

Objective 3. Estimate the economic advantages of using a multi-tactic approach for management of insect and nematode pests in organic squash production.

Yield. Results indicated no significant differences in total marketable yield between treated and untreated squash seeds. However, total marketable yield was highest in the marigold treatment, followed by the cowpea, and the mixed treatment (P= 0.0001, Figure 1). There was significantly less yield harvested in the M-Pede and the negative control treatments.

Overall, the techniques developed from this project enhance the quality of the soil and are useful to Florida squash growers including the organic and conventional farming industry. These integrated approaches preserve and restore over exploited habitats, help protect watersheds from pesticide contamination, and improve soil health. Additionally, these techniques help maintain and increase crop profitability.

Goals and Outcomes Achieved:
Please refer to the "Expected Measurable Outcomes" section of your proposal. list each expected measurable outcome from that section and explain what progress you have made toward achieving each one.

If outcome measures were long term, summarize the progress that has been made towards achievement. Every expected measurable outcome listed in your original project proposal must be addressed in this section.

Provide a comparison of actual accomplishments with the goals established for the project.

Clearly convey completion of achieving outcomes by illustrating baseline data that has gathered to date and showing the progress toward achieving set targets.

Highlight the major successes of the project in quantifiable terms.

Goal #	Goal	Measure	Target	Accomplishments
1	Develop an efficient sustainable pest management program that combines cultural practices (cover crops, and companion planting) and biological control (use of *P. penetrans*) for management of insect and nematode pests in squash production.	Densities of insect pests (aphids, whiteflies, and melonworms), root-knot nematodes (*M. javanica*), and beneficial arthropods present in the cover crops, squash, and companion plants will be measured and evaluated before planting, during plant development, and at the termination of cover crop/squash + companion plant rotations.	Reduce the densities of insect pests and root-knot nematodes by 60% and increase the numbers of beneficial arthropods present in the squash and companion plants by 50%.	The techniques developed from this project enhance the quality of the soil and are useful to Florida squash growers including the organic and conventional farming industry. These integrated approaches increased significantly the numbers of natural enemies when the companion plants were present, whereas the numbers of insect pests were reduced. Additionally, these techniques help maintain and increase crop profitability. The goal was achieved and the target was accomplished.
2	Determine *P. penetrans* level of infectivity and its ability to prevail in the soil after cover crop/squash + companion plant rotations.	Number of *P. penetrans* infecting 100 *M. javanica* second-stage juveniles and number of *P. penetrans* endospores present in 100-cm^3 soil samples collected per experimental plot.	Reduce the population of nematodes *M. javanica* by 70% and increase the prevalence of *P. penetrans* by 30% in the soil.	The goal was achieved. A reduction in the population of *M. javanica* was observed (~50%) in the squash when seeds coated with *P. penetrans* were used.
3	Demonstrate the economic advantages of using a sustainable multi-tactic program for management of insect and nematode pests in organic	Densities of insect pests, *M. javanica*, and beneficial arthropods in the squash and companion plants will be evaluated, squash	To increase farmer profitability by 50%	There was significantly less total marketable yield harvested in the M-Pede and the negative control treatments compared to the treatments including

	squash production.	plants health (disease incidence), costs incur in pest management and farm operation, and squash total and marketable yield will also be assessed.		marigolds. The goal was achieved and the target was accomplished.
4	Develop and disseminate educational materials on sustainable management of insect and nematode pests in squash farming as part of an extension program.	Number of people in attendance to field days and meeting presentations. Number of extension agents trained by the project personnel and number of peer-reviewed and non-peer-reviewed publications.	Improve educational programs to cucurbit producers in Florida, as well as other southern states like Georgia. Provide new knowledge about the efficiency of *P. penetrans* for management of *M. javanica* and possible adoption of this bacterial biological control agent by squash growers.	One field day was conducted during the Cucurbit Growers Workshop in Marianna, FL. Approximately 40 growers attended. Our results were presented during the 2017 Florida Entomological Society in San Juan, Puerto Rico (July 2017) and during the 2017 Entomological Society of America (ESA) meeting in Denver, CO (November 2017). The goal was achieved and the target was accomplished.

The major successes of the project include the significant increase of natural enemies in squash plants planted next to African marigolds. Immature and adult stages of *Orius* sp. and big-eyed bugs were recorded in high numbers in marigolds flowers and leaves. They were frequently observed moving from the marigold flowers towards the squash plants. Low numbers of silverleaf whiteflies and thrips were observed in those squash plants. Also, squash plants in treatments including marigolds showed higher total marketable yields compared to the rest of the treatments.

Beneficiaries:

Please refer to the "Potential Impact" section of your proposal. Any beneficiary group mentioned in that section must be addressed.

Provide a description of the groups and other operations that benefited from the completion of this project's accomplishments. What specialty crop stakeholders benefitted from this project? How did they benefit, and how were they made aware of project results? How many of them are there?

Clearly state the quantitative data that concerns the beneficiaries effected by the project's accomplishments and/or the potential economic impact of the project.

Approximately 40 growers from the Florida Cucurbit Growers Association and the Florida Fruit Vegetable and Growers Association are aware of the techniques developed in this project. All growers shared similar concerns regarding the increase of silverleaf whitefly numbers and whitefly-transmitted viruses in cucurbits in the last 5 to 10 years. They attended to the field day conducted in Marianna, FL in 2017 and acknowledged the need for this type of new integrated approaches in cucurbit production.

The techniques developed in this project has the potential to impact at least 600 cucurbit growers. Approximately 200 squash farmers in Florida and an additional 400 growers who produce other types of cucurbits including watermelons, cucumber and cantaloupe. The vast majority of growers are impacted by aphids and whiteflies as well as nematode problems. Pest management strategies developed from this project

can be adopted not only by squash growers using sustainable organic farming systems but also conventional growers. Peer-reviewed and newsletter publications resulting from this project (in the short future) will contribute to the dissemination and adoption of the developed approaches.

We aimed to generate a cost-effective pest management program available to farmers against insect pests and *M. javanica* populations in squash production. We reached farmers and researchers from other states and other countries by presenting part of our preliminary results at the 2016 International Congress of Entomology (ICE; Orlando, FL) and the 2017 Entomological Society of America Meeting (ESA; Denver, CO). The presentation of our results in entomological meeting already reached and impacted scientist working on similar approaches in other states in the countries where cucurbit crops are grown (e.g. California, Alabama). Young scientists and early professionals are aware of the techniques implemented in this project and acknowledged the importance of integration of disciplines and strategies in pest management programs.

Lessons Learned:

Please refer to the "Work Plan" and "Expected Measurable Outcomes" sections of your proposal, as well as the "Problems and Delays" sections of your annual reports. Any problems and delays, any changes which had to be made to the work plan, and any project goals which could not be achieved must be addressed in this section.

Offer insights into the lessons learned, illustrating the positive and negative results of implementing this project, by the project staff as a result of completing this project.

Describe unexpected outcomes or results that occurred as a result of implementing this project.

If the goals or outcomes measured were not achieved, identify and share the lessons learned to help others expedite problem solving.

Lessons learned should draw upon positive experiences (e.g. good ideas that improve project efficiency or save money} and negative experiences (e.g. lessons learned about what did not go well and what needs to be changed}.

Refugia increased natural enemies but only in selected plots where African marigolds were present, movement of the natural enemy populations towards the squash was observed. Populations of minute pirate bugs (*Orius* spp.) were the most commonly found in the squash plants followed by adult lady beetles. *Orius* spp. seemed to be related with the numbers of thrips found in the marigolds; however, the numbers of thrips did not increase in the squash planted next to the African marigolds.

Despite being attractive to multiple predatory species, cowpeas harbored high numbers of aphids that can become a reservoir for secondary infestation, fungal and viral diseases. Thus, the use of cowpeas intercropped with squash under these conditions was not identified as viable strategy for squash pest management. Reason why, sweet Alyssum ‘Tall White’ (Urban Farmer LLC., Westfield, IN) was used during the last two squash season as the second companion plant instead of cowpeas.

The applications of Entrust® on positive control treatments (twice during each season) had no apparent effect on the silverleaf whitefly and cowpea aphid populations attacking the squash. Thus, M-Pede was used instead in the last two squash season. The applications of M-Pede® reduced the numbers of whiteflies and aphids on positive control treatments. Additionally, the use of Entrust® in the positive control

treatment had no apparent effect on parasitoid populations which made it a preferred option for control of lepidopteran pests during the experiments.

The increased numbers of natural enemies and the applications of Entrust in the positive controls had no apparent effect on CuLCrV incidence. This is consistent with the lack of significant differences in the incidence of whitefly-transmitted viruses across treatments. Moreover, DAS-ELISA showed low incidence of all four aphid-transmitted viruses (potyviruses). Reports of potyviruses established in the experimental station have been negative or rare for a few years now; however, the constant presence of various aphid species in the squash and other crops in the area requires constant monitoring to avoid sudden disease infestations.

Results showed no significant differences in the Reproductive Factor (RF) of *M. javanica* between treated and untreated squash seeds. Low numbers initial numbers of *M. javanica* in the experimental field may explain lack of significant differences across treatments.

Total marketable yields were significantly lower (approximately 50% lower) during fall seasons compared to spring seasons. CuLCrV and melonworms appeared to have an effect on fruit production during fall seasons (Figure 2).

Total funding expended to date. $ 126,456.00

Please list the percentage of the project that has been completed at this time. 100%

Signature: Oscar Liburd

Print Name and Title: Oscar E. Liburd, Professor of Entomology

Date 06/13/2018

FDACS Employee signature:

Date received:

Project (17): Development of integrated strategies for management of Tomato chlorotic spot virus (TCSV) and other tospoviruses in commercial tomato production

Contact Person

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Project Partners

University of Florida - Tropical Research and Education Center; Department of Plant Pathology; UF/IFAS Miami-Dade County Extension, Hendry County Extension.

Partners - DiMare, Kern Carpenter Farm, and many tomato growers in south Florida.

Project Summary

A. The initial purpose of the project including the specific issue, problem, or need that was addressed by the project

South Florida tomato growers have experienced a severe outbreak of *Tomato chlorotic spot virus* (TCSV), an emerging tospovirus since the fall of 2014. TCSV has caused significant yield losses to tomato growers as its infection incites necrosis of leaves, stunts plant growth, and renders the fruit unmarketable. In December 2014, more than 30% tomato plants in some fields of Homestead, FL were infected with TCSV and the infected plants had to be rogued out from fields attempting to reduce the spread of the virus. The worst case was seen from a 50-acre field that the tomato plantings were abandoned completely due to severe infection of TCSV. Losses of tomato yield could reach as high as nearly 100% based on observations in our field trial conducted in the spring of 2015 in Homestead, FL. Initial symptoms of TCSV such as chlorotic spots and necrotic lesions were typically seen in October 2014 in tomato fields of Homestead, FL, only three weeks after transplanting. Following the initial symptoms, if the plants survived then symptoms of stunting, bronzing, necrosis, and deformation appeared on plants. Early infection of TCSV in young tomato plants resulted in severe stunting with few or no flowers or fruit, and eventually death of the plant. Fruit from infected plants, if any, showed necrotic rings thus is often unmarketable.

TCSV is analogous to TSWV, GRSV and other known tospoviruses. The genome of TCSV consists of large (L), medium (M) and small (S) RNA segments. The L RNA is negative sense that encodes a multifunction protein (L) including a domain of RNA-dependent RNA polymerase. The M RNA is ambisense and encodes a precursor for two glycoproteins (GN and GC) and a non-structural movement protein (NSm). The S RNA is also ambisense, but encodes a nucleocapsid (N) protein and a non-structural silencing suppressor protein (NSs). Tomato plants in South Florida can be infected with TCSV as well as other two known tospoviruses, i.e. *Groundnut ring spot virus* (GRSV) and *Tomato spotted wilt virus* (TSWV). However, the most concerned problem in the growing season 2014-2015 of South Florida was TCSV. Testing of samples of symptomatic tomato plants has confirmed that all of them were unexclusively infected with TCSV. Clearly, TCSV has become a major problem of tospovirus threatening commercial tomato production in South Florida. Thus, this proposal is specifically geared towards the development of integrated strategies for management of TCSV in commercial tomato production of Florida. Strategies developed in this proposal should be also applicable to management of GRSV and TSWV.

Detection of TCSV is complicated and improved assays are desperately needed for more reliable and faster identification. In South Florida, tomato plants could be infected by any of three tospoviruses TCSV, GRSV and TSWV, in some cases in mixed infection. These tospoviruses share approximately 80% sequence identities, but infected tomato plants display undistinguishable symptoms. Currently, dipstick, ELISA, and RT-PCR are the assays available for TCSV detection from plants. However, the ELISA and dipstick assays are not specific for TCSV and often detect TSWV, resulting in false positives for TCSV diagnoses. RT-PCR can be virus-specific depending on the primers used, but it is time consuming and more expensive than the other two assays. Furthermore, RT-PCR is sensitive to plant contaminants, which may result in false negatives. Because symptoms of TCSV resemble those of other tospoviruses, there is an urgent need for a rapid, reliable and specific assay for TCSV diagnosis.

Similar to other tospoviruses, TCSV is known to be exclusively transmitted by thrips. Western flower thrips (*Frankliniella occidentalis*) and common blossom thrips (*F. schultzei*) are confirmed to be the main vectors of TCSV in South Florida. It is unknown if other species of thrips transmit TCSV in Florida. *F. occidentalis* is a major vector of many tospoviruses including TCSV, and is widespread in the USA and worldwide. It is reported that *F. schultzei* is not widespread in Florida and the USA. However, *F. schultzei* was reported in vegetable production areas of South Florida where TCSV was detected. The potential of other thrips species to transmit TCSV in Florida has not been determined. Vegetable fields in Florida and the southeastern US are commonly inhabited by many species of thrips including Florida flower thrips (*F. bispinosa*), melon thrips (*Thrips palmi*), tobacco thrips (*F. fusca*), and eastern flower thrips (*F. tritici*) that are reported to transmit tospoviruses. It is important to understand the role of these commonly present species of thrips in transmitting TCSV in Florida.

The known hosts of TCSV are primarily in solanaceous family including vegetables (tomato, pepper, eggplant, tomatillo), ornamentals (tobacco, petunia) and weed (jimson). Non-solanaceous hosts of TCSV that are known, to date, are lettuce and impatiens. Most recently, TCSV was also detected from annual vinca (also known as Madagascar periwinkle, *Catharanthus roseus*) in South Florida in 2014, and from two ornamental crops porcelainflower or waxflower (*Hoya wayetii*) and false Christmas cactus (*Schlumbergera truncata*) in Central Florida in 2014. The recent identification of plant species as the natural hosts of TCSV in Florida implicates that TCSV exists both in vegetables and ornamental crops, studies of epidemiology and management of TCSV have to be on both groups of crops as they often share production space in Florida. Considering the establishment and spread of TCSV in South Florida, where vegetable fields, ornamental plant nurseries, tropical and subtropical fruit groves, and transplant producers are in close proximity, many crops and weeds may be potential reservoirs or hosts of TCSV.

There is a great concern that TCSV could spread beyond South Florida to other areas due to the widespread presence of thrips vectors and transplant producers in this area. As matter of fact, TCSV was

reported from Ohio infecting tomatoes in a commercial high tunnel in the summer of 2013. This is the second report of TCSV in the USA, highlighting great potential of this virus spreading to other U.S. states. Appropriate management measures must be taken in vegetable and ornamental fields to not only reduce losses to those crops, but to restrict the movement of TCSV into transplants being shipped throughout the eastern US.

B. **Important and timeliness of this project**

This project proposed to develop integrated strategies for management of the devastating disease caused by TCSV as well as other tospoviruses. As stated previously, TCSV is a recently discovered tospovirus in the USA, and after only a couple of years, it became epidemic and a major concern in commercial tomato production in South Florida. In November of 2014, DiMare Inc., a major tomato grower in Florida and in the eastern USA, approached the UF/IFAS Tropical Research and Education Center (TREC) and UF/IFAS Miami-Dade County Extension in Homestead, FL for assistance to control this disease problem. Meanwhile, other tomato growers such as Kern Carpenter and Ralph Jordan expressed their desperate needs for strategies to manage this disease. They reported a disease incidence of 10 to 30% in their tomato fields in Homestead. Since a large number of plants have to be rogued and fruit from survived infected plants is unmarketable, TCSV has caused significant losses in tomato production. However, the development of effective management tools has been hindered because TCSV is new to the USA, and therefore, little is known about its biology and epidemiology relative to Florida and the USA.

In response to the crisis caused by TCSV, a team consisting of faculty at UF/IFAS TREC (Shouan Zhang, Vegetable Plant Pathology; Dakshina Seal, Vegetable IPM) and UF/IFAS Miami-Dade (Qingren Wang) and Hendry County Extension (Eugene McAvoy) quickly responded to promote awareness of TCSV and made recommendations to tomato growers attempting to minimize the impact of TCSV. On December 10, 2014, the team visited growers' fields with Jim Husk, the farm manager from DiMare Inc., surveyed on TCSV disease incidence, collected samples of tomato plants, other vegetables and weeds, and monitored populations of thrips. Within a week, a factsheet entitled “Update of Tomato Pests and Management” focusing on TCSV was developed and disseminated to all vegetable growers in Miami-Dade County and South Florida for awareness of this outbreak and strategies growers could take. A workshop entitled “Strategy and Practical Approaches to Managing Field Pests” was held January 7, 2015 in Homestead, FL to convey relevant information currently available on TCSV and other tospoviruses to the growers, and recommendations were provided for potential strategies to manage this new disease outbreak based on a similar tospovirus TSWV. Three field days were organized at UF/IFAS TREC on January 14, March 19, and April 2, 2015 and one on the farm of DiMare, Inc. in Homestead on February 12, 2015 to showcase potential strategies for managing thrips and reducing incidence of TCSV infestations.

To provide potentially quick and best strategies for management of TCSV in tomato production, in collaboration with the industry, a tomato cultivar trial was conducted in the spring of 2015 at UF/IFAS TREC in Homestead, FL to evaluate host resistance to TCSV under commercial production conditions of South Florida. Fourteen TSWV-resistant cultivars of tomato were included in this trial with a commercial standard cultivar FL 47 as a susceptible control. Symptoms of TCSV appeared three weeks after transplanting (WAT). All tested tomato cultivars were resistant or highly tolerant to TCSV in this field trial. Six weeks after transplanting, two cultivars had the lowest incidences (1.8%) of TCSV, six had incidences of 5-10%, and six had incidences of 10-15%. Disease incidences in FL 47 were as high as 91%.

In another field trial, eight insecticides applied individually and another six treatments containing rotations or tank mixes of different insecticides were evaluated at UF/IFAS TREC in Homestead, FL for their effects on thrips and incidence of TCSV symptomatic plants. Compared to the untreated control,

Radiant and Exirel applied individually as weekly foliar sprays significantly ($P < 0.05$) reduced TCSV incidence 6 WAT. All other treatments, regardless of whether they were single applications, rotations, or tank mixes of multiple insecticides, were ineffective and gave the same results as the untreated control. Sprays of some insecticides showed suppression in populations of flower thrips adults, but the population densities fluctuated over time. Preliminary data from this field trial indicated that the use of pesticides alone will not be sufficient to reduce the incidence of TCSV symptomatic plants and hence yield losses, that will be acceptable for commercial tomato production. Hence, integrated strategies have to be developed for successful management of TCSV.

In addition, South Florida is currently the only area in the USA where TCSV is established and causing annual epidemics in tomatoes. This makes Homestead of Miami-Dade County, FL the ideal location to resolve many of the biological and ecological questions concerning TCSV as well as the best location to conduct field trials of TCSV under commercial production conditions. It is only a matter of time before TCSV is found outside Florida. Data generated by the proposed research may delay its movement out of Florida on transplants, and will be beneficial in helping other states where TCSV may eventually establish.

Project Approach

Objective 1. Develop rapid and reliable assays for specific diagnosis of TCSV, which can be used to identify TCSV in plant samples from growers as well as in biological studies on TCSV.

Tasks - A rapid and accurate method of alternative isothermal amplification, called recombinase polymerase amplification (RPA), will be developed for the use to specifically and rapidly detect TCSV from plant samples. Through multiple sequence analysis from representative isolates of TCSV, GRSV, and TSWV, a conserved sequence region identical to the currently known TCSV isolates, but with significant divergence from GRSV and TSWV, will be used to design primers that will produce an amplicon specific for TCSV in an RT-RPA. The RPA can be conducted in a water bath at a constant temperature of 37-42°C. The assays will be developed by Polston and her biological scientist during January 1 - June 30, 2016, and will be verified during July1- December 31, 2016. (Polston has successfully developed RPA and RT-RPA assays for other viruses). Zhang and the postdoc to be hired will also be involved in this research during the same time.

Accomplishments - We have identified an extraction protocol that works well, but are trying to develop one that is faster. Also, we have tested several primers but to date have not identified a pair that will work as well as we would like in the RT-RPA. We continue working on this in a hope that specific primers that work well for development of the RT-RPA for specifically detecting TCSV from plants.

Objective 2. Determine if other vegetable crops, ornamental plants, tropical fruit crops and weeds are hosts of TCSV, and investigate if other thrips species rather than those currently known are vectors of TCSV.

Tasks- Host range experiments will be primarily performed by Polston and the biological scientist during the period of two years (January 1, 2016-December 31, 2017) with a focus in Year 1 (January 1 - December 31, 2016). Zhang and the postdoc at TREC in Homestead will also be conducting this research

during January 1, 2016-December 31, 2017. Weed and indicator host species will be grown from seeds produced from virus-free source plants and certain plants will be propagated from virus-free stock plants in the greenhouses at department of Plant pathology, University of Florida in Gainesville, FL and at UF/IFAS TREC in Homestead, FL. Plants of each species or cultivar will be mechanically inoculated at the stage of two to three true leaves. Plant of each species or cultivar will be also mock inoculated with buffer. Inoculated plants will be examined weekly for symptom development. The presence of target virus in leaves of inoculated plants will be determined 3-4 weeks after inoculation using the TCSV-specific RT- RPA assay to be developed and described in Objective 1.

Investigation of TCSV vectors will be primarily conducted by Seal and his research technician at UF/IFAS TREC in Homestead, FL. This will be primarily performed during the two years of this project (January 1, 2016 - December 31, 2017) with an emphasis in Year 1 (January 1, 2016 - December 31, 2016). Transmission ability and rates of TCSV will be determined by collecting live adult thrips from fields with plants confirmed to be infected with the virus. Thrips will be maintained in containers with green bean pods. Colonies will be maintained at 25 ± 1°C, 50 - 80% RH, and a photoperiod of 16 h light and 8 h darkness, and will be maintained on different crops depending on thrips species. Tomato, pepper and bean plants will be grown in a greenhouse equipped with thrips-proof net, and will be tested to ensure they are TCSV-free prior to use. Females and males of each thrips species will be confined in cages containing these plants for 48 h when plants are moved out of the cage after thrips are killed by insecticides. Plants will be closely monitored for development of typical symptoms of TCSV, and also will be confirmed by RT-RPA assays.

Adults of each thrips species will be maintained in separate containers and allowed to oviposit on bean pods before they are removed. First instar larvae will be collected from bean pods shortly after emergence and placed on symptomatic leaves of TCSV-infected tomato `Florida 47' source plants for acquisition and transmission experiments. Infection of source plants with TCSV will be confirmed by RT-RPA assays prior to use in transmission experiments. A single adult thrips from a larva feeding on infected source plants will be moved to a single leaf disc of tomato `Florida 47'. Adults will be removed from leaf discs after 2 days. Thrips and leaf discs will be tested for TCSV by either RT-RPA. Acquisition and transmission of TCSV by thrips species will be compared. The detection of TCSV from thrips and leaf disc will be assisted by the Zhang and Polston laboratories.

Accomplishments -

We found that TCSV was able to infect several orchid species. TCSV was detected in large concentric ringspots of orchids (*Phaleanopsis spp.*). Viable isolates of TCSV were obtained from these lesions by inoculation to *Nicotiana benthamiana*. These lesions appeared in the spring in Homestead in orchid houses that were located downwind of tomato fields. The infection appeared to be restricted to the tissues in and around the lesions. Extensive testing of non-symptomatic leaves indicted that TCSV was not present in those leaves. This suggests that the orchids are resistant to TCSV but do produce a lesion at the point of inoculation which then expands. The TCSV isolate from orchid was highly pathogenic in tomato, pepper and *Nicotiana benthamiana* (an experimental host). However, we were unable to successfully infect orchid leaves with the TCSV by mechanical inoculation, even after many attempts with different buffers and different methods. We suspect that inoculation occurs by thrips which are leaving tomato fields in the spring which then land in the plant houses, feed and transmit TCSV to the orchids. Since the infection is not systemic, growers only need to remove symptomatic leaves in order to make the plant marketable. Observed incidences of lesions were relatively low (less than 5%). For these reasons, TCSV does not appear to be a serious problem for producers of these orchids.

TCSV was first detected in the USA in snapbean plants in a greenhouse in Homestead, FL in 2017 by the Zhang laboratory. This expands the host range of TCSV in the USA. As snapbean is a large acreage vegetable crop in Miami-Dade County and Florida, this will pose a huge challenge to bean growers in this area. Management of TCSV needs to consider snapbean as a potential reservoir of TCSV.

Given the wide identified host range that includes plants like pepper, lettuce, long beans, impatients other than tomatoes, a preliminary screening of alternative hosts was conducted. Thirteen weed species were collected from around the tomato fields and tested for the presence of TCSV by RT-PCR. Purslane (Portulaca oleracea), a common weed species in south Florida, was detected positive against TCSV in 7 out of 16 samples (43.75%) and was confirmed to be an alternate host of TCSV.

The Seal laboratory conducted survey on thrips in tomato fields in Homestead, FL to determine weed hosts of thrips. All samples with thrips belonging to different species which will be identified to species level.

Objective 3. Develop integrated strategies for management of TCSV in commercial tomato production based on the data produced in Objectives 1 - 2.

Tasks - Field trials will be conducted on tomato to verify if any of the recommendations that are used to manage TSWV are effective in management of TCSV. This will include evaluation tomato cultivars for host resistance, the use of plant activator (ASM), and the efficacy tests of insecticides for thrips suppression and disease management. Evaluation of other cultural practices will also be conducted in Homestead, FL once the host range of TCSV is determined in Florida. Field trials will be established on the farm at UF/IFAS TREC in Homestead, FL to evaluate the effect of host weed removal, use of UV-reflective mulch, and use of non-host crops as a barrier, cover crops on reducing TCSV incidence and suppressing thrips. This will be primarily conducted by Zhang, his postdoc and Seal, his technician with the help of Wang and his OPS worker. During Year 1 (January 1 - December 31, 2016), research will focus on evaluating the effect of each individual strategies, and an integrated program will be developed in Year 2 (January 1 - December 31, 2017) based on results from trials being conducted currently and in Year 1.

Evaluating tomato cultivars for resistance to TCSV. Tomato cultivars listed in the 2014-2015 Vegetable Production Handbook for Florida and EDIS, and those recently released from seed companies will be collected and evaluated in field trials at UF/IFAS TREC in Homestead, FL for their performance against TCSV. The trials will focus on tomato cultivars known resistant to TSWV including Brickyard, BHN602, BHN 640, Quincy, Richmond, SV 7631TD, and Volante. A trial consisting of 10-15 tomato cultivars will be conducted each growing season at the research farm of UF/IFAS TREC in Homestead, FL where TCSV became established and is a threat to the tomato production. To ensure success of the field trials, tomato seedlings will be tested for TCSV prior to transplanting to verify that they are virus-free. After symptoms of TCSV develop, incidence of the disease will be rated every 3-4 days. Tomato fruit will be harvested, graded and yields will be recorded. Other horticultural traits such as maturity time and fruit quality (firmness, taste, pH) that drive grower selection of cultivars will be also investigated after harvest. This will be primarily conducted by Zhang, his postdoc with the help of Wang and Seal and their technicians in Year 1, January 1-December 31, 2016.

Evaluating insecticides for thrips and TCSV. Selective insecticides including spinetoram, cyantraniliprole, acetamiprid, flonicamid, methomyl, rosemary oil, and peppermint oil that are recommended on fruiting vegetables for management of thrips with low toxicity to natural enemies will be evaluated on tomato (cv. FL47 or Sanibel, susceptible to TCSV and the most common cultivars in Homestead) for their efficacy in controlling thrips that transmit TCSV and in reducing TCSV incidence. Composition, abundance of thrips species will be monitored weekly and TCSV incidence every 3-4 days

for evaluating insecticides, and will be compared to the standard chemical and non-treated control plots. This will be conducted by Seal ad his technician with the help of Zhang and Wang in Year 1, January 1-December 31, 2016.

Effect of acibenzolar-S-methyl on TCSV. Actigard (Syngenta, Greensboro, NC), a product of plant activator acibenzolar-S-methyl used routinely in tomato for management of bacterial spot, was proved to be effective in reducing incidence of TSWV. Field trials will be conducted to evaluate Actigard for its potential to reduce TCSV incidence. Field experiments on black mulch without insecticides will be primarily conducted by Zhang and his postdoc in Homestead, FL in Years 1 (January 1-December 31, 2016). Actigard will be applied as one spray 1 week prior to transplanting, six sprays at 1-week intervals starting immediately after transplanting, and then once every 2 weeks for rest of the season. Plants without treatment will be included as an untreated control. TCSV incidence will be recorded every 3-4 days after symptoms appears. Data of disease incidence will be analyzed using analysis of variation (ANOVA).

Development of integrated strategies. Integrated effects of insecticides, use of Actigard, other cultural practices such as UV-reflective mulch, cover crops, weed host removal on thrips and TCSV incidence will be evaluated by Zhang, his postdoc & Seal, his technician in Years 2, January 1 - December 31, 2017 with assistance of Wang and McAvoy. For instance, experiments will be designed as a split-split plot with black or UV-reflective mulch as the whole plot treatments, Actigard or no Actigard as the split plot treatments, and insecticides as the split-split plots. Actigard will be applied as described previously. Insecticide treatments will include spinetoram (Radiant), cyantraniliprole (Exirel), and alternating or tank mix applications of each spinetoram, cyantraniliprole with other insecticides based on the data from a field trial in the spring of 2015 in Homestead, FL. Design of insecticide treatments will be a RCB with four replications. Disease incidence of TCSV will be determined by inspecting plants in each plot every 3-4 days. The area under the disease progress curve (AUDPC) will be calculated.

Population densities of thrips will be monitored weekly by randomly sampling 10 flowers per 35-ft plot. Tomato flowers will be placed in vials containing 70% alcohol and transported to the laboratory for processing. Thrips adults will be extracted and identified to species under a stereo-dissecting microscope, and numbers of thrips larvae will also be recorded. Mature tomatoes from healthy plants will be harvested from each plot and graded for marketable yields according to the U.S. Department of Agriculture standards. An analysis of variance (ANOVA) for a split-split plot RCB design will be performed to determine the effect of UV-reflective mulch, Actigard, insecticides, and other agricultural practices on TCSV disease, thrips populations and tomato yields.

Accomplishments -

We compared three backgrounds of tomato that possessed the *SW5* locus (one was heterozygous and the other two were homozygous) for resistance to TCSV and an isolate of TSWV. We determined that the single dominant *Sw5* locus that confers resistance to TSWV also confers resistance to TCSV. Plants containing the *Sw5* locus, whether in a heterogeneous or homogeneous state, exhibited similar local lesion responses to both TSWV and TCSV inoculation under greenhouse conditions. Plants inoculated with TCSV lacked typical systemic symptoms 14 and 21 days post-inoculation which was the same response to TSWV. Known susceptible backgrounds lacking the Sw5 locus (sw5/sw5) exhibited local and systemic symptoms typical of tospoviruses. TCSV did present more severe symptoms than the isolate of TSWV used in these studies.

During the 2015-2016 growing season, Zhang and his group, with help of McAvoy in collecting seed of tomato varieties with TSWV resistance from seed companies, conducted two field trials in south Florida to evaluate tomato varieties for resistance to TCSV, and other trials to

determine the effect of UV-reflective plastic mulch, plant activator acibenzolar-S-methyl (ASM, Actigard®) alone and in combination on TCSV. Results from variety trials indicate that tomato varieties with known resistance to TSWV (*w-5* gene) were proved to be also resistant/tolerant to TCSV in south Florida. UV-reflective plastic mulch and Actigard reduced disease incidence, but not significantly. This may be due to the El Nino which resulted in more days in this season that were cloudy and rainy than normal seasons. During the 2016-2017 growing season, Zhang group continued conducting field trials in Homestead, FL to evaluate tomato cultivars for resistance to TCSV, to determine the effect of UV-reflective plastic mulch, plant activator acibenzolar-S-methyl (ASM, Actigard®) on TCSV. Due to TCSV incidence was low (<5 %) in the testing fields and most infection occurred on northern/eastern edges of the fields, data collected from the field trials were not reliable and convincing. This work needs to be repeated in the 2017-2018 season. However, tomato yield from plots covered with UV-reflective plastic beds was significantly increased by >30% compared to the black plastic.

Seal group conducted three efficacy trials in this season. In the first trial, seven insecticide treatments were evaluated including three rates of one new insecticide BAS45001 (under registration) which provided high levels of thrips suppression (ca. 70% mortality). Efficacy of this new insecticide was compared with other registered insecticides spirotetramat (Movento®, Bayer CropScience), spinetoram (Radiant®, Dow AgroSciences), tolfenpyrad (Torac®, Nichino America) and a nontreated control. BAS45001, Torac and Movento significantly ($P < 0.05$) reduced thrips population as compared to the nontreated control.

In the second trial, insecticide treatments evaluated were: 1. tolfenpyrad (Torac®, 21 oz/acre); 2. tolfenpyrad (21 oz/acre) followed by methomyl (Lannate®LV, 48 fl.oz/acre); 3. tolfenpyrad (21 oz/acre) followed by spinetoram (Radiant®, 8.0 fl oz/acre); 4. spinetoram (8 fl oz/acre); and 5. a nontreated control. All insecticide treatments significantly reduced thrips population as compared to the nontreated control.

In the third trial, three insecticides were evaluated to control thrips and their transmitted TCSV. The treatments were: 1. cyazypyr (13.5 oz, Verimark, Dupont Crop Protection) applied at planting in furrow; 2. imidacloprid (10.5 oz/acre, Admire®, Bayer CropScience) applied at planting in furrow; 3. imidacloprid (1.5 oz/acre) applied at planting in furrow followed by cyazypyr (13.5 oz/acre) applied 2 weeks after planting through irrigation drip; 4. cyantraniliprole (20 oz/acre, Exirel) applied on foliage; and 5. a nontreated control. All treatments reduced thrips populations when compared with the nontreated control. Mean numbers of TCSV infected plants were only significantly fewer in plots treated with soil applications of Verimark and Admire.

Objective 4. Promote awareness of TCSV and other tospoviruses, and educate tomato growers on integrated management of these tospoviruses.

Tasks - The Extension component including educating tomato growers and other clientele is an important part of this proposal. This task of extension will be led by Wang and McAvoy, Zhang and Seal will also be involved in outreach activities during the period of two years, January 1, 2016- December 31, 2017. Promoting awareness of TCSV in the USA is imperative as TCSV has become a significant threat to tomato industry in South Florida and potentially in the eastern Unites States. Prior to the development of integrated management program during Year 1, January 1 - December 31, 2016, the team will deliver presentations at extension workshops and meetings, publish extension articles, factsheets and newsletters on TCSV and its potential of TCSV spreading to other regions. Some extension article(s) about TCSV and its management will be posted on UF/IFAS TREC website and Miami-Dade County

Extension website, which are extensively used by local growers and extension specialists in South Florida. The extension faculty on this team routinely speak at vegetable grower meetings, which will provide additional opportunities for outreach education. Research information will also be delivered to tomato growers, other specialty vegetable producers, industry representatives, regulators, and other researchers through seminars, grower magazines, newsletters, extension publications, press releases, web postings, and presentations at meetings including the annual meetings of Florida Tomato Institute, Florida State Horticultural Society, American Phytopathological Society, Entomological Society of America, and American Society for Horticultural Sciences.

We will make the protocol available to private, state and federal plant disease diagnostic laboratories so that they can improve their detection of TCSV. Currently these diagnostic labs do not have a rapid assay for specific detection of TCSV, and can only use assays that detect tospoviruses in general, but cannot distinguish among the different virus species. This assay will improve the capacity of our diagnostic laboratories and thereby improve our management of TCSV.

A number of field days and demonstrations on sites of field trials will be conducted to demonstrate the strategies for effective management of TCSV once the strategies are developed. Zhang and Seal are also state extension experts in FL in addition to their research appointments; Wang and McAvoy are county and regional extension agents on vegetable crops in South Florida, respectively. McAvoy maintains a biweekly South Florida Vegetable Pest and Disease Hotline with access to over 2,000 individuals and businesses, and provides up-to-date information on disease occurrence on over 120,000 acres of farm land representing all major South Florida vegetable production areas. Findings from this project will be sent to the Hotline and disseminated to growers and the vegetable industry. The PIs have ready access to county extension faculty, tomato growers and other stakeholders, allowing us to facilitate dissemination of research findings to the end users. We will inform the Miami-Dade County Extension Vegetable Advisory Committee and solicit their comments in order to elucidate their perceptions and concerns. Questionnaires will be administered before and after these outreach events to determine the effectiveness in educating the growers concerning identification and management of TCSV. Follow-up surveys will be carried out to assess the impact of the implementation and the adoption of changes in farming practices. Summaries of research findings and outreach activities will be distributed during these events.

An EDIS on TCSV was published by Polston and Zhang groups in 2013 for keeping informed growers, researchers, extension agents, and chemical industry of this important viral disease. We will refine and update current management recommendations in this EDIS and other Extension publications in Year 2, January 1 - December 31, 2017 based upon research findings and outreach programs, and disseminate the information through grower meetings, UF/IFAS TREC program websites, articles in popular and trade magazines, and updating extension publications such as EDIS and newsletters. As TCSV is new to Florida and the USA, it is imperative to determine the important inoculum sources, to identify the thrips vectors, and to modify and update integrated management strategies for control particularly with the introduction of new insecticides. It is also anticipated that this proposed project will provide educational and training opportunities to graduate students in the academic areas including invasive plant pathogen, molecular identification, chemical and biological disease control.

Accomplishments - The team worked closely to educate growers for managing TCSV. We have conducted six field days and five workshops on TCSV. We showcased the strategies for effective management of TCSV on-site and also in handouts. A total of 155 participants attended

the field days, 187 for the workshops, and 29 for meetings with key growers. A post-event survey was conducted for each of these activities. The result indicated that, on average, the weighted overall rating was 4.3 in knowledge gain, 4.4 in practice change, and 4.3 in satisfaction on a scale of 1-5 (low-high). As a result, growers in south Florida gained knowledge of TCSV and its management. For example, DiMare, a large tomato grower in Florida and the east US states for many years, reported that they did not suffer the same from TCSV in 2015-2016 season as did in 2014-2015. This is partly because they learnt a lot about TCSV during training we conducted and started the program at the very beginning for their tomato plantings. EDIS update are in progress.

In addition, the results of this project were disseminated through refereed papers, non-refereed conference publications and oral and poster presentations at professional meetings. Five papers were published in refereed journals, four non-refereed papers were published, and four oral presentations were made at state, regional and national, international conference meetings.

Goals and Outcomes Achieved

Goal 1. Develop rapid and reliable assays for specific diagnosis of TCSV, which can be used to identify TCSV in plant samples from growers as well as in biological studies on TCSV.

Performance Measure

The recombinase polymerase amplification (RPA) assay has emerged as a novel alternative isothermal amplification method for the detection of nucleic acid. In this project, a reverse transcript (RT) -RPA will be developed for rapid and reliable detection of TCSV and other tospovirus from plants. The RT-RPA assay was reported to take only 20 min to perform and requires a constant temperature of 37-42°C. We will design and evaluate a range of primers that are specific for TCSV in the presence of TSWV and GRSV. These primers will be tested using several different RNA extraction protocols in order to develop a rapid, sensitive and reliable protocol. Once developed will we use this protocol to detect TCSV in Goals 2-4. We will also make the protocol available to diagnostic labs so that they can improve their detection of TCSV. Currently diagnostic labs do not have a rapid assay for specific detection of TCSV, and can only use assays that detect tospoviruses in general, but cannot distinguish among the different virus species.

Target

A novel detection method - the RT-RPA assay will be developed as an isothermal amplification method for rapid and accurate detection of TCSV and other tospoviruses. We anticipate that by using this new detection method, we will be able to specifically detect the viruses from plant samples within one day.

Accomplishments -

We have tested several primers but to date have not identified a pair that will work as well as we would like in the RT-RPA. We continue working on this in a hope that specific primers that work well for development of the RT-RPA for specifically detecting TCSV from plants.

Instead, a method of loop-mediated isothermal amplification (LAMP) has been developed to specifically and rapidly detect TCSV from plant samples. Through multiple sequence analysis from representative isolates of TCSV, GRSV and TSWV, a conserved sequence region (240 nt in the N gene - S RNA segment) to all known TCSV isolates has been identified and used to design specific primers. In addition, primers for species-specific detection in RT-PCR for TCSV, GRSV and TSWV were also developed for confirmation and validation of the LAMP technology on species-specific detection and its sensitivity as described below. Specificity tests showed that the LAMP assay was specific to TCSV, without any cross reaction to TSWV or health tomato samples. Once the specificity determined, we were interested in determining the sensitivity of the newly developed LAMP for virus detection. Using serial dilutions (10-fold) of purified RNA (2 ug/ul) as templates, comparative sensitivity of RT-PCR and RT-LAMP assays were evaluated using purified RNA preparation. In this test, we found that RT-LAMP assay (10^7) was ten times more sensitive than RT-PCR (10^6) for TCSV detection. In order to make LAMP technology for rapid detection of field sample by extension specialists and growers, using a diluted crude sample would be an ideal situation. To simplify the process of RT-LAMP detection, crude tissue extract preparation was used for RT-LAMP and RT-PCR assays. Crude tissue extract from TCSV-infected tomato was prepared in ten-fold serial dilutions from 1:100 to $1:10^5$. Similar to using purified RNA as templates, LAMP assay (10^4) was also ten times sensitive than RT-PCR (10^3) for TCSV detection.

Goal 2. Determine if other vegetable crops, ornamental plants, tropical fruit crops and weeds are hosts of TCSV, and investigate if other thrips species rather than those currently known are vectors of TCSV.

Performance Measure

In host range experiments, virus-free plants of each species or cultivar will be mechanically inoculated at the stage of 2-3 true leaves in the greenhouses with plants being mock inoculated as controls. Inoculated plants will be examined weekly for TCSV symptom development. The presence of target virus in leaves of inoculated plants will be determined 4-6 weeks after inoculation using the TCSV-specific RT-RPA as described in Objective 1.

Transmission ability and rates of TCSV will be determined. Colonies of thrips will be maintained on different crops depending on thrips species. Females and males of each thrips species will be confined in cages containing these plants for 48 h. Plants will be closely monitored for development of typical symptoms of TCSV, and the presence of TCSV will be confirmed by RT-RPA assays. Adults of each thrips species will be maintained in separate containers and allowed to oviposit on bean pods before they are removed. First instar larvae will be collected from bean pods shortly after emergence and placed on symptomatic leaves of TCSV-infected tomato `Florida 47' source plants for acquisition and transmission experiments. A single adult thrips from a larva feeding on infected source plants will be moved to a single leaf disc of tomato `Florida 47' placed on agar. Adults will be removed from leaf discs after 2 days. Identification of thrips will be confirmed. Thrips and leaf discs will be tested for TCSV by RT-RPA. Acquisition and transmission of TCSV by thrips species will be compared.

Target

For host range of TCSV, we will determine other host species of TCSV in Florida including vegetable, ornamental, tropical fruit crops and weeds with emphasis on those in the solanaceous family. For vectors of TCSV, focus will be placed on the following species of thrips that commonly inhibited in Florida vegetable plantings: Florida flower thrips (*F. bispinosa*), tobacco thrips (*F. fusca*), melon thrips (*Thrips palmi*), and eastern flower thrips (*F. tritici*) for their capacity to transmit TCSV and the efficacy.

Accomplishments-

We found that TCSV was able to infect several orchid species. TCSV was detected in large concentric ringspots of orchids (*Phaleanopsis spp.*). Viable isolates of TCSV were obtained from these lesions by inoculation to *Nicotiana benthamiana*. These lesions appeared in the spring in Homestead in orchid houses that were located downwind of tomato fields. The infection appeared to be restricted to the tissues in and around the lesions. Extensive testing of non-symptomatic leaves indicted that TCSV was not present in those leaves. This suggests that the orchids are resistant to TCSV but do produce a lesion at the point of inoculation which then expands. The TCSV isolate from orchid was highly pathogenic in tomato, pepper and *Nicotiana benthamiana* (an experimental host). However, we were unable to successfully infect orchid leaves with the TCSV by mechanical inoculation, even after many attempts with different buffers and different methods. We suspect that inoculation occurs by thrips which are leaving tomato fields in the spring which then land in the plant houses, feed and transmit TCSV to the orchids. Since the infection is not systemic, growers only need to remove symptomatic leaves in order to make the plant marketable. Observed incidences of lesions were relatively low (less than 5%). For these reasons, TCSV does not appear to be a serious problem for producers of these orchids.

TCSV was first detected in the USA in snapbean plants in a greenhouse in Homestead, FL in 2017 by the Zhang laboratory. This expands the host range of TCSV in the USA. As snapbean is a large acreage vegetable crop in Miami-Dade County and Florida, this will pose a huge challenge to bean growers in this area. Management of TCSV needs to consider snapbean as a potential reservoir of TCSV.

Among the weeds commonly present in south Florida vegetable fields, purslane (Portulaca oleracea) was detected positive against TCSV in 7 out of 16 samples (43.75%) and was confirmed to be an alternate host of TCSV.

The Seal laboratory conducted survey on thrips in tomato fields in Homestead, FL to determine weed hosts of thrips. All samples with thrips belonging to different species which will be identified to species level. Relationship between TCSV infection and thrips population will be determined. For a specific species of thrips, its role in transmitting TCSV is in progress.

Goal 3. Develop integrated strategies for management of TCSV in commercial tomato production based on the data produced in Objectives 1 - 2.

Performance Measure

Field trials will be conducted to evaluate tomato TSWV-resistant cultivars for their resistance to TCSV, insecticides available for managing thrips and TCSV incidence, and other practices for their effects on TCSV incidence including host weed removal, use of UV-reflective mulch, use of non-host crops as a barrier, cover crops, and intercropping with fast growing non-host crops. In addition, a plant activator acibenzolar-S-methyl (Actigard) effective in reducing TSWV incidence, will also be evaluated for reducing symptom development of TCSV. An integrated program will be developed based on the individual strategies stated above for improved management of TCSV in South Florida.

Target

Develop integrated management strategies for TCSV in Florida including use of host resistance, plant activator ASM, and insecticides for thrips suppression and eventually TCSV reduction. Effects of other cultural practices will also be determined on TCSV including the use of UV-reflective mulch, host weed removal, non-host cover crops. We anticipated that a reduction of 60-70% or above will be achieved in TCSV disease incidence by using the integrated management program to be developed in this project.

Accomplishments -

During the 2015-2016 growing season, Zhang and his group, with help of McAvoy in collecting seed of tomato varieties with TSWV resistance from seed companies, conducted two field trials in south Florida to evaluate tomato varieties for resistance to TCSV, and other trials to determine the effect of UV-reflective plastic mulch, plant activator acibenzolar-S-methyl (ASM, Actigard®) alone and in combination on TCSV. Results from variety trials indicate that tomato varieties with known resistance to TSWV (*w-5* gene) were proved to be also resistant/tolerant to TCSV in south Florida. UV-reflective plastic mulch and Actigard reduced disease incidence, but not significantly. This may be due to the El Nino which resulted in more days in this season that were cloudy and rainy than normal seasons. During the 2016-2017 growing season, Zhang group continued conducting field trials in Homestead, FL to evaluate tomato cultivars for resistance to TCSV, to determine the effect of UV-reflective plastic mulch, plant activator acibenzolar-S-methyl (ASM, Actigard®) on TCSV. Due to TCSV incidence was low (<5 %) in the testing fields and most infection occurred on northern/eastern edges of the fields, data collected from the field trials were not reliable and convincing. This work needs to be repeated in the 2017-2018 season. However, tomato yield from plots covered with UV-reflective plastic beds was significantly increased by >30% compared to the black plastic.

Seal group conducted three efficacy trials in this season. In the first trial, seven insecticide treatments were evaluated including three rates of one new insecticide BAS45001 (under registration) which provided high levels of thrips suppression (ca. 70% mortality). Efficacy of this new insecticide was compared with other registered insecticides spirotetramat (Movento®, Bayer CropScience), spinetoram (Radiant®, Dow AgroSciences), tolfenpyrad (Torac®, Nichino America) and a nontreated control. BAS45001, Torac and Movento significantly ($P < 0.05$) reduced thrips population as compared to the nontreated control.

In the second trial, insecticide treatments evaluated were: 1. tolfenpyrad (Torac®, 21 oz/acre); 2. tolfenpyrad (21 oz/acre) followed by methomyl (Lannate®LV, 48 fl.oz/acre); 3. tolfenpyrad (21 oz/acre) followed by spinetoram (Radiant®, 8.0 fl oz/acre); 4. spinetoram (8 fl oz/acre); and 5. a nontreated control. All insecticide treatments significantly reduced thrips population as compared to the nontreated control.

In the third trial, three insecticides were evaluated to control thrips and their transmitted TCSV. The treatments were: 1. cyazypyr (13.5 oz, Verimark, Dupont Crop Protection) applied at planting in furrow; 2. imidacloprid (10.5 oz/acre, Admire®, Bayer CropScience) applied at planting in furrow; 3. imidacloprid (1.5 oz/acre) applied at planting in furrow followed by cyazypyr (13.5 oz/acre) applied 2 weeks after planting through irrigation drip; 4. cyantraniliprole (20 oz/acre, Exirel) applied on foliage; and 5. a nontreated control. All treatments reduced thrips populations when compared with the nontreated control. Mean numbers of TCSV infected plants were only significantly fewer in plots treated with soil applications of Verimark and Admire.

Goal 4. Promote awareness of TCSV and other tospoviruses, and educate tomato growers on integrated management of these tospoviruses.

Performance Measure

A series of outreach activities such as workshops and field days will be carried out each year on sites of field trials where effective strategies for managing TCSV are developed to showcase growers and other stakeholders the tools for effective management of TCSV. Results from this research will be delivered to tomato growers, industry representatives, regulators, other researchers and extension specialists

through seminars, grower magazines, newsletters, extension publications, press releases, web postings, and presentations at meetings including the annual meetings of American Phytopathological Society, Entomological Society of America, American Horticultural Society, Florida Tomato Institute, and the Florida State Horticultural Society. Questionnaires will be administered before and after these outreach events to determine the effectiveness in educating the growers concerning management of TCSV. Summaries of research findings and other relevant information will be distributed during these events. We will refine and update current management recommendations based upon research findings and disseminate through grower meetings, UF/IFAS TREC program websites, articles in popular and trade magazines, and updating extension publications such as newsletters and EDIS (Electronic Data Information System).

Target

Information developed in this project on integrated management programs for control of TCSV will be provided to growers immediately for implementation. Led by county Extension agents, tomato growers, other specialty vegetable producers, crop advisors and consultants will be trained through the train-the-trainer program to correctly identify the diseases caused by TCSV and other tospoviruses by themselves based on field symptoms. We anticipate that the growers are able to identify 70% of TCSV symptoms they may encounter in commercial tomato fields. Also, at the end of the project, they will be capable of adopting the integrated strategies for successful management of TCSV in Florida.

Accomplishments -

The team worked closely to educate growers for managing TCSV. We have conducted six field days and five workshops on TCSV. We showcased the strategies for effective management of TCSV on-site and also in handouts. A total of 155 participants attended the field days, 187 for the workshops, and 29 for meetings with key growers. A post-event survey was conducted for each of these activities. The result indicated that, on average, the weighted overall rating was 4.3 in knowledge gain, 4.4 in practice change, and 4.3 in satisfaction on a scale of 1-5 (low-high). As a result, growers in south Florida gained knowledge of TCSV and its management. For example, DiMare, a large tomato grower in Florida and the east US states for many years, reported that they did not suffer the same from TCSV in 2015-2016 season as did in 2014-2015. This is partly because they learnt a lot about TCSV during training we conducted and started the program at the very beginning for their tomato plantings. EDIS update are in progress.

In addition, the results of this project were disseminated through refereed papers, non-refereed conference publications and oral and poster presentations at professional meetings. Five papers were published in refereed journals, four non-refereed papers were published, and four oral presentations were made at state, regional and national, international conference meetings.

Beneficiaries

1. **Who are the specialty crop beneficiaries of the project?**

The primary beneficiaries of this project are tomato growers in Florida. Producers of tomato transplants in South Florida are also the beneficiaries of the project as tomato transplants may play an important role in disseminating TCSV. The detection assay (RT-LAMP) developed in this project can be used to specifically detect TCSV from transplants prior to shipment to the east coast U.S. states. Besides tomato, to date, we do not know how many other crops are being affected by TCSV because little is known about the host range of TCSV yet. Plants that are likely to be infected, based on greenhouse experiments, are

pepper, eggplant, tomatillo, lettuce, and a number of ornamentals including petunias, impatiens, annual vinca (*Catharanthus roseus*), porcelainflower or waxflower (*Hoya wayetii*), and false Christmas cactus (*Schlumbergera truncate*). Apparently, there are likely to be many more hosts of TCSV. Therefore, growers producing transplants of these vegetable and ornamental crops are also the beneficiaries of this project.

2. How many specialty crop beneficiaries will the project benefit?

It is estimated that hundreds of growers in Florida including primarily vegetables and some ornamental growers benefited from this project, even though we do not know how many growers have been affected by TCSV because it is unknown, to date, how many crops are infected by the virus. However, we know most tomato growers, if not all, in Homestead and Miami-Dade County have been affected as well as many in other counties in South Florida such as Palm Beach County, the largest tomato production area in Florida. Pepper growers are also affected in Miami-Dade County by TCSV. In April 2015, a sample of bell pepper from a local grower was diagnosed to be infected with TCSV. Snapbean growers are potentially affected by TCSV. Growers of other susceptible crops - lettuce, eggplant, tomatillo, and growers of a number of bedding plants - petunias, impatiens, and other ornamental crops including annual vinca, *Hoya wayetii* and *Schlumbergera truncate* are also likely to be affected. Producers of transplants of TCSV-susceptible crops which are marketed through large box stores (K-Mart, Home Depot, etc …) also benefit from this project.

3. How will the project benefit the specialty crop beneficiaries?

This proposed project developed a rapid and reliable method (RT-LAMP) for detecting TCSV from plant samples, and integrated strategies for managing the disease in tomato and other susceptible crops in Florida. This benefits growers of tomato and other susceptible crops to TCSV through providing cost-effective strategies for reducing the damage to crops by TCSV. Thus, the successful completion of this project results in increased productivity, profitability and competitiveness of vegetable/ornamental/transplant growers in South Florida. For example, many tomato growers in Miami-Dade County adopted TCSV resistant varieties and used UV-reflective plastic mulch in tomato production. The incidence of TCSV has significantly been reduced since 2016 in most tomato fields in Homestead, FL. Based on reports from major tomato growers, the disease was reduced by 60-80% after the strategies were taken. In addition, growers and homeowners in other areas of Florida and the eastern USA are likely to benefit by having access to the technology which will be developed in this project once the virus is introduced and established in these regions. Improved management in South Florida reduces the rate at which TCSV and its vectors are moved within and outside of Florida.

4. What is the potential economic impact of the project if it can be estimated?

Miami-Dade County is an important contributor to the Florida tomato industry. In 2006, approximately 9.3% of the total tomato revenue of Florida came from Miami-Dade County with the value of $45.8 million. A study conducted by the University of Florida indicated that tomato growers in the Miami-Dade County are likely to regain a competitive edge in the market for winter vegetables versus other distant producers. The study predicted that tomato production is likely to double over the next coming years with the majority of the expansion occurring at the expense of other vegetables. In fact, the acreage (3,809 acres) of tomato harvested in Miami-Dade County in 2012 increased slightly from 2007 (3,667 acres). However, tomato growers in South Florida particularly in Miami-Dade County are facing increasing challenges posed by tospoviruses such as TCSV (as described above), which was recently discovered in South Florida and became epidemic in the fall of 2014 in the area.

Since the growing season of 2014-2015, TCSV is the dominant virus in commercial tomato fields across South Florida. In Homestead, FL, TCSV resulted in losses of about 30% plants in many tomato fields, and some tomato plantings were abandoned due to the heavy TCSV infection. In the spring of 2015, 100% tomato plants could be infected with TCSV, which is devastating tomato plantings of U-Pick operations. For example, a large tomato producer grows a total of 800 acres of tomato each season in Homestead, FL. In the 2014-2015 season, they experienced 30-50% yield losses due to TCSV. Since the 2015-2016 season, because of attending our training programs, they developed a management program and start the program right after transplanting. The incident of TCSV has been significantly reduced, save about 20% of the total yield. Tomato yield in South Florida averages about 30K pounds per acre. According to the average price is $12/box (25 lbs), this saved them about $4.6 million in these two seasons. In the 2017-2018 season, tomato growers used metalized plastic to reduce insects for virus infection, and to increase tomato yield. Based on our field trials during the 2016-2017 season, tomato yield was increased by approximately 30% on metalized plastic compared to black plastic. According to the average price was $37.95/carton (= 28 lbs. See Gene McAvoy - "FL Veg, Tomato May Miss Sky High Market As Imports Increase" sent via South Florida Vegetable Pest and Disease Hotline. December 18, 2017) for early plantings of 200 acres, this would save them about $2.4 million in the 2017-2018 season.

Considering the significant losses it has inflicted only two years after its discovery, there is great potential that TCSV will likely spread to other regions of Florida and to other states in the eastern USA. TCSV was also detected in Ohio from tomato fruit produced in a commercial high tunnel in 2013 and from New York in 2017. It is impossible at this time to estimate the damage caused by the establishment of TCSV in other states, but it is definitely a considerable impact. This project greatly benefited the tomato industry by developing integrated management strategies for TCSV, and potentially for other TCSV susceptible crops in Florida as well as in other eastern states of the USA.

5. **If applicable, how will the project have a multi-state (benefitting two more or states) or national impact?**

The main purpose of this proposed project was to develop an integrated management program to minimize the damage caused by TCSV which will provide Florida growers cost-effective and reliable recommendations for the management of TCSV. This significantly reduced yield losses to TCSV in crops of Florida as well as other states of the USA. A reduction in incidence of TCSV-infected plants help reduce the incidence of the export of TCSV-infected plants to the other eastern US states from the transplant production facilities in South Florida. This in turn helps reduce the chances that TCSV will be introduced into other US states. If introduction and establishment of TCSV do occur in other states, the integrated management program and information on biology of TCSV developed from this project can greatly aid these states in their response to TCSV.

Lessons Learned

A postdoctoral associate on this project had to leave the position due to emergent family issues in August 2016. Every effort has been made trying to find another qualified one as quickly as possible. Fortunately, Dr. Bind Poudel was identified and came to work on this project on January 2, 2018. This was the best we could do to refill the position ensuring to achieve the project goals. Eventually we were able to be on target to complete the project goals.

Another problem we encountered was low TCSV incidence in the field trials in 2017. The overall incidence of TCSV in our trials was <5% in our field trials and most of the growers' fields in Homestead, FL. This is attributed to knowledge about TCSV and its control gained by the growers as a result of training and education we have provided in the past few years. This reflects that our training and educational activities were effective and play an important role in TCSV management in south Florida, which we are very happy to see. However, for research purpose, we have to find a way to continue this project. We found an area near the National Everglade National Park showing heavy TCSV infection in tomato plants. The incidence of TCSV was about 12-47% in the 2017-2018 season. We have contacted the grower about this, and he agreed to collaborate with us on this research by providing us the fields in that area in a hope that the field trials can be successfully conducted in the near future, if necessary.

Project (18): Students Based Production of Specialty Vegetable Crops in Southern Florida

Project Summary

The project has addressed educational needs in the areas of production, sustainability, and environmental preservation in the South Florida Vegetable Industry. South Florida is a major United States source of winter vegetables, and its production area is very diverse in nature. The techniques and practices are very unique to the area, and the educational program at South Dade Senior is a direct reflection of this area. Students in the agriculture program learn these techniques in a "hands-on" environment, and take the skills into the South Florida industry.

Education is the foundation of the agriculture industry, and this project addresses needs at the very bottom of the spectrum by exposing young people to the concepts and needs of agricultural production. The project allows students to see the importance of agriculture, and the role South Florida plays in the United States agricultural industry as a whole. It allows us to expose and equip the next generation of agriculture industry members to a skill set that will allow them to be successful and productive.

Currently, the average age of production crop farmers is 50+, and the need to infuse new "blood" is at the forefront of our industry. This project is a recruiting tool for that purpose, and the exposure students need to cultivate their future.

Project Approach

During the 2015-2016 school year, students have participated in many crop production practices. These practices were land prep and planning for Thanksgiving 2015 market, supply ordering, and IPM focus points. Students were instrumental in planning the optimal plant date for green beans and squash. upon picking date students were actively involved with the planting, cultivation, IPM, and harvest planning of the crops. The same was done for the Christmas market in all aspects. Crops were sold through Five Brothers Produce, with help from a former working in their sales office, to many retail chains throughout the US and Canada. The total production for the year was 1072 boxes of zucchini, 212 boxes of yellow squash, and 702 boxes of green beans. The highlight of the year was harvesting 509 boxes of green beans on a 1.9 acre planting that netted approximately $2,500 for the program. The program enrollment for the year 207 students in which every student had exposure to the work involved through the project.

In addition to the large crops produced, students also planted, cultivated, and sold produce from the "Buccaneer Farms Farmer's Market". Crops included tomatoes, cabbage, Bok Choy, bell peppers, strawberries, and various lettuces. The crops were sold locally to the students, staff, and community.

There were many partners that were involved in the project as well. Diamond R fertilizer, Helena Chemical, and Clifton seeds provided technical and operational support to student. Their advice and mentoring helped in many ways. Students also received support from the UF Tropical research and Education Center with research. 29 students also participated in the center's annual cabbage trial study on black rot by helping plan and plant the trial in the field.

Goals and Outcomes Achieved

Expose and educate students to the specialty crop industry of South Florida through hands-on activity in technology, BMP, crop sciences, and IPM.
207 students were enrolled in the program, were exposed to the entire spectrum of the project.

Increase the number of specialty crop varieties grown by students in the agricultural program at South Dade Senior High by 50%.
Students have increased the variety of crops grown from 12 to 17. This is slightly under the desired goal, but will be easily attained with better planning

Reduce the amount of chemical used by implementing a revised IPM plan that uses minimal chemical pesticides, promotes irrigation management, healthy plant varieties, and helps protect bee populations in the area.

Chemical usage has been reduced with varied results. Due to weather events and growing conditions, crops received heavy disease pressure which reduced yields. The usage of newer plant varieties has helped because of better disease resistance, but the weather made it difficult to reduce chemicals. We tried, but to reduce down to 50% chemical reduction was not cost effective. December 2015 saw historic rainfall in southern Florida, and the entire industry suffered.

Improve student achievement in agricultural processes and certification. (FNGLA FCHP and State Pesticide Licensing)

2015-16 school year showed an increased from 5 to 9 students passing industry certification, and more interest in pesticide licensing. Student progression in horticultural class scores has improved by 23%, and the overall production of crops has improved by 35% from the previous year.

Beneficiaries

The beneficiaries of the project are the students and faculty of South Dade Senior High as well as the countless people who will be buying the crops throughout the local community and southeast United States. The faculty, students, and staff of the school were all educated in what our program does in the way of production and marketing. Partners Helena chemical, Clifton Seeds, and the University of Florida were able to pass on expertise, and expose their companies to prospective employees that could further their business success. The trial work done in collaboration with the UF TREC is passed on throughout the cabbage industry to help produce better performing crops, and to improve efficiency of the market. Our partners were able to see their products put to use in our field, and were encourage to "stop by" whenever possible provide feedback.

Lessons Learned

Agriculture is challenge just by its nature. Changing climates and weather issues are always a problem, which we have seen during this period of report. It both helped and hurt our overall outcomes in regards to production and IPM development, but helped sales price because of the low supply in the market. What we were able to produce was sold at a better price. The market was a challenge well. Students were educated in "what a difference a week makes" in regards to sales windows. One week beans were selling for $38 per box, a week later they were $22.

There were also paperwork challenges on our end. Bookkeeping and processing they correct paperwork with the school site treasurer has been hard to navigate. the school board has many policies that make it hard to get things done in a timely manner. This is not what you want in an agricultural production area because most of the time, time is of the essence. Delaying something a week because of having to wait on a signature or authorization is counter productive, but is standard procedure unfortunately. Communication and planning have been improved because of this, but emergencies will still be a problem.

Networking with stakeholders is also improving because of the student involvement and mentoring by community partners.

Contact

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Project (19): Improving Safety of Specialty Crops Sold at Farmers' Markets in Florida

Contact Person

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Project Partners

University of Florida / Institute of Food and Agricultural Sciences (UF/IFAS) -primary

Florida Organic Growers Assoc (FOG) -partner

Sharon Yeago, LLC.-partner

Project Summary

Farmers' markets have steadily grown with increasing public interest in healthy diet and local community support. With their steady growth, farmers' markets have become an important source of revenues for farmers, particularly those with small-sized operations. Commodities marketed through farmer's markets can vary; however, fresh vegetables, fruits, and nuts are the three major commodities sold through direct marketing channels, which account for 65% of the total sales of locally grown food.

As the number of farmers' markets has surged, concerns over safety of fresh produce sold at farmers' markets have also increased. FDA has reported that 17.3% of total foodborne outbreaks and 35.9% of total foodborne illness were linked to fresh produce. In fact, some of the recent foodborne outbreaks have been directly associated with fresh produce sold at farmers' markets. Despite the potential safety risks associated with farmers' markets, few studies have assessed the microbiological safety of fresh produce from farmers' markets. Therefore, it would be highly critical to assess microbiological risk factors associated with fresh produce sold at farmers' markets, and to develop an educational curriculum that can extend to direct marketing venues. This project addressed food safety issues related to specialty crops (fruits and vegetables) sold at Florida farmers' markets by: (1) assessing microbiological risks of produce and (2) developing an effective food safety education program specifically designed for small-scale produce growers who sell their fresh produce at farmers' markets and managers of markets whose main commodities are fresh produce. The findings from research and extension activities helped establish strategies to improve the safety of specialty crops grown and sold by small-sized fresh produce farms. Our project have found that the markets whose managers and vendors were trained from extension program show higher rate of food safety practice implementation than markets that did not participate in the training. Training program developed from this study will improve marketability and competitiveness of Florida specialty crops grown by underserved small-sized farmers.

Project Approach

In this project, we have analyzed the microbiological quality of 501 specialty crop samples including leafy green, spinach, berries, and tomatoes. Out of 501 samples, 351 samples were collected from 9 farmers' markets in North and Central Florida, and the other 150 samples were collected from 12 local supermarkets. Each market was visited 3 to 7 times between Jul 2016 and Mar 2018 for sample collection. For each visit, the number of total vendors, the number of produce vendors, and weather condition of the day of sampling were recorded for each market. All samples were prepared according to the FDA BAM method, and analyzed for the presence of coliforms, generic E. coli, and target pathogens (Salmonella, E. coli O157:H7 and Listeria). Coliforms and generic E. coli were identified and enumerated using 3M Petrifilm E. coli/coliform count plates based on the AOAC Official Method, and the presence of foodborne pathogens were identified by qPCR assay kits. Positive sample from qPCR were confirmed by selective plating. Additionally, samples were tested for shiga toxin-producing E. coli (STEC) other than O157:H7 using xMAP-based multiple assay. Once microbiological analysis was completed, statistical analysis was performed to compare the prevalence of total coliforms, generic E. coli, and foodborne pathogens among the various product types and market types. Research findings were presented in multiple regional, national, and international conferences, and also published in a peer-reviewed journal.

We also developed and delivered food safety educational program for farmers market produce vendors and managers. We organized the project advisory group for curriculum development and workshop planning, which included UF/IFAS faculty and extension specialists and agents, Sharon Yeago, who is a national leader in Community Farmers' Markets, members from Florida Organic Growers Association. The education curriculum was developed to cover food safety practices, effective marketing based on consumer trend change, and food regulations. A training manual "Food Safety Practices for Farmers' Markets" (384-page) was developed for the training, 120 copies of which have been distributed to produce growers and market market managers to date. During the project period, 8 trainings were provided: 3 trainings for produce growers/vendors and market managers ("Best Practices at farmers markets - improving food safety and market growth") and 5 trainings for specialty crop-based cottage food operators ("Understanding Florida Cottage Food Law"). Additionally, at the end of project, 38 farmers' markets were visited to survey market and vendor practices. The survey included 17 markets whose manager or multiple vendors have attended the training and 21 markets whose managers have not participated in the training. We compared the practices between these two groups of markets to measure the impact of training programs developed in the project.

Goals and Outcomes Achieved

Goal 1: (Research) Conduct comprehensive microbiological assessment on fresh produce sold at Florida farmers markets to determine the prevalence of foodborne pathogens and indicator bacteria.

Measurable outcomes and progress achieved:

1. Obtain objective measures of typical microbial loads on produce sold at farmers markets.

We found that total coliform was present with a significantly higher occurrence rate in farmers' market produce samples (51.4%) than supermarket samples (25.8%) (P-value<0.01). Generic E. coli occurrence was low in farmers market produce samples (2.0%), and the level of total coliform found in farmers market produce is generally low (0.5 to 2.0 log CFU/mL). In general, foodborne pathogen prevalence was very low in farmers' market produce samples.Listeria monocytogenes was detected only farmers' market samples (1.1%), and Salmonella was detected in both market samples with 0.6% and 0.7% of occurrence rates in farmers market samples and supermarket samples, respectively. No E. coli O157:H7 was detected in this study. When analyzed by the commodity type, leafy green samples had the highest prevalence of total coliforms (73%) in farmers market samples. Leafy green and spinach showed significantly higher prevalence in farmers market samples than in supermarket samples (73.0% vs 30.6% and 57.1 vs 17.6%, respectively).

2. Determine the areas of highest risk that will allow targeted focus on specific produce types and market conditions.

The results of this project suggest that practices of farmers' market produce vendors either at the market or at the farm as well as outdoor market condition may have a significant impact on the microbiological quality of produce sold at farmers' markets. Since it is hard to provide a temperature control for displaying fresh produce or any food products in an open outdoor market setting as farmers' markets, it would be beneficial to provide vendors with the information on best practices for food safety. Additionally, public education on safe produce handling practices including the importance of washing produce before consumption will be critical to prevent produce-related foodborne illnesses.

Goal 2: (Extension) Develop education materials that will address food safety practices and effective management strategies specifically designed for farmers' market managers and produce vendors, and deliver materials through direct (farmers' market manager/vendor training) and indirect (extension agent training, web-communication) channels.

Measurable outcomes and progress achieved:

1. Train 30 Extension agents and 50 farmers' market managers/vendors on farmers' market-specific food safety issues and GAP, who will further train 200 small specialty crop growers.

During the project period, a total of 8 educational workshops, including 2 in-service training for extension agents and 6 workshops for farmers market managers and produce growers/vendors were provided. As a result, 45 extension agents and 178 farmers' market managers, produce growers/vendors, and specialty-crop based cottage food operators got directly trained, which is much higher number than expected outcome. Additionally, 5 additional county-based programs were created

by trained extension agents, through which approximately 150 additional small specialty crop growers were additionally trained. According to the exit survey given at the workshops, 100% of participants said the workshop was very satisfactory (88%) or satisfactory (12%), and 100% of respondents self-assessed that their knowledge on the topic was improved after the workshop with 75% indicating significant improvement. Participants' self-assessment of improved understanding was supported by the results from pre- and post-tests that showed the workshop attendees' knowledge was improved from 59% to 74% on average.

2. Strengthen the overall Florida agricultural industry by raising food safety awareness and compliance of all growers with the regulations regardless of their sizes (long-term).

According to the follow-up survey given to workshop participants at their 1-year time point after the workshop, 31% of participants have improved their practices at the farm, and 67% changed their safety practices at the market based on the information they learned at the workshop. In addition, 40% of market managers responded that they have already changed or plan to change their market management strategy based on their learning. Moreover, 83% of respondents said they use the educational packet from the workshop as their resource for further information.

In addition to the follow-up survey, We also visited farmers' markets in Florida to observe their on-site practices to assess the impact of the educational programs. In total, 38 markets were visited, of which 17 markets with market managers who attended my educational workshop. The findings from the observation survey show higher rates in food safety practices at the markets whose managers participated in the educational workshop.

Beneficiaries

Primary beneficiaries of the proposed project are fresh produce vendors, managers and consumers at farmer' markets as well as any small to mid-sized produce growers, especially those using direct marketing venues. These small to mid-sized prdouce growers have traditionally been underserved in that they have limited resources for good agricultural practices (GAP) training and food safety education. Understanding food safety risks associated with specialty crops sold at farmers' markets (research) and developing and delivering food safety education materials specifically addressing farmers' market-associated issues (extension) help reduce the risk of foodborne illness associated with fresh produce, which consequently enhance the marketability and competitiveness of specialty crops grown by small to mid-sized farmers.

Our results show that, as a result of participating in the educational workshop, participants' knowledge on food safety practices and regulations has increased. Also, our market survey results found that food safety practices at the markets whose managers participated in the educational workshop are implemented better than markets whose manager did not participate. This project is believed to strengthen the overall Florida fresh produce industry by raising the level of food safety awareness and compliance of growers of all sizes.

Overall, the number of beneficiaries is estimated at 158 growers and market managers who either participated in a training, received a copy of the course manual directly, or were included in the survey of farmers' markets.

Lessons Learned

There were a few changes and additional work added for research component. Microbiological analytical method for foodborne pathogens had to change from originally proposed method due to intensive labor and time. Original method was to detect foodborne pathogens using selective plating, and to confirm the positive result with qPCR. However, we changed the method to run qPCR first as the main identification tool and used selective plating as a confirmation method. We also focused on O157:H7 serotype only as a target STEC, but had to rerun produce samples with a FDA BAM rapid method (xMAP technology-based Luminex multiplex system) to further confirm the presence/absence of other major STEC serotypes ("big 6"). Additionally, per peer review suggestions, we had to compare other qPCR to other types of rapid detection methods, which was not a part of original work plan.

In extension component, we decided to add the survey of markets to assess the impact of the developed training programs. This was not a part of the original work plan, but helped measure the practice and behavior changes in the markets whose managers and vendors were trained.

Even though additional tasks were added to the project, we were able to complete the project within the budget and the given project period. It would have saved a little more time and increased work efficiency if these tasks were included with the original work plan; however, it did not significantly affect the project completion.

Total Funding Expended: $141,761.62

Percentage of Project Completed: 100

Project (20): Food Safety Recall Workshops

Contact Person

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Project Partners

Florida Specialty Crop Foundation

Florida Fruit & Vegetable Association

McDermott, Will & Emery

Project Summary

With the implementation of the Food Safety Modernization Act, fruit and vegetable producers face new scrutiny on food safety issues. The FDA now focuses on prevention of microbial contamination, and will be able to issue mandatory recalls and also inspect production facilities. These facilities will also need to have a food safety plan.

The purpose of this grant was to educate and prepare producers to be ready in the event of a food safety incident and/or recall. Topics covered included: the importance of traceability; what to expect during a recall; developing a checklist; liability and legal issues related to food safety; and developing a crisis communication plan.

In addition to the two workshops "Are You Ready for a Recall", complete notebooks with reference materials, presentations and a crisis communication template were made available to attendees. Additionally, a 4-page insert was printed and distributed via Florida Grower magazine to over 10,000 fruit and vegetable readers and information was placed on the GrowingProduce.com website.

This project was not previously funded by a grant.

Project Approach

Two workshops were presented, entitled "Are You Ready for a Recall?". The first was held in Belle Glade, FL on October 26, 2016 and the second was held in Wimauma, FL on February 21, 2017.

Primary topics of the workshop were:

1) Overview: Why We're Here

2) Understanding the Recall Process

3) Supplier/Retail Expectations

4) Communicating in a Crisis

5) Recall Lessons Learned

6) Limiting Liability

An extensive binder of presentation and resource materials was given to each attendee, including operational checklists, reference sheets, a template crisis communication plan, etc.

For the February 2017 workshop, a spokesperson from Publix was able to provide valuable input to the group on retail expectations for food safety recalls. (He replaced the speaker from Markon at the October session.) Additionally, the videographer captured the presentation and has processed the videos and they are available on the FFVA website for audiences to view. Pre- and post-tests were conducted for both workshops.

A 4-page insert highlighting major points from the workshops was developed. It was inserted into the June 2017 Florida Grower magazine, which is sent to 10,096 growers, shippers, consultants and others allied to the field of specialty crop production in Florida. This widely disseminated the information developed by the grant to specialty crop producers in Florida. Additionally, a digital ad ran in growingproduce.com for both American Vegetable Grower and American Fruit Grower to direct them to the information. There are 7,900 average monthly visitors to those sites.

The insert is also available on the FFVA website. Overruns were printed to use in upcoming meetings.

As a result of the interest in some of the legal issues and responsibilities of growers, the law firm McDermott, Will and Emery sponsored two additional workshops entitled "Food Law Boot Camp". One was held in August 2017 at the Gulf Coast Research Center and one was held in October 2017 at the Everglades Research and Education Center. The 4-page insert which summarized the results of the earlier workshop were provided to these attendees.

All items in the Work Plan have been completed and exceeded. Only specialty crop producers participated in the workshop (or received the Florida Grower magazine).

Goals and Outcomes Achieved

For the October session, both a pre-test and a post-test were given to measure the increase in knowledge as a result of participating in the workshop. Additionally, a survey was sent out to attendees to measure the value of the information as well as the speaker presentations. All speakers were rated extremely highly for their presentations. When asked if they learned new information, 88% said yes.

For three questions, all survey participants said yes:

1) Will the information presented help you do your job better?

2) Did the workshop meet your expectations?

3) Would you recommend this workshop to others?

When asked to rate the useful of the binders they were given with the workshop materials, 100% of the participants rated it as excellent.

For the pre-test and post-test, a definite increase in knowledge was measured:

There were 11 questions on the test. On seven questions the number of correct answers increased between the pre- and post-tests. On six of the questions, the percentage increase in knowledge ranged from 31% to 68% increase.

Perhaps more importantly, when asked "How likely are you to go back to your company and develop a crisis communication plan, or if you currently have one, review it to ensure it's up to date?", on the pre-test 69% indicated very likely, 23% as somewhat likely, 8% as neutral. On the post-test, 84% indicated very likely and 16% showed somewhat likely.

For the February session, tests were also given. Based on pre- and post-test results, there is a significant increase in knowledge of food safety recall processes, as well as intention to either develop or update existing food safety recall plans. Between the pre-test and the post-test, there was a 78% increase in knowledge. At the end of the session, 81% of respondents indicated they intended to either develop a crisis communication plan, or review an existing on to make sure it is up to date.

Both measures outlined in the Expected Measurable Outcomes were exceeded.

Beneficiaries

The October 2016 workshop hosted 29 participants; the February 2017 workshop hosted 27 participants. The 4-page insert in the Florida Grower magazine was sent to 10,096 subscribers who are Florida fruit and vegetable growers, packers, shippers, processors and stakeholders. The GrowingProduce.com placements achieved 82,729 gross impressions. Both the insert and the videos are available on the www.ffva.com website, which had 1,982 unique users this month.

Lessons Learned

The lesson we learned is that there is a definite interest in food safety issues particularly related to recalls, and the audiences were looking for more information on what the expectations and responsibilities are. They were also interested in improving or developing the crisis management plans. They found direct input from marketers such as Markon and Publix provided very valuable insight into the process.

And we also learned there was enough interest that the law firm hosted two additional workshops to provide more in-depth discussion and question-and-answer sessions on legal aspects of the food safety recall process.

Funding Expended to Date and Project Progress

The project is 100% complete, with 15,750.80 expended to date.

FINAL PERFORMANCE REPORT

AWARD YEARS 2018 FORWARD

A Final Performance Report must be received within 90 days after the end of the grant agreement. You are required to report on the administration of the agreement and each project approved within the agreement. The completed Final Performance Report will be posted to the AMS website.

GRANT INFORMATION

AGREEMENT

AMS Agreement Number:	USDA-AMS-SCBGP-2015 GM02370			
Period of Performance:	**Start Date:**	1/1/2016	**End Date:**	9/15/2018
Award Amount:	$130,387.50			

RECIPIENT

Recipient Organization Name:	University of Florida, IFAS
Recipient's Point of Contact	
Name:	Joyce Jackson
Phone:	863-956-5897
Email:	jaj@ufl.edu

REPORT

Report Type:	Final report
Date Report is Submitted:	10/31/2018

GRANT ADMINISTRATION

If funds were used for grant administration, indicate the amount of funding expended from the beginning of the grant to the end of the reporting period covered by this report. Also, indicate the amount charged as indirect expenses versus the amount charged as direct expenses.

Amount Requested	Direct and/or Indirect Expended to Date

FINAL PROJECT REPORT TEMPLATE

Final Performance Reports must illustrate the completion of each project within the grant agreement. Each project shall be outlined as separate project profiles. You will report on projects in the same order they were submitted in the approved application and subsequent amendments.

PROJECT INFORMATION

Project Title	Production of young Tahiti lime and three triploid lime hybrids on four citrus greening tolerant rootstocks and a lime standard rootstock
Recipient Organization Name:	University of Florida, IFAS

Period of Performance:	Start Date:	1/1/2016	End Date:	9/15/2018

Recipient's Project Contact	
Name:	Jonathan H. Crane
Phone:	786-217-9271
Email:	jhcr@ulf.edu

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Provide enough information for the reader to understand the importance or context of the project. This section may draw from the background and justification contained in the approved project proposal.

Citrus greening [*Candidatus* Liberibacter asiaticus (Las)] and canker (*Xanthomonsas axonopodis* pv. *citri*) has effectively eliminated about 500,000 acres of Florida citrus. 'Tahiti' lime (*Citrus latifolia*; syn. *C. aurantifolia*) is susceptible to citrus greening however several investigations rate 'Tahiti' lime as moderately tolerant (little or no symptoms) to greening compared to orange, grapefruit, and some mandarins and tangelos. South Florida is the ideal climatic location to grow 'Tahiti' limes and prior to citrus canker had a thriving 3,500-acre industry. To re-establish the Florida lime industry a combination of a tolerant scion (e.g., 'Tahiti' lime) and rootstock (four are being tested) need to be evaluated if a sustainable lime production industry is to be re-established.

Citrus rootstocks have a profound impact on citrus tree disease tolerance and rootstocks and rootstock-interstock combinations vary in their tolerance to citrus greening and affect the scion growth and fruit production. Recently UF/IFAS and USDA-ARS have developed citrus greening tolerant rootstocks. There is great interest in replanting and re-establishing a lime industry in south Florida. However, guidance on which citrus greening tolerant rootstock to use is lacking. The purpose of this investigation was to establish a lime scion (variety) – rootstock planting and investigate their tolerance to citrus greening, insect attack, and tree growth and production.

ACTIVITIES PERFORMED

Address the below sections as they relate to the entire project's period of performance.

OBJECTIVES

Provide the approved project's objectives.

#	Objective	Completed?	
		Yes	No*
1	Establish a field demonstration grove of 'Tahiti' lime and three superior seedless lime triploid hybrids with commercial potential on four citrus greening tolerant rootstocks plus an industry standard rootstock at the UF/IFAS Tropical Research and Education Center, Homestead.	X	
2	Monitor Asian citrus psyllid (ACP; the disease vector) activity and citrus greening infection (time to first detection and spread rate) on the different lime scion and rootstock combinations. Monitor plant growth and development, including plant nutrition (leaf sampling), time to first flowering, and fruit production. If trees begin producing within two years obtain yield and fruit quality information) of the fruit produced within the first two years will be determined.	X	

3	Gather economic data to develop an enterprise budget that analyze the economic viability of establishment and maintenance of specialty acid citrus.	X	
4	Utilize the demonstration plantings in Extension programs to advise growers on lime and other acid citrus planting establishment and production.	X	

**If no is selected for any of the listed objectives, you must expand upon this in the challenges and lessons learned sections.*

ACCOMPLISHMENTS

List your accomplishments for the project's period of performance, including the impact they had on the project's beneficiaries, and indicate how these accomplishments assist in the fulfillment of your project's objective(s), outcome(s), and/or indicator(s).

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Established experimental lime citrus scion and citrus greening rootstock planting	Trees all successfully established. Six trees of 239 died due to Hurricane Irma (Sept. 2017); all other trees recovered. (OUTCOME 2 as identified in proposal)
2	Document growth and plant nutrient content of the lime scion-rootstock combinations	Determined lime scions on some rootstocks (e.g., C11) were grew faster (e.g., C11) than on others (e.g., C33). Young trees on C11 and TL rootstocks flowered before trees on all other rootstocks. Rootstock sprouting was a greater problem on trees grafted to US-802, US-897 and US-942 than tree grafted to CM and SO+50-7. In general, there were no practical differences in plant nutrient uptake among scion-rootstocks. For all scions, two rootstocks (C11 and TL) demonstrated ealier flowering, and the C11 rootstock demonstrated faster growth. (OUTCOME 2)
3	Experimental planting monitored for ACP and other insects throughout project	ACP were detected in the planting soon after planting (123 times) but ACP was only detected once in 12 months on 83% of trees. The ACP pest control program was successful for about 12 months. (OUTCOME 2)
4	Citrus greening monitoring and spread over time	The ACP control program was successful for about 12 months as all trees were citrus greening free from March 2017 to March 2018. Citrus greening was detected on 23 trees in March 2018. (OUTCOME 2)
5	Develop an enterprise budget for lime production	Twelve months of data on the cost of establishment and maintenance was obtained. This will provide potential producers with economic decision information. The budget for establishing new groves was estimated at $10,180 per acre, inclusive of all inputs, including installing micro-irrigation, drilling, plant, planting labor, fertilizers, pesticides, etc. This budget was presented at all extension activities and published in the proceedings of the Florida State Horticultural Society. (OUTCOME 3)

6	Extend project results to potential commercial lime growers	A field-day and seminar was offered and well attended exposing potential producers to the performance characteristics of these new rootstocks and scions. An extension publication has been completed and accepted in the proceedings of the Florida State Horticulture Society. Attendance at these extension presentations totaled 62, short of our goal of 200. However, the response to the surveys given at these events was encouraging. 40%of attendees at the field day responded to surveys. Of those, 57% were not currently growing limes. The 43% who were currently growing limes had a total of 55 acres collectively. 100% of respondents who did not currently grow limes indicated interest in establishing groves. Furthermore, 100%of respondents indicated that the event had been worthwhile to attend, and were interested in attending future presentations. 60% found the information to have been relayed 'clearly' with 40% responding that information was relayed 'somewhat clearly'.(OUTCOME 1)

CHALLENGES AND DEVELOPMENTS

Provide any challenges to the completion of your project or any positive developments outside of the project's original intent that you experienced during this project. Also, provide the corrective actions you took to address these issues. If you did not attain an approved objectives, outcome(s), and/or indicator(s), provide an explanation in the Corrective Actions column.

#	Challenge or Development	Corrective Action or Project Change
1	Seven months post planting Hurricane Irma caused all trees to lean west with some trunk, limb, stem and leaf damage. Six trees eventually succumbed to their storm damage.	All trees were re-set and staked and additional fertilizer was provided. The loss of six out of 239 trees will not substantially affect the trial.

LESSONS LEARNED

Provide recommendations or advice that others may use to improve their performance in implementing similar projects.

Obtaining the plants for this trial took more time than anticipated. If possible (and due to the economics of business this may be impossible), order but do not buy plants prior to obtaining the grant funds. This can be risky.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Describe your plans for continuing the project (sustainability; capacity building) and/or disseminating the project results.

We applied for and were granted continued funding for two more years. Furthermore, we plan on selling some of the produce from the planting to re-invest in maintenance chemicals and repairs to continue the planting and data collection. An Extension grower workshop-field day was held at UF/IFAS TREC and attended by 27 participants. A post-program survey found all participants indicated their interest in growing limes increased, that all the information provided was somewhat to very clear, and that they were interested in attending more programs pertaining to our scion-rootstock project. An extension presentation on results of the project was offered at the Annual meeting of the Florida State Horticultural Society (June, 2018) and a journal article for their proceedings has been accepted for publication (Crane, J.H. et al., 2018. First Year Results of a Trial of 'Tahiti' Lime and Three Lime-hybrid Scions Grafted to Five Citrus Rootstocks Established at TREC. Proc. Fla. State Hort. Soc. (accepted).

BENEFICIARIES

Number of project beneficiaries: potentially 2,000_____Enter Number of Project Beneficiaries

OUTCOME(S) AND INDICTATOR(S)/SUB-INDICATOR(S)

Provide the results of the project outcome(s) and indicator(s) as approved in your application and project proposal. The results of the outcome(s) and indicator(s) will be used to evaluate the performance of the Program on a national level.

OUTCOME MEASURE(S)

Select the Outcome Measure(s) that were approved for your project.

- ☐ **Outcome 1**: Enhance the competitiveness of specialty crops through increased sales
- ☐ **Outcome 2**: Enhance the competitiveness of specialty crops through increased consumption
- ☐ **Outcome 3**: Enhance the competitiveness of specialty crops through increased access
- ❒ **Outcome 4**: Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources
- ❒ **Outcome 5**: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems
- ☐ **Outcome 6**: Enhance the competitiveness of specialty crops through increasing the number of viable technologies to improve food safety
- ☐ **Outcome 7**: Enhance the competitiveness of specialty crops through increased understanding of the ecology of threats to food safety from microbial and chemical sources
- ❒ **Outcome 8**: Enhance the competitiveness of specialty crops through enhancing or improving the economy as a result of specialty crop development

OUTCOME INDICATOR(S)

Provide the indicator approved for your project and the related quantifiable result. If you have multiple outcomes and/or indicators, repeat this for each outcome/indicator (add more rows as needed).

#	Outcome and Indicator	Quantifiable Results
1	Outcome 4 and 5: Use of citrus greening tolerant rootstocks and interest in alternative crops	The use of citrus greening tolerant rootstocks offer the possibility of maintaining crop yields and quality. Twenty-seven local commercial fruit growers participated in a field day-seminar and increased their knowledge on the availability of citrus greening rootstocks. A post-program survey found all participants indicated their interest in growing limes increased, that all the information provided was somewhat to very clear, and that they were interested in attending more programs pertaining to our scion-rootstock project.
2	Outcome 8: Economic analysis of a specialty crop	Data was collected and analyzed for the establishment of a new lime grove was accomplished. Total costs of establishing a new lime grove in year one was $10,209.77. This information may be used by potential producers in their feasibility analysis for this crop.

DATA COLLECTION

Explain what data was collected, how it was collected, the evaluation methods used, and how the data was analyzed to derive the quantifiable indicator.

Plant growth and development: Tree height and trunk diameter were periodically measured, and leaf nutrient content was analyzed. The commencement of flowering was recorded over time and the amount of rootstock sprouting taken periodically over a 12-month period. Plant height, trunk diameter and leaf nutrient data was analyzed using linear mixed model to account for blocking associated with row and Tukey's multiple comparison method was used for pairwise comparisons of means. A Fisher's exact test was used to analyze the flowering and rootstock sprouting data.

Pest and disease management: Trees were inspected weekly to bi-weekly for the presence of insect pests (e.g., Asian citrus psyllids, ACP – citrus greening vector) and beneficial insects. Total number of ACP pest detections and relative amounts of other insect pests and beneficial insects were noted. Tri-monthly each tree was screened for citrus greening bacteria. Screening results are reported as totals.

Economics: The infrastructure costs (e.g., irrigation system, labor) to establish and maintain a lime grove for 12-months was recorded and analyzed.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel		
Fringe Benefits		
Travel		
Equipment		

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Supplies		
Contractual		
Other		
Direct Costs Sub-Total		
Indirect Costs		
Total Federal Costs		

PROGRAM INCOME (IF APPLICABLE) - **NOT APPLICABLE**

Source/Nature (i.e., registration fees)	Amount Approved in Budget	Actual Amount Earned
1.		
2.		
3.		
Total Program Income Earned		

Use of Program Income *Describe how the earned program income was used to further the objectives of this project.*
NA

ADDITIONAL INFORMATION

Provide additional information available (i.e., publications, websites, photographs) that is not applicable to any of the prior sections.

Lime scion-rootstock planting at TREC, one year later, March 2018.

Example of rootstock sprouting on TL-US-802 rootstock.

Project (22): Promoting specialty crops through agritourism: Producer professional development

Contact Person

Joy Rumble, 352-273-1663, jnrumble@ufl.edu

Project Partners

University of Florida

IFAS Communications

IFAS Extension

Project Summary

The public is generally very disconnected from their food sources and may be unaware or uninterested in addressing problems facing agricultural industries: invasive species, population pressure, climate change, immigration, and even market pressures from international competition. Estimates suggest that fewer than 2% of Americans currently live on farms and only a small percentage are involved in agriculture or natural resource jobs. However, interest in eating local food is growing. Therefore, agritourism offers an opportunity to reconnect consumers and producers to promote these relationships in the long term, as agritourist customers, are generally highly satisfied and intend to return to agricultural destinations. Agritourism can also offer additional revenue for producers.

For the purposes of this project, agritourism was defined as tourism at a broad range of agricultural facilities with an emphasis on specialty crops. Agritourism and agritainment (agriculture and entertainment) are all different ways to describe similar types of happenings on farms and at production facilities. Events include a variety of activities that showcase the products and sometimes the acts of production to public audiences. Audiences may come on a drop-in basis as with tourists or locals who visit pumpkin patches, u-pick opportunities, or wine tastings. School and other stakeholder groups are another potential audience for more or less structured tours. Often, agritourism offer consumer the opportunity to buy raw products or derivatives such as citrus candles straight from the producer, thus increasing the sales of local products.

The purpose of this project was to understand consumers' awareness and preferences for agritourism, while also helping specialty crop producers to improve or develop agritourism ventures. Through this project a statewide plan for agritourism on specialty crop operations was developed.

This project aligns with the following FDACS funding priorities:

- Immediate benefit to the public or industry
- Expand child and adult knowledge of nutritional benefits, improve access, and increase consumption of Florida specialty crops
- Increase sales and marketability and drive demand for commercially-grown specialty crops.

This project was important and timely because it has the potential to provide specialty crop producers with added benefits, especially economic, and is timely because of the intersection of increasing pressures on producer with growing interest by consumers. Producer are faced with numerous challenges that threaten their ability to remain in the industry, such as pests, urban sprawl, decreased profits, weather, regulation, and succession, among others. Agritourism provide3s an opportunity for producers to diversify and potentially ensure longevity while also providing cultural and economic benefits to their local surroundings. Agritourism in particular can bring social, environmental, and economic benefits to the participating farms, including addressing labor shortages, preserving heritage sites, and adapting to climate change effects.

Agritourism is a growing business that can help specialty crop producers promote their products, establish long term relationships with customers, and add additional revenue to their businesses. The number of agritourism operations in Florida nearly tripled within five years, increasing from 281 operations in 2007 to 724 operations in 2012. Additionally, income from these pursuits increased from $11.4 million in 2007 to $15.7 million in 2012. Florida Extension recommends agritourism as a valuable addition to the core business of producers. In Tennessee, a state with a $54.2 million annual agritourism industry, producers have reported 9.3% increased sales as a result of agritourism. Agritourism offers an opportunity for specialty crops, often smaller, less-well-known operations, to get their products in front of consumers who would otherwise not go out of their way to try new things.

Additionally, with the growing interest in local food and food origin among consumers, agritourism is uniquely positioned to provide consumers with an outlet to see when, where, and how their food is produced. In Florida, agritourism sites not only have the opportunity to reach local residents or state residents looking for a staycation, but also the more than 97 million people who visit Florida each year. Agritourism combines Florida's two largest industries (tourism and agriculture) to offer great benefit not only to the state's economy, but also the owners of agritourism sites and those who visit them.

This project was not a continuation of a previous grant.

Project Approach

The activities performed and any significant results/accomplishments/conclusions/recommendations or each objective of the grant project are outlined below.

Objective 1: To understand consumers' preferences for and awareness of Florida agritourism opportunities.

Activities:

- 10 focus groups were completed in 5 different regions of Florida)(Northwest – Pensacola, Northeast – Jacksonville, Central – Orlando, South Central – Sarasota, South – West Palm Beach)
- The audio recordings of the focus groups were transcribed verbatim.
- Focus group data was analyzed.
- A report of the findings was drafted and finalized. The report can be found at http://www.piecenter.com/issues/food/local-food under the dropdown titled "Promoting specialty crops through agritourism: Producer professional development"
- An overview of the focus groups and preliminary data was delivered to extension agents attending the 2016 Extension Professional Associations of Florida Conference.
- A presentation of the focus group results was given to a group of 30 producers attending an extension program association with the Florida Agricultural Network.
- A presentation of the focus group results was given to at the South Florida and Central Florida agritourism workshops.
- A presentation of the focus group results was given at an agritourism conference in Quincy, FL. There were approximately 40 producers and industry representatives in attendance.

Significant Results / accomplishments / conclusions / recommendations:

- Participants were not familiar with the term agritourism.
- Participants were not sure whether they had participated in agritourism, although many had.
- Participants were not familiar with agritourism in their local areas.
- Participants were enthusiastic about future agritourism visits.
- Participants want to visit agritourism sites with family and friends, or other social groups, and especially with children and out-of-town visitors.
- Participants wanted activities to be affordable, generally $10-$20 per person through this could vary.
- Participants wanted activities with 1-2 hours' drive.
- Participants mostly make a plan to visit agritourism venues, though some will visit spontaneously, especially if they see roadside signs.
- Participants wanted a mix of self-guided and guided activities.
- The most important amenities desired were clean, non-portable restrooms, shade or air conditioning. And kid-friendly activities.
- Participants were interested in leisurely or moderately strenuous activities for the most part.

- Participants were interested in a range of agritourism activities, thought ”smelly”, dangerous, or controversial activities were not of interest to some participants.
- The opportunity to learn and just to have a new experience were the top reasons people visit agritourism venues.
- Participants wanted to learn about particular crops and products, including how to grow or raise their own, as well as history of agriculture and the area, contemporary issues such as hydroponics and genetically modified organisms, the source of food and products, and the environment in general.
- Participants had previously heard of agritourism opportunities by word of mouth or the internet, but indicated they wanted to learn about them via local publications and television most frequently. This may be influenced by the overall age of focus group participants.
- Participants could not offer a better term than agritourism to describe the activities discussed, nor did they prefer options we offered such as agritainment or farm/rural tourism.

Objective 2: To provide professional development for specialty crop producers participating in or interested in participating in agritourism.

Activities:

- The workshop in south Florida was held on June 15th and 16th. We had between 50 and 60 registered attendees, which included specialty crop producers currently involved in agritourism, specialty crop producers, interested in diversifying into agritourism, and non-profit and state and local government representatives. The following topics were discussed:
    - Agritourism’s importance to the local economy (county commissioner)
    - State of agritourism in Florida (Florida Agritourism Association)
    - Agritourism as a connector (IFAS Extension)
    - Marketing Developments (Martin County Tourism Development)
    - What consumers want (Focus group data)
    - Best Practices from neighbors to the north (out of state speaker from North Carolina, agritourism operator and member of NAFDMA)
    - Agritourism rules and liability (county staff, Florida Farm Bureau, Department of Health, local agritourism owner/operator)
    - Funding resources (USDA Rural Development, PNC Agricultural Banking, Farm Service Agency)
    - Business planning for agritourism (UF / IFAS)
    - Your successful agritourism operation (local agritourism owner/operator)
    - Farm to table operation that hosts dinners and events. Growing many specialty crops including a variety of fruits, vegetables, and herbs.
    - Farm in the city that offers live music, art exhibits, and field trips. The events are complemented by the local food grown on the farm. They grow a variety of seasonal vegetables, fruits and herbs.
    - Winery that hosts tastings, live music, and events. Many of the grapes used are grown on site.

- The workshop in central Florida was held September 26-27. We had 63 registered attendees which included specialty crop producers currently involved in agritourism, specialty crop producers interested in diversifying into agritourism, and non-profit and local government representatives. The following topics were discussed:
    - Agritourism's importance to the local economy (county commissioner)
    - State of agritourism in Florida (Florida Agritourism Association)
    - FDACS marketing of agritourism (FDACS)
    - What consumers want (focus group data)
    - Learning from others (panel discussion of four local agritourism owners / operators)
    - Lessons in collaboration (out-of-state speaker from California, Sonoma County Farm Trails)
    - Service providers panel discussion (Visit Florida, Florida Agritourism Association, FDACS, Polk County Zoning, out-of-state speaker)
    - Create a plan of action – Participants broke out into groups and discussed needs for additional training and resources.
    - Bus tour stops included:
        - A citrus grove that offers citrus and citrus products to visitors. They also sell Florida themed gifts and offer grove tours. In addition to citrus, they also have a small peach orchard.
        - A crop maze that sells pumpkins and offers a variety of education al events and activities.

Significant results / accomplishments / conclusions / recommendations:

- Specialty crop producers participating in agritourism expressed a need for more guidance and support on the following topics:
    - Business planning
    - Navigating and interpreting regulations
    - Marketing
    - Rural/urban interface
    - Crisis and risk communication and responding to negative reviews
- The evaluation results from the south Florida workshop show:
    - Before the workshop 18.8% of responding attendees were aware of Florida agritourism legislation, after the workshop 81.3% were aware.
    - Before the workshop, 0% of responding attendees were aware of the agritourism landscape in Martin County, after the workshop 93.8% were aware.
    - Before the workshop, 0% of responding attendees were aware of the marketing opportunities for Martin County agritourism operations, afterward 100% were knowledgeable.
    - Before the workshop, 0% of responding attendees were knowledgeable about consumers' agritourism preferences, afterward 93.8% were knowledgeable.
    - Before the workshop, 12.6% of responding attendees were knowledgeable about best practices for agritourism, afterward 93.8% were knowledgeable.

    - Before the workshop, 0% of responding attendees were knowledgeable about the liability associated with agritourism, afterward 93.8% were.
    - Before the workshop, 6.3% of responding attendees were familiar with funding opportunities associated with agritourism, afterward 81.3% were familiar.
    - Before the workshop, 12.5% of responding attendees were knowledgeable about business planning for agritourism, afterward 100% were knowledgeable.
    - 87.6% of responding attendees reported that they would use the knowledge gained during the workshop in their business.
- Evaluation results from the Central Florida workshop show
    - Before the workshop, 22.9% of responding attendees were knowledgeable about the Florida agritourism legislation, afterward 77.2% were knowledgeable.
    - Before the workshop, 25.7% of responding attendees were knowledgeable about the agritourism landscape in Florida, afterward 91.4% were knowledgeable.
    - Before the workshop, 22.9% of responding attendees were aware of marketing opportunities for agritourism operations, afterward 77.2% were knowledgeable.
    - Before the workshop, 22.8% of responding attendees were knowledgeable about best practices for agritourism, afterward 71.4% were knowledgeable.
    - Before the workshop, 34.3% of responding attendees were knowledgeable about liability associated with agritourism, afterward 80% were.
    - Before the workshop, 8.6% of responding attendees were familiar with funding opportunities for my agritourism operation, afterward 42.9% were familiar.

Objective 3: To develop a comprehensive strategic plan to launch a statewide agritourism initiative for specialty crops

Activities:

- The results of objective 1, feedback gained at the workshops, as well as the situation and benchmarks of agritourism in Florida in other states was compiled to inform the strategic plan.
- The strategic plan was drafted and finalized and can be found at http://wwwpiecenter.com/issues/food/local-food under the dropdown titled "Promoting specialty crops through agritourism: Producer professional development."

Significant results / accomplishments / conclusions / recommendations:

The strategic plan identified the following goals and tactics:

Goals

- Extension agents express intent to support agritourism operations.
- Clearly defined roles are established among organizations supporting agritourism in Florida
- Extension agents offer operator education throughout the state on an annual basis.
- Increase the number of agritourism operations in Florida by 10%
- Develop and implement statewide initiative and/or brand to promote Florida agritourism.
- Florida residents and visitors are active participants in agritourism.
- Florida residents and visitors are knowledgeable and aware of Florida agritourism

Tactics

- Meeting of key players in organizations supporting agritourism in Florida

- Link to other supporting organizations.
- Training and development of extension agents to be able to train agritourism operators in the skills necessary to run a successful agritourism business.
- Training and development of agritourism operators.
- Utilization of existing materials
- Develop a branding and marketing plan for Florida agritourism.
- Lobby to support agritourism in the state tourism budget.

Objective 4: To develop and disseminate outreach materials based on the findings and feedback from components 1 and 2.

Activities:

- Two EDIS documents have been drafted and submitted for publication. They should be published within the next couple of months.
- The trade publication appeared in the December / January issue of FloridAgriculture. This is the publications of the Florida Farm Bureau Federation and reaches a readership of over 140,000 households. These include producer and consumer households. The piece was written so that it will appeal to specialty crop producers who participate in or would like to participate in agritourism.
- An in-service training was held in January 2018. 41 people participated. These included current and future agritourism operators, extension personnel, agritourism service providers and affiliated organizations. The strategic plan was presented during the webinar. Following the webinar, participants were sent electronic copies of the focus group report, the strategic plan, and the insert that appeared in the FloridAgriculture magazine. Representatives from FDACS and the Florida Agritourism association were present at the IST, so a special trip was not made to meet with these organizations.

Significant results/accomplishments/conclusions/recommendations:

- Several agritourism operators followed up with the researchers after the in-service training to get more information.
- As a result of the in-service training and materials produced through this project, there seems to be a renewed interest among those integrally involved in agritourism to collaborate and work toward a common goal of improving and increasing agritourism in Florida.

Ensuring benefit to specialty crops:

Agritourism has the potential to benefit both specialty crop producers and producers of non-specialty crops. To ensure that funds for this project only benefitted specialty crops, several precautions were taken. During the focus group with consumers, we asked the consumers to tell us what they thought agritourism was and what their preferences for agritourism were. The discussion was mainly focused on the components of agritourism operations (amenities, education, resources, marketing, etc.) and reference to the types of operations were included only when respondents were read the legal definition of agritourism from the state legislature. The report of the findings was shared at the workshops but was not widely distributed to the agricultural industry. When selecting speakers for the agritourism workshops, we took great care in selecting speakers who either were directly involved in

specialty crops or who could speak to the unique characteristics of specialty crop producers. Throughout the project we began to realize that many agritourism operators are involved in specialty crops even if the specialty crop isn't the main focus of their agritourism operation. For example, they may have a crop maze, but also sell pumpkins or another specialty crops or grow a specialty crop when their agritourism operation is in the off-season. This was not something we anticipated when we first applied for the project but was an encouraging finding. Additionally, the tour stops during the workshops were operations that were focused on specialty crops or supplemented their agritourism operations with specialty crop production. Through the course of the project, we realized that the statewide plan for agritourism operators growing specialty crops was not very different from a plan covering all agritourism operators statewide. They all need the same things. The plan is very broad and is focused on big picture goals and tactics that could be applicable to all agritourism operators. We tried to limit the benefit to all agritourism operations by disseminating the plan only to those attending in service trainings and making it available online. Lastly, the insert in the FloridAgriculture magazine could have reached a variety of agricultural producers, but had the benefit of reaching consumers as well. We felt that this publication outlet could help us reach the largest number of specialty crop producers, while also reaching consumers to attend agritourism operations. The farms visited on the tours during the workshops were also featured in the publication to help reinforce specialty crop agritourism operations or operations supplemented by specialty crops.

Significant contributions and role project partners:

- The PI and research staff worked to speak with organizations contributing to agritourism in Florida to understand the unique challenges and opportunities specific to agritourism in Florida. In addition, the researchers collected data from consumers, helped plan and facilitate the workshops, and compiled the statewide plan.
- UF/IFAS Extension was instrumental in helping to plan, organize, host, and facilitate the two agritourism workshops. Additionally, extension offices were also used to conduct the consumer focus groups.
- UF/IFAS communications was instrumental in leading strategic planning sessions to inform the strategic plan.

Goals and Outcomes Achieved

Goal 1: Increase specialty crop producers' knowledge of agritourism opportunities and strategies

Performance Measure: Percent of knowledge gain regarding agritourism opportunities and strategies

Benchmark – Benchmark will be a measure of producers reported knowledge of agritourism opportunities/strategies before the professional development workshop

Target – 40% increase in knowledge

Result – Before the professional development workshops, 20% of specialty crop producers reported that they were knowledgeable of agritourism opportunities and strategies. After the professional development workshops 84% of specialty crop producers reported that they were knowledgeable. The

target for this goal was met with a 64 percentage point gain in number of producers who said they were knowledgeable.

Goal 2: Increase specialty crop producers' intent to adopt agritourism opportunities / strategies

The target was a 30% increase in intent to adopt agritourism opportunities / strategies.

Result / Accomplishment – Before the workshops started, producers were unaware of the opportunities or strategies to be discussed. Therefore, they were unable to report intent and the benchmark was 0. After the workshops, 82% of specialty crop producers expressed intent to use what they had learned about agritourism opportunities and strategies in their business. The target was met.

Goal 3: Encourage incorporation of the agritourism strategic plan into extension programming.

The target was to achieve 35% intent to incorporate at least one element of the strategic plan into programming. 63% of participants attending the in-service training reported intent to apply what they had learned in the webinar into their programming. The target was met.

Beneficiaries

Florida Agritourism Operations – In the 2012 Census of Agriculture, there were an estimated 724 agritourism operations in Florida. It is unknown how many of these still exist and if the number has increased or decreased. Additionally, it is unknown how many of the operations include specialty crops or are supplemented by specialty crops. Originally researchers estimated that at least 50% included specialty crops. However, researchers estimate that the number is probably much higher based on conversations with the operators they interacted with during the workshops. These operators may have benefited by gaining an understanding of consumer awareness and preferences for agritourism, having a strategic plan to guide their business and the industry, and from resources and conversations presented at the agritourism conferences. Agritourism operators may have learned of the results of the project from the workshops, the insert in the FloridAgriculture magazine, in service training, or from the project web page.

Florida Specialty Crop Producers – Florida has 1,614 fresh vegetable operations, 3,194 fresh fruit operations, 3,639 citrus operations, 1,598 berry operations, and 1,436 nut operations which could have benefitted from this research. These producers could have benefitted by gaining an understanding of consumer awareness and preferences for agritourism, having a strategic plan to guide their businesses and the industry and from resources and conversations presented at the agritourism conferences. Specialty crop producers may have learned of the results of the project from the workshops, the insert in the FloridAgriculture magazine, in-service training, or from the project webpage.

Florida residents and visitors – the more than 20 million Florida residents and more than 40 million Florida visitors are also beneficiaries of this project. The individuals may benefit from a better organized, marketed, and consumer oriented agritourism industry in the state. Additionally, they may benefit from improvements made at individual operations and the additional of more operations. Florida residents and visitors may have received the project results when disseminated in the FloridAgriculture magazine.

Lessons Learned

Problems or delays:

- There was a delay in both professional development workshops in order to better align with producers availability during the off-season.
- The analysis and report of the focus group findings was delayed due to workshop planning.
- The development of the strategic plan was supposed to begin in December 2016. Due to the holidays and finishing the completion of focus group analysis, the development began in January 2017.
- The EDIS documents were submitted for publication before the end of the grant, but were not officially published yet at the close of the grant.

Project goals not achieved:

- All goals were achieved

Lessons Learned

- Many of the agritourism producers encountered during this project were involved in specialty crop production in some capacity. They either featured specialty crops as part of their agritourism operations or they supplemented their agritourism operation with specialty crops. This was an encouraging conclusion.
- Identifying, targeting, and disseminating information to only agritourism operators with specialty crops difficult. Most channels of dissemination include large groups of groups of agritourism operators or agricultural producers. There is no good way to identify and target just specialty crop producers involved in agritourism.
- For the professional development workshops, operators really wanted more time to talk to and learn from each other.

Total Funding Expended: $71,940.95

Percentage of Project Completed: 100

FINAL PROJECT REPORT TEMPLATE

Final Performance Reports must illustrate the completion of each project within the grant agreement. Each project shall be outlined as separate project profiles. You will report on projects in the same order they were submitted in the approved State Plan and subsequent amendments.

FDACS CONTRACT NUMBER: 022911

Project Title

Exploring and identifying edamame (vegetable soybean) suitable for cultivation as a high value crop for the fresh food market of Florida

PROJECT IMPACT AND FINDINGS

Research has demonstrated that consumer demand for locally grown food is rapidly increasing in Florida. Edamame (vegetable soybean) is a potential source for locally grown food to meet demand of fresh food for Florida consumer. Edamame is a long-established vegetable crop in many countries of Asia, but demand is exploding in the US as a healthy snack or vegetable, or as salad component. The demand is increasing due to the health benefit that includes low density lipoprotein (LDL) cholesterol levels and reduces the risk of cardiovascular diseases, cancer. Despite being a high value crop and a favorable environment for production, there are no known edamame producers in Florida, likely due to lack of information on yield, performance and specific varieties adapted for the Florida environment. The major objective of the current project was to evaluate different edamame genotypes potentially suited for growing condition of Florida. University of Florida led the research project and identified several edamame genotypes with suitable maturity and could potentially grow in Florida. The project team initially evaluated 119 edamame genotypes of different origins and identified 19 genotypes for in depth morphological and quality characterization. The researchers collected data on different yield contributing traits, days to different growth stages, colorimetric measurements of greenness, and protein and lipid content on 19 edamame genotypes at two locations (Plant Science Research and Education Unit, Citra and West Florida Research and Education Center, Jay) in Florida. Based on extensive evaluation of those 19 genotypes, we believe that there are 3-4 genotypes which could potentially be used for the local Florida market. Based on our data (days to R1 and R6, height, nodes/plant, pods/plant, fresh pod yield at R6, 1,-2, and -3 seeded pods/plant, 100-green seed weight, total protein content, and total lipid concentration), genotypes PI614832, PI584470, and PI633424 can be grown in North-western regions of Florida, while PI417206 is suitable from growing in both North-western and North-Central Florida. The findings need to be further confirmed by evaluating those 3-4 genotypes in farmers' field, and by developing proper irrigation and other management practices. The preliminary findings were presented at a scientific conference, Southern ASA, 2018, in Jacksonville Florida. Currently, we are preparing a scientific manuscript and an extension publication for the scientific, extension and grower communities of Florida. Because Edamame has generated considerable media attention and interest in recent years, Florida producers could benefit from the growing consumer awareness of this crop. Edamame will be profitable for small-scale growers who are willing to develop or cultivate a market through local farmers markets, other direct markets, or for wholesale to smaller distributors. Also growers promoting an organically grown product could have a marketing edge. This research helped to establish a basic knowledge on the production and variety of edamame for Florida growers.

BENEFICIARIES

Number of project beneficiaries: There are no known edamame producers in Florida currently. So, it is difficult to estimate the exact number of growers who will be benefitted from the current research. However, our study has established a baseline for growing suitable variety under Florida conditions. So, now growers in Florida have option to grow edamame for local market. Based on the increasing demand of consumption in the past decade in the USA, there will be need of a large increase in production of edamame to meet future demand. Because of high value in the market, the findings of the present research will motivate growers towards edamame cultivation in Florida.

ACTIVITIES PERFORMED

OBJECTIVES

Provide the approved project's objectives.

#	Objective	Completed?	
		Yes	No*
1	Evaluate the yield and production potential of the edamame cultivars in Florida	Yes	
2	Evaluate each variety for nutritional content and taste potential	Yes	
4			

Further advancement of selected edamame genotypes in different cropping systems will advance the long-tern objective of project.

ACCOMPLISHMENTS

Accomplishment/Impact	Relevance to Objective, Outcome, and/or Indicator
Selected 3-4 edamame genotypes could potentially be grown for Florida fresh food market	Related to objective 1 and 2 of the project. These 3-4 genotypes could be further evaluated in growers' field to demonstrate potential opportunity of growing edamame in Florida and increase the likelihood of potential income of the growers.
Results presented at Southern ASA conference	The findings of the research project were reached to a large, regional scientific community

CHALLENGES

Challenge	Corrective Actions
Lack of seed supply	The initially seed amount received from USDA repository and AVRDC was small and necessitated a seed increase. This took the project team some time to increase seed for larger plot evaluations.
Weather condition	Due to heavy rain in most of the growing season in 2017, we couldn't arrange field day for the growers. However, if the project is extended for 3rd year, the field day could be arranged.

LESSONS LEARNED

We have a quantity of seeds of the selected lines for future study. It would have been better if we started this project with a sufficient amount of seed from different edamame genotypes.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

We are currently working of preparing a manuscript and an extension publication for the dissemination of findings to growers, extension personnel, consultants and other researchers.

OUTCOME(S) AND INDICATOR(S)/SUB-INDICATOR(S)

- ➔ Establish successful edamame trials in North-Central and North-Western Florida and achieve high yield in those trials which measures the production potential of Edamame under Florida growing conditions which we never had any information before establishing the present research.
- ➔ Identified high yield potential three edamame varieties (PI614832, PI584470, and PI633424) for North-Western Florida and variety PI4172064 for both North-Western and North-Central regions of Florida, The pods and green seeds of these edamame varieties could potential be supplied to Florida Fresh Food market.
- ➔ The identified four varieties have high protein content and low lipid concentration which is required for Edamame fresh food market.
- ➔ It is difficult to establish a cropping system in a two years study, especially with a new crop which didn't have any prior information for Florida growing condition. However, the trials were established in field locations where cotton and corn was planted in previous year. Nonetheless, four verities produced yield more than 10,000 kg/ha, 100-green seed weight >30 g, high protein content (>40%) and low lipid concentration which are required for edamame market and make product profitable. The results indicate that these verities can potentially be suited to current grain-legume or cotton-legume cropping patter in Florida. However, we still need to conduct research to understand how much nitrogen fixation is occurring in the soil and also how the cropping pattern is helping to change the insect and pest dynamics in the following crops.
- ➔ We couldn't organize field days in two locations due to very bad weather conditions in 2017. In June, Plant Science Research and Education Unit (PSREU), Citra, FL and West Florida Research and Education Center (WFREC), Jay, FL received 330 mm and 510 mm rainfall which is higher than 30 years of average rainfall. In August, PSREU and WFREC received 180 and 260mm rainfall, respectively, while PSREU received 280mm rainfall in September. These rainfalls are higher than long-tern rainfall pattern. Due to inclined weather condition, we failed to organize field day. In 2016, we did preliminary screening of germplasm and didn't have enough big plots (due to short of seed amount) for grower's demonstration.
- ➔ We presented preliminary results at Southern ASA conference in 2018 at Jacksonville, Florida. We currently preparing manuscript and will be submitted to Agronomy Journal for review by end of March, 2019. We will publish an EDIS extension publication of identified four edamame varieties by June, 2019.

Outcome Measure(s)

Select the Outcome Measure(s) that were approved for your project.

- ☐ **Outcome 1**: Enhance the competitiveness of specialty crops through increased sales
- ☐ **Outcome 2**: Enhance the competitiveness of specialty crops through increased consumption
- ☑ **Outcome 3**: Enhance the competitiveness of specialty crops through increased access
- ☐ **Outcome 4**: Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources
- ☑ **Outcome 5**: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems
- ☑ **Outcome 6**: Enhance the competitiveness of specialty crops through increasing the number of viable technologies to improve food safety
- ☐ **Outcome 7**: Enhance the competitiveness of specialty crops through increased understanding of the ecology of threats to food safety from microbial and chemical sources
- ☑ **Outcome 8**: Enhance the competitiveness of specialty crops through enhancing or improving the economy as a result of specialty crop development

OUTCOME INDICATOR(S)

- ➔ Established successful edamame trials in North-Central and North-Western Florida
- ➔ Identified potentially suitable genotypes of edamame for the production in Central and Western Florida
- ➔ Identified suitable edamame genotypes with high food quality (protein, lipid, etc) for human consumption
- ➔ Knowledge on potential production of Edamame in Florida grain-legume or cotton-legume production system

- presented results in conference to a large and diversified set of audience
- Preparation of scientific and extension publication in 2019.

DATA COLLECTION

The experiment was laid out in an RCBD design in two locations, Citra and Jay, Florida. The following data were collected:

- Emergence: % field emergence was counted after three weeks of planting by comparing number of emerged seedling to number of seeds planted
- Time to R1 days: Calculated from planting to 50% of plants in a plot at R1 stage (beginning of flowering).
- Time to R6 days: Calculated from planting to 50% of plants in a plot at R6 stage.
- Height at R6: Measured from soil surface to plant apex (expressed in cm).
- Number of nodes/plant at R6 stage: 10 plants/plot (middle two rows) were used used to count number of nodes/plant.
- Number of pods/plant at R6 stage: 10 plants/plot (middle two rows) were harvested and counted number of pods/plant.
- Number of seeds/pod (one-, two-, three-seeded pods) at R6 stage: Pods of harvested 10 plants were classified and counted for number of pods/plant.
- Pod fresh weight at R6 stage/plant: Fresh weight of the pods with seeds was recorded. The pods were shelled to determine shell and green seed fresh weights. The fresh weight of 100 green seeds was recorded and number of seeds per pod was computed. All seed yield data were expressed on fresh weight basis. Single harvest was performed based on the maturity of varieties (when varieties reach at R6 stage)
- The CR-410 chroma meter, hand held color measuring instrument, was used to check color and quality of pods at harvest time (R6 stage) of different genotypes to compare among varieties and seasons.
- Total protein and lipid analysis: Fresh green seeds harvested at the R6 stage were oven dried and grinded. The grinded seed samples were sent to UF forage evaluation support laboratory for total protein estimation. The seed samples were also sent to UF mass spectrometry research and education center for total lipid estimation.

The collected data were subjected to statistical analysis by considering genotypes as fixed effect, and location and replications as random effect.

CONTACT PERSON

Contact Person for the Project Md Babar

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FEDERAL PROJECT EXPENDITURES TO DATE

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$75,310.74	$72,703.41
Fringe Benefits	$10,829.90	$11,886.70
Travel	$1,437.00	$1,458.91
Equipment	$6,142.00	$6,142.06
Supplies	$3,678.02	$859.83
Contractual	0	0
Other	$10,659.84	$13,383.90
Direct Costs Sub-Total	$108,057.50	$106,434.81
Indirect Costs		

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Total Federal Costs	$106,434.81	

PROGRAM INCOME: NONE

Source/Nature (i.e., registration fees)	Amount Approved in Budget	Actual Amount Earned
Total Program Income Earned		

ADDITIONAL INFORMATION

We are attaching poster which was presented at 2018 Southern ASA conference

Project (24): Determination of a Crop Coefficient for Florida Peach Production

Contact Person

Lincoln Zotarelli, Associate Professor, 352-273-4949, lzota@ufl.edu

Project Partners

University of Florida

Project Summary

This project developed peach tree crop coefficients produced in subtropical climates. Although there are existing crop coefficients, these were derived in the high desert environments of California. The application of these existing crop coefficients to Florida peach orchards overestimate the amount of irrigation required, resulting in excessive irrigation water application and excessive drainage since peaches are relatively shallowly rooted. In addition, excess irrigation can exacerbate fertilize leaching beyond the root zone. Thus, to be efficient with water and fertilizer resources, peach crop coefficients are needed. The results from this project may be applicable for Florida and the coastal zones along the Gulf coast.

Peach acreage in Florida continues to increase as growers seek alternatives to citrus-greening infested groves. The greatest growth is in central and south central Florida, where growers are using citrus irrigation strategies and applying them to peaches. Since peaches are deciduous and citrus are evergreen crop, the irrigation scheduling used for citrus cannot be applied to peaches. Proper irrigation scheduling using evapotranspiration is dependent upon accurate crop coefficients for tree phenology and fruit development in humid climates. Without these accurate crop coefficients for peaches and the climate in which it is grown, irrigation scheduling will be imprecise.

An initial delay occurred because the funding was not available when originally thought and that delayed the acquisition of equipment needed to start the project. Despite of it all activities were performed achieving all the goals originally proposed in the grant.

Project Approach

Goal 1: Determine the daily tree water use (ETc) by measuring the soil water content (Θ) and calculating reference evapotranspiration (ETo) over a range of soil moistures to calculate a monthly peach crop coefficient (Kc) over three growing seasons

1. Activity: Soil characterization via baseline soil sampling prior to sensor installation.

Soil sampling, soil characteristic curve were performed in Apr. 2016 prior sensors installation to collect and analyze soil moisture content.

2. Activity: Soil sensor installation within and between tree rows as described in Morgan et al. (2006).

Soils sensors were installed in Aug. 2016 and volumetric water content was measured very 15 min daily to estimate tree water consumption based on the daily change of soil moisture content.

3. Activity: Tree growth measurements including trunk cross-sectional area, pruning weight (winter and summer) and fruit size and yield will be measured.

Tree growth measurements were collected in winter 2016-17 and summer (2017-2018) to analyze tree growth. Fruit yield was collected in spring 2017 and 2018.

Goal 2: Determine the relationship of the soil water uptake as soils dry after irrigation to separate water drainage through the soil profile from tree uptake.

1. Activity: Soils properties characterized in Goal 1 were used in calculations of soil water drainage.

Soil water content was collected daily and data was used to for calculations of soil water drainage, tree water consumption and soil water depletion starting Aug. 2016 due to a delay in fundings to buy the soil moisture sensors needed to perform this activity.

2. Activity: Soil water drainage through the soil profile to 80 cm will be measured after each irrigation set to 50% of available water depletion. Data from soil water content and sensors will be recorded daily to a data logger and downloaded on a weekly basis.

Soil water drainage was collected starting Aug. 2016 on a daily basis except during short periods of times were sensors needed to be re-calibrated due to a malfunction.

Goal 3: Determine the water needs (actual tree evapotranspiration) for young peach trees based on tree size and reference evapotranspiration using large weighing lysimeters.

1. Activity: Establish young peach trees in large weighing lysimeters at UF-MREC, Apopka, FL in 7 gallon pots.

Grafted peach trees ‘TropicBeauty’ x ‘Flordaguard’ were transplanted on 25 gal containers since the beginning of the project (Mar. 2016) to avoid root constrain. Additionally border trees were planted in separated 15 x 20 ft between trees and between rows respectively to simulate commercial conditions.

2. Activity: Measure and collect data on daily water evapotranspiration for trees planted in large weighing lysimeters.

Tree actual evapotranspiration was collected every 30 min. daily from the lysimeters starting in Mar. 2016 except for short periods of times were sensors were damaged by lightning strikes, power lost, or hurricane damages.

3. Activity: Monthly measurements of tree height, projected canopy area, and trunk circumference at three locations. Trees will be defoliated in December of each year, thus canopy area will only be collected when leaves are present on the tree

Tree height, projected canopy area, and trunk circumference were collected every 21 days staring in Mar. 2016 except from December 15 to February 28 of each year because the trees were dormant the trees were leafless.

Goal 4: Establish baseline information on current irrigation practices and disseminate research results to growers, stakeholders and water management districts.

1. Activity: Survey growers to establish baseline irrigation practices for subtropical peach production.

A survey was conducted in May 2018 in the "Annual Stone Fruit Field Day" the survey was conducted to peach growers to establish base line information on irrigation practices to address issues and possible areas were improvement can be achieved.

2. Activity: Present preliminary research results to existing and potential growers to aid in development of irrigation strategies for subtropical peaches.

Preliminary results of this research was presented in Feb. 2018 at the Southern Region American Society of Horticultural Sciences (SR-ASHS) conference, May 2018 at the "Annual Stone Fruit Field Day", June 2018 at the Florida Society of Horticultural Sciences (FSHS) conference and July 2018 at the American Society of Horticultural Sciences (ASHS) 2018 conference.

3. Activity: Post information and progress results online through the UF Stone Fruit Extension webpage or blog site to inform stakeholders.

Information was posted online on the "Stone Fruit at the University of Florida" about preliminary results delivered on the "Annual Stone Fruit Field Day" in May 2018.

4. Activity: Publish information via extension documents or peer-reviewed journal articles to distribute preliminary data.

Feb. 2018 - Determine water requirement for peach trees in a humid climate (Abstract). SR-ASHS 2018. In press

Apr. 2018 - Irrigation Practices for Peaches in Florida (EDIS publication) is currently available online at http://edis.ifas.ufl.edu/hs1316

June 2018 - Determine water requirement for young peach trees in humid climates. FSHS 2018 paper would be submitted to the HortTechnology Journal in the Following months.

July 2018 - Estimation of a Crop Coefficient for Low-Chill Peach Trees in Subtropical Humid Climates. ASHS 2018. paper will be submitted to the HortScience Journal in the following months. (draft is attached)

Goals and Outcomes Achieved

Goal 1:

Determine the daily tree water use and evaporation (ETc) by measuring the soil water content (Θ) and calculating reference evapotranspiration (ETo) over a range of soil moistures to calculate a peach crop coefficient (Kc) over three growing seasons that was correlated with key phenological stages. The original goal was achieved despite the delay on the start of the project.

Daily ETc and ETo were used to calculate monthly peach crop coefficients based on the tree phenology. Kcs for the flowering, fruit growth, vegetative growth and dormancy months (key phenological stages) were calculated.

The average daily ETo and ETc from Sep.2016 to July 2017 were:

Month	ETo (mm)	ETc(mm)

Sep-16	3.68	2.49
Oct-16	2.75	1.97
Nov-16	1.74	1.23
Dec-16	1.34	0.85
Jan-17	1.49	0.95
Feb-17	2.16	1.37
Mar-17	2.70	1.70
Apr-17	3.64	2.93
May-17	4.28	3.39
Jun-17	3.62	2.14
Jul-17	4.13	2.77
Aug-17	4.01	2.12
Sep-17	3.30	1.80
Oct-17	2.54	1.71
Nov-17	1.78	1.28
Dec-17	1.27	0.63
Jan-18	1.52	0.75
Feb-18	2.29	1.18
Mar-18	2.57	1.70
Apr-18	3.37	2.47
May-18	3.47	2.37
Jun-18	4.08	2.44
Jul-18	3.81	2.49

Goal 2:

Determine the relationship of the soil water uptake as soils dry after irrigation to separate water drainage through the soil profile from tree uptake.

A soil characteristic curve was done prior the start of the project to establish soil field capacity soil permanent wilting point and daily soil moisture content was used to establish a soil water depletion coefficient of this soil type to calculate tree water uptake and soil drainage. Ks was calculated on a daily basis to develop a linear equation to calculate Ks based on soil water content. The original goal was achieved despite the delay on the start of the project.

The Ks equation for the Arredondo fine sand were the study was conducted had a statistically significant at ($p< 0.001$) the r2 was 0.54

Ks =1.070- (0.00978 *ASWD%)

Goal 3:

Determine the water needs (actual tree evapotranspiration) for young peach trees based on tree size and reference evapotranspiration using large weighing lysimeters.

Actual tree evapotranspiration ETa was measured daily and correlated to tree size to establish a water requirement (L/day) and crop coefficients for young peach trees for over two years after transplant. The final results not only give a recommendation but allow the growers to calculate daily water requirement for young peach trees based on trunk cross sectional area (TCSA) and projected canopy area (PCA).

The PCA and TCSA regressed against the N-ETa had a statistically significant relation ($P<0.001$) in both cases. The R-squared for PCA regression was 0.91 and 0.96 for the regression of TCSA at 15 and 30 cm height from the ground. Moreover, the Kc for the first year after transplant ranged from 0.15 to 0.75 from Apr. 2016 to Mar. 2017, from 0.36 to 0.76 from Apr. 2017 to July 2018.

Goal 4:

Establish baseline information on current irrigation practices and disseminate research results to growers, stakeholders and water management districts.

A survey was conducted to establish a base line information about current irrigation practices and possible areas to improve recommendations. Preliminary information about irrigation strategies was shared with growers and extension agents on a field day, state and national conferences. The main findings of this project was published in the University of Florida Extension website EDIS (Electronic Data Information Source) publication available free online.

The results from the conducted survey were:

1) The survey covered 13 counties of FL where peaches are grown.

2) The most popular varieties are 23% 'Tropic Beauty', 13% 'UF Best', 10% 'UF Sun', 10% FL Prince, 7% 'UF One' , 7% 'Tropic snow'.

3) On average participants of the survey manage/owned 12 acres.

4) 54% of the growers answered they are planing on expand their operation in the next year.

5) 55% of growers use micro-sprinkler irrigation, 18% drip irrigation, 9% overhead irrigation.

6) 56% use visual appreciation to assess if the orchard should be irrigated, 19% irrigate based on a calendar or schedule, 6% use soil moisture sensors, 6% use reference evapotranspiration.

7) 31% the growers use emitters ranging from 10 to 15 gph.

8) on average the growers irrigate 3 days per week.

9) The irrigation run time was on average 2 hours per day.

10) Since this project started 13% of growers have modified/change (improved) the irrigation system in the orchard, 29% have change the running time of an irrigation event, 25% of them have modified the number of days they irrigate per week, and 8% have changed the method they use to decide if irrigation is needed.

11) For frost protection 50% of the survey participants use micro-sprinklers, 7% overhead sprinklers, 7% use both systems and 29% do not use frost protection.

Beneficiaries

The information generated in this project benefited (and will benefit) growers throughout the southeastern U.S. in states that are growing low- and mid-chill peach varieties with melting and non-melting flesh textures. In the Florida, Georgia, and South Carolina peach industries, there are over 100 growers.

The growers in the southeastern U.S. benefited from this project through extension agents, faculty, students and industry personnel that assisted to the Florida (Florida Society of Horticultural Sciences Annual Meeting 2018), regional (South Region-Amer. Soc. Hort. Sci. 2018) and national (ASHS annual meeting 2018) conferences because all the preliminary results were presented there.

Results of this project were presented on the field day and handouts were distributed to over 100 people that assisted to the "Annual Stone Fruit Field Day" in May 2018.

Growers, Extension agents and general public were aware of the conferences trough the web-pages of each association (SR_ASHS), (FSHS) and (ASHS) and the "Stone Fruit at the University of Florida" that promoted the "Annual Stone Fruit Field Day".

The gross value of current bearing peach acreage is estimated about $5 million dollars from about 2,000 acres planted in Florida. The Florida industry will benefit of from this research reducing up to 27% water usage compared to the FAO recommendations with an estimated water savings of 14 inches (380,156 gallons/acre/yr) or 760 million gallons/yr of water that are currently over applied in Florida peach orchards.

Lessons Learned

At the begging of the project a delay on the project occurred because funding was not available to buy equipment. This delayed the start of the project 6 months from Feb. 2016 to Aug. 2016. Additionally, lightning strikes, power losses and hurricane damages cause equipment malfunction in Fall 2016, Spring 2017, Fall 2017 for short period of times but did not affected the overall data collection.

Possible improvement of activities would be to collaborate with other departments or faculty members when conducting research since most growers are not willing to participate on them perhaps a multidisciplinary approach can lead to better results having more growers willing to complete a survey.

Total Funding Expended: $95,358.18

Percentage of Project Completed: 100

FINAL PERFORMANCE REPORT

FINAL PROJECT REPORT TEMPLATE

Final Performance Reports must illustrate the completion of each project within the grant agreement. Each project shall be outlined as separate project profiles. You will report on projects in the same order they were submitted in the approved State Plan and subsequent amendments.

FDACS CONTRACT NUMBER:

022897

PROJECT TITLE

Provide the project's title. (Must be the same title used in the approved State Plan or amendment.)

Reducing reliance on neonicotinoid insecticides for Florida tomato growers

PROJECT IMPACT AND FINDINGS

Include a summary of the project results of 250 words or less, suitable for dissemination to the public. A statement of results provides a brief description of your project, its success, and/or lessons learned. A statement of results should:

1. *Include the name of the organization that led and executed the project (State department of agriculture or subrecipient);*
2. *Capture the project's purpose and activities completed;*
3. *Outline the outcomes and indicators achieved, results/important findings and lessons learned; and*
4. *Highlight the target population reached and products developed (including intended use and audience).*

The vegetable entomology lab at the University of Florida's Gulf Coast Research and Education Center carried out field trials, insecticide resistance bioassays, and a grower survey to evaluate the potential of biopesticides to reduce reliance among Florida tomato growers on neonicotinoid insecticides for management of *Bemisia tabaci*, the sweetpotato whitefly, and *Tomato yellow leaf curl virus* (TYLCV), which the whitefly transmits. Alternatives to neonicotinoids are sought because of the presence of resistant whitefly populations in the state, and because of potential pollinator health concerns. The biopesticides evaluated have the advantage that they do not kill in a way that selects for resistance. Neonicotinoid insecticides are most commonly used during the first five weeks of the crop, when infection with TYLCV drastically reduces yield. M-Pede, an insecticidal soap, demonstrated the ability to reduce virus incidence on a level comparable to dinotefuran, one of the most effective neonicotinoids. M-Pede and Suffoil-X, a mineral oil, demonstrated the ability to reduce numbers of whitefly nymphs. Of twenty-three field populations tested, four were highly resistant to imidacloprid. Four generations without exposure to imidacloprid were needed for susceptibility to return. Approximately 67% of Florida's tomato growers incorporate biopesticides in their insecticide rotations. The results of the project have been presented at five scientific meetings, including two national meetings, and four industry/grower meetings. The results will support efforts by growers and researchers to design and implement management plans for whiteflies in tomatoes and other crops that mitigate the onset of insecticide resistance while protecting yields and pollinators.

BENEFICIARIES

Number of project beneficiaries: 1000

ACTIVITIES PERFORMED

OBJECTIVES

Provide the approved project's objectives.

#	Objective	Completed?	
		Yes	No*
1	Evaluate alternatives to neonicotinoid insecticides for early season protection of tomato from whiteflies and Tomato yellow leaf curl virus, including virus-tolerant tomato varieties and reflective mulch combined with early season applications of biorational insecticides.	yes	
2	Determine the length of time or number of generations that a tolerant whitefly population must be kept free from exposure to neonicotinoids before it regains susceptibility to the material.	yes	
3	Collect information from Florida tomato growers on the use of conventional and biorational insecticides for management of whiteflies and other pests. Document increased understanding of the potential of biorational insecticides through the collection of baseline and follow up survey information over a two year period.	yes	
4			

**If no is selected for any of the listed objectives, you must expand upon this in the challenges and lessons learned sections.*

ACCOMPLISHMENTS

List your accomplishments for the project's period of performance, including the impact they had on the project's beneficiaries, and indicate how these accomplishments assist in the fulfillment of your project's objective(s), outcome(s), and/or indicator(s).

Accomplishment/Impact	Relevance to Objective, Outcome, and/or Indicator
Accomplishment: Four field trials were carried out comparing biopesticides to neonicotinoids for control of whiteflies and whitefly-transmitted virus during the first five-week treatment window in tomato. **Impacts:** Trials in 2016 confirmed that weekly applications of M-Pede, an insecticidal soap, could reduce numbers of whitefly nymphs and eggs and reduce virus on a level commensurate with neonicotinoid treatments. Suffoil-X, a mineral oil, was useful in some trials for reducing whitefly nymph numbers, but not eggs and not virus. Surround, a kaolin clay product, was not	These trials demonstrated that biopesticides are available that can be used in conjunction with or as an alternative to neonicotinoids for managing whiteflies and the viruses they transmit. Insecticidal soap was the only product that reduced virus transmission, however other biopesticides demonstrated the ability to reduce whitefly numbers. In this way they are important insecticide resistance management tools for managing whiteflies, because they control the pest via mechanisms that do not select for resistance. These trials contribute to objective 1 of the project, reducing growers' reliance on neonicotinoid

effective in reducing whitefly numbers and may be attractive to whiteflies. Trials in 2017 focused on rotations of biopesticides in comparison with neonicotinoids and other conventional insecticides. Biopesticide rotations in the spring of 2017 reduced whitefly eggs, nymphs and virus by ~54 % compared to the untreated control. In the fall 2017 trial, whitefly egg and nymph numbers were not statistically different in biopesticide and conventional rotations, both of which were significantly lower than the untreated on most sample dates. The biopesticide rotation failed to control virus that season, which was very high (96.7% in the untreated control).	insecticides. The results of these trials confirm the necessity to continue to evaluate biopesticides and refine their use in insecticide rotations.
Accomplishment: 1. Twenty-three populations of whiteflies collected from commercial tomato fields were tested for susceptibility to imidacloprid. 2. The number of generations needed for resistant populations of whitefly to regain susceptibility to imidacloprid was documented.	The results of our resistance monitoring of field populations of whiteflies are consistent with surveys from previous years that confirm resistance to imidacloprid (Admire Pro) and thiamethoxam (Platinum) is variable across populations in the state. There are highly resistant populations, but resistance is not uniform. These two commonly used neonicotinoid insecticides continue to be effective in many vegetable producing areas of the state. In the process of screening field populations, four populations were detected with high levels of resistance to imidacloprid, that is LC50s of 100 ppm or greater. These populations were maintained in separate growth rooms and tested each generation. A decline in resistance was measured in each generation, and after four generations the LC50s were around 20 ppm or less, indicating susceptibility to imidacloprid. These results are promising in that they indicate lack of exposure to imidacloprid will result in loss of resistance each generation. However they also suggest that highly resistant whitefly populations may pass two or three generations between cropping cycles during the summer in Florida and still retain some degree of tolerance at the beginning of the fall cropping cycle.
Accomplishment: Baseline information on biopesticide use among Florida's tomato growers was collected. Impact: 72% of growers interviewed use at least one biopesticide. 67% use insecticidal soap and 39% use azadirachtin products. 28% use mineral oils, and 17% use microbial insecticides.	The results of the survey indicate that growers are open to using biopesticides and in many instances are already incorporating them into their insecticide rotations. These results confirm the need for ongoing evaluations of biopesticides to refine their placement in management plans for whiteflies and other pests. The ongoing revisions of conventional insecticide uses by the EPA add urgency to the need to provide alternate tools to Florida's vegetable growers.

CHALLENGES

If you experienced any challenges during the project's period of performance, provide a listing of them below. Also, provide the corrective actions you took to address these issues. If you did not attain the approved outcome(s) and indicator(s), provide an explanation in the Corrective Actions column.

Challenge	Corrective Actions
NA	

LESSONS LEARNED

Provide recommendations or advice that others may use to improve their performance in implementing similar projects. NA.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Describe your plans for continuing the project (sustainability; capacity building) and/or disseminating the project results.
Resistance monitoring. We have received funding from the USDA NIFA Specialty Crop Research Initiative to continue resistance monitoring. We will build on what we have been able to learn with support from SCBP by including molecular techniques and focusing on the resistance status of early season whitefly populations. We know some populations are resistant after exposure to a full season of insecticides. We will be exploring the resistance status of early season populations to determine how well resistance management guidelines are working across the state.

Biopesticide evaluations. We have continued to incorporate biopesticide treatments in our efficacy trials focusing on combinations of biopesticides that kill mechanically (soaps and oils) with microbial insecticides.

Dissemination of results. We have presented the results of this research at the annual meetings of the Florida Tomato Institute (2017, 2018), the Florida Entomological Society (2018), the Entomological Society of America (2017, 2018), the Biopesticides East Conference (2017), the Florida Ag Expo (2017), and the Tomato Disease Workshop (2017), in addition to smaller grower meetings in Manatee county (2018). We are working on two separate manuscripts to publish the resistance monitoring data and biopesticide data in peer review journals. We plan to publish the results of the grower survey in the 2019 Proceedings of the Florida Tomato Institute.

OUTCOME(S) AND INDICATOR(S)/SUB-INDICATOR(S)

Provide the results of the project outcome(s) and indicator(s) as approved in your State Plan and project proposal. The results of the outcome(s) and indicator(s) will be used to evaluate the performance of the SCBGP on a national level.

We have documented reduction in virus incidence from biopesticide treatments that is commensurate with reduction due to dinotefuran, imidacloprid and thiamethoxam, commonly used neonicotinoid insecticides. In addition we have collected baseline information documenting widespread use of biopesticides among Florida's tomato growers. Reduced reliance on neonicotinoid insecticides provides Florida tomato growers with more options when managing whiteflies, including options that will not contribute to the development of insecticide resistance. More effective sprays will reduce inputs and enhance the sustainability of the crop.

OUTCOME MEASURE(S)

Select the Outcome Measure(s) that were approved for your project.

- ☐ **Outcome 1**: Enhance the competitiveness of specialty crops through increased sales
- ☐ **Outcome 2**: Enhance the competitiveness of specialty crops through increased consumption
- ☐ **Outcome 3**: Enhance the competitiveness of specialty crops through increased access
- ☑ **Outcome 4**: Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources

- ☐ **Outcome 5**: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems
- ☐ **Outcome 6**: Enhance the competitiveness of specialty crops through increasing the number of viable technologies to improve food safety
- ☐ **Outcome 7**: Enhance the competitiveness of specialty crops through increased understanding of the ecology of threats to food safety from microbial and chemical sources
- ☐ **Outcome 8**: Enhance the competitiveness of specialty crops through enhancing or improving the economy as a result of specialty crop development

OUTCOME INDICATOR(S)

Provide the indicator approved for your project and the related quantifiable result. If you have multiple outcomes and/or indicators, repeat this for each outcome/indicator.

Our target was to identify at least one biopesticide that could contribute to the reduction of transmission of Tomato yellow leaf curl virus, and we found that material in M-Pede, an insecticidal soap.

A second target was to increase grower awareness of the use of biopesticides. That has been more difficult to measure, but we did determine that 67% of Florida tomato growers were using biopesticides in their insecticide rotations. In addition, we estimate that we presented information on biopesticides to at least 50 growers in total when the audiences at the 2017 ag Expo, 2017 and 2018 Tomato Institute meeting, and 2018 Manatee County grower meetings are taken in to account.

DATA COLLECTION

Explain what data was collected, how it was collected, the evaluation methods used, and how the data was analyzed to derive the quantifiable indicator.

Data on biopesticide use for reduction in incidence of TYLCV was collected by carrying out field trials with tomato at GCREC. Tomatoes are grown in plots that consist of three rows of 14 plants on raised plastic mulch covered beds with drip irrigation. Insecticide treatments are applied via drip for soil treatments and with a high clearance sprayer for foliar treatments. Each treatment is replicated four times in a randomized complete block design. Starting three weeks after planting, leaf samples are collected and the number of whitefly eggs and nymphs per leaf are counted using a stereomicroscope. As soon as symptoms of virus appear, data are collected each week on the number of plants per plot with virus symptoms. Yield data are collected twice at the end of the season. Analysis of variance is used to determine if treatment differences exist in numbers of whitefly eggs and nymphs, virus incidence, and yield. A means separation test is then used to compare treatment differences.

CONTACT PERSON

Contact Person for the Project Hugh A. Smith

Telephone Number 813 419 6588
Email Address hughasmith@ufl.edu

FEDERAL PROJECT EXPENDITURES TO DATE

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	67000	61981

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Fringe Benefits	3618	7150
Travel	0	
Equipment	0	
Supplies	9600	9600
Contractual		
Other		
Direct Costs Sub-Total	80,218	79,704
Indirect Costs	0	0
Total Federal Costs	80,218	79,704

PROGRAM INCOME

Source/Nature (i.e., registration fees)	Amount Approved in Budget	Actual Amount Earned
Total Program Income Earned		

ADDITIONAL INFORMATION

Provide additional information available (i.e., publications, websites, photographs) that is not applicable to any of the prior sections.

Be sure to include any documents, publications, or other attachments referenced throughout the report. If the attachments are large, the State Department of Agriculture should consider combining them as an appendix to the full report and submitting the appendix as a separate file.

Project 26: Food Safety Modernization Act (FSMA) Training Program for Florida Growers

Contact Person:

Name the Contact Person for the Project, including telephone number and email address.

Keith R. Schneider, Food Science and Human Nutrition, 352-294-3910, keiths29@ufl.edu

Project Partners:

This project was conducted by the University of Florida, specifically the Food Science and Human Nutrition Department, IFAS Extension personnel and FDACS. These groups all worked together to train extension educators, regulators and most importantly farmers.

Project Summary:

Effective food safety standards, practices and education are critical to sustain and safeguard Florida's high level of specialty crop production. It is essential to establish, utilize and maintain food safety guidelines to enhance food safety at every phase of agriculture production. A high priority placed on food safety education results in the safest product possible and enhanced consumer protection.

In order for Florida specialty crops to remain competitive and to ensure their safe consumption, many practices must be followed to prevent/reduce microbial contamination. With the finalization of the FSMA rules, there are practices that must be implemented to comply with these new regulations; thus Florida farmers and packers face a significant challenge. Florida farmers must be trained on the new practices and understand the new regulatory paradigm in order to remain operationally viable. While FSMA does exempt many smaller operations from various aspects of some it's rules, once the program becomes widespread, produce buyers will require the same safety procedures of all growers, regardless of size. We have observed this in many other programs (e.g., GAPs and HACCP) where on-farm auditors (acting on behalf of buyers) requiring at least one person at each operation to be trained in these food safety programs.

The goal of this educational program was to initiate training immediately in order to assist Florida growers and packers effectively learn the food safety practices required under the new food safety regulations, specifically the new Produce Safety Rule (PSR). The UF food safety extension team has led food safety training efforts in the state for over a decade, and has a unique perspective of, and responsibility to, our stakeholders and others to develop programs for Florida. With over 48,000 commercial farms (with nearly a third dedicated to the production of fruit and vegetables) and over 280 commodities, the need for produce safety training was and remains, truly staggering. Even with the 2-, 3- and 4-year implementation

time line for large, medium and small farms respectively, training programs like the one undertaken, were needed to meet the demands of the state's farmers.

Project Approach:

The approach to this grant can be broken down into two specific goals. They were 1) to adapt existing food safety educational materials and training (targeting delivery directly to the stakeholders but also to state regulators and Florida extension faculty who will partner in the training) based firmly on the requirements associated with FSMA, specifically the PSR; and 2) deliver training, and educational materials statewide to producers, packers and repackers of fresh fruits and vegetables.

Goal 1 was focused on the development and delivery of an effective, FSMA-compliant (as administrated by the Produce Safety Alliance or PSA) educational curricula and materials for delivery to and use by specialty crop growers and packers, and associated stakeholders. This goal's target was to deliver education materials and specific training that would educate farms, packers, extension personnel and regulators (i.e., the stakeholders) in the area of produce safety. To this end a series of fact sheets (currently accessible on edis.ifas.ufl.edu) were updated and/or modified. This series, Food Safety on the Farm, has been updated and the eight associated fact sheets are either published or are in the review process awaiting peer review. All work on this project has been completed. Another aspect of goal 1 was the development of commodity specific content. The tomato 'Comparing FSMA and T-GAPS' slide set was created and presented in an all-day training workshop presented at the Annual Tomato Food Safety Workshop held in conjunction with the Florida Tomato Committee's annual meeting in Naples, FL (Sept. 5th, 2017). Over 100 attendees were certified by the Produce Safety Alliance on the PSR in that one workshop only.

Goal 2 was designed to increase the knowledge of specialty crop growers and packers about the food safety requirements related to the PSR, and to a lesser extent the Preventive Controls Rule (PCR) for Human Food. With changes in the regulation, where packing operations were (re)classified as farms, the need for PCR training diminished and the need for PSR training increased. The original plan was to conduct a total of 7 workshops (3 and 4 in years 1 and 2, respectively). At each workshop, pre-and post-tests and course evaluations were conducted to assess attendee's knowledge gain. The long-term outcome extending beyond the grant period is that farmers will still be farming. In 2016, 5 trainings were conducted, 2017, 23 trainings were conducted and in 2018, 11 additional trainings were conducted. These trainings were often held in conjunction with other programs and agencies leveraging the funding of several programs, resulting in a number of actual trainings that far exceeded our originally planned seven. In addition to training producers and packers, we trained 10 additional trainers, increasing the number of available trainers in the state. These included extension and FDACS personnel.

This increased cadre of trainer were then utilized to conduct additional trainings and to secure more funding for training in the state of Florida. Beyond the original training group, workshops in 2017 and 2018 routinely utilized extension agents and FDACS personnel to help conduct trainings. This expanded training group, I believe, enhanced the training experience by bringing a wider range of experience and professional background to our workshops.

Goals and Outcomes Achieved:

Our goals for this project were to finalized curricula comprising a course agenda, associated slide sets, reference materials, Florida Cooperative Extension factsheets, case study statements, and pre-and post-tests. With the initial GAPs workshop held in Palmetto in May 2016, until the last PSA train-the-trainer event in Tallahassee in July 2018, the outcomes from this training grant have greatly surpassed expectations. Specifically, the set goals for this project were great exceeded during the term of this project. We had originally planned for a series of seven workshops. Within this grant and our partnerships with other personnel and programs, nearly 40 training events were conducted. Provide a comparison of actual accomplishments with the goals established for the project.

Beneficiaries:

Florida growers and packers were the direct beneficiaries of this project. Secondary stakeholders that benefited were equipment suppliers, retailers and consumers of these products. The more farmers we keep farming, the better for the agriculture industry in Florida. Ultimately the production of safe produce benefits the entire state, its valuable agricultural economy and the “Fresh from Florida” brand, as well as Florida's industry partners and markets, both nationally and internationally

In the trainings conducted, approximately 900 individuals were trained. Of these, 750 were from Florida; and these represent approximately 460 unique operations that include farms, retail establishments, food services operations, commodity boards/grower associations, auditor, government and University personnel (including University of Florida, Florida A&M, and Florida International).

According to co-PI Danyluk (in conjunction with this grant and her and another USDA-funded project she currently serves as PI), the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS data estimates 3,267 farms in Florida covered by the Produce Safety Rule. We estimate we have trained 426 farms (13.04%) over the last three years. This leaves approximately 2,841 farm operations that require training. We are unsure how many of these operations may have received PSA

training from other sources. While there still exists a large group of individuals that need to be trained, the cadre of trainers produced by this program and others is well situated to meet this demand.

Lessons Learned:

The delivery of the standardized PSA curricula commenced starting Sept 2016, after the release of the official training curricula prepared by the Produce Safety Alliance. One GAPs workshop was held prior to the new program. One lesson learn during start of the program is that the official training material and requirements of buyers did not always match up. Care was taken to separate the legal requirements and business (audit) requirements as not to confuse the workshop attendees.

PI/Co-PIs Schneider, Goodrich-Schneider and Danyluk utilized their train-the-trainer status to train staff, extension specialists and extension agents to expand the training team. The delivery of programs statewide required significant travel commitment and the additional instructor base provided a cadre of trainers who were able to conduct well over the originally estimated number of workshops. Additionally, in our best professional opinion, face-to-face meetings with growers and packers were and still are the most effective means of communicating these requirements and rules. After three years of conducting workshops, we do not feel that online instruction is the best training option for growers and packers. Antidotally, we also found that training workshops with more instructors helped keep the students engaged. Changing speakers, with their various lecturing styles kept the interest level in the workshop high. This is reflected in the evaluations conducted.

Total funding expended to date. $99,587.47

Please list the percentage of the project that has been completed at this time. 100%

Signature:

Print Name and Title: Keith R. Schneider, Professor

Date: 10/23/18

FDACS Employee signature:

Date received:

Project (27): Moving towards implementation of the Decision Support System for late blight

Contact Person

Dr. Pamela Roberts

Phone: 239-658-3400

E-mail: pdr@ufl.edu

Project Partners

Southwest Florida Research and Education Center, University of Florida - IFAS

Florida Specialty Crop Foundation

Glades Crop Care

Partners: commercial tomato and potato growers

Project Summary

This project addressed the precision timing of fungicide applications to manage late blight, caused by Phytophthora infestans, on tomato and potato in Florida using the USAblight Decision Support System (USAblight DSS) at http://usablight.org/dss. The USAblight DSS uses site-specific weather and weather forecasts to recommend fungicide applications based upon current and forecasted favorable environmental conditions; therefore growers are aided in deciding the best time and interval to apply fungicides. Additionally, the USAblight DSS uses the inputs of tomato and potato varieties, the pathogen genotyping, date of most recent fungicide application, and fungicide weathering. Preliminary data generated in spring 2014 in tomato test plots in Immokalee showed a 40% reduction in the number of fungicide applications using the USAblight DSS while maintaining the same level of late blight management. This grant is needed to expand and validate testing of USAblight in Florida and cooperate with growers to test USAblight DSS in their fields.

Late blight on winter tomato and potato crops can cause variable but still significant yield losses (up to 50% in some fields) depending upon environmental conditions at specific farms. Additionally, the fungicides required for late blight control can increase pest management costs up to $300/acre since

growers spray on a preventative basis and may not be able to use fungicides in the optimum timing which reduced the efficacy of fungicide applications targeting this variable pathogen.

The USAblight DSS will assist growers in optimizing the timing of fungicide applications and improve or maintain the level of disease control while reducing the inputs; most notably the number of fungicide sprays. In turn, this should retard the selection of fungicide-resistant pathogens and decrease production costs. Growers will use the DSS to determine when and how often to spray and also aid in fungicide selection as the USAblight DSS includes fungicide weathering in the model. The USAblight DSS is recently available and preliminary testing in research plots at SWFREC showed the potential for its use in Florida production.

The project is timely since the technology now exists that makes it easy and convenient for growers to use the DSS. It can be accessed through the web and uses text messaging to keep the grower informed of when upcoming sprays are recommended.

Our previously funded block grants have provided the necessary background knowledge of late blight in Florida to get us to this point: focusing on improved field disease management. Previous grants established the pathogen genotype in different seasons which is crucial to understanding the biological and field attributes of the pathogen, such as fungicide sensitivity. Additionally, through our previously funded projects, we increased our understanding of the pathogen's survival, lack of initial inoculum from endemic sources, and the predominant genotypes in Florida which all contributes to field management.

We conducted an initial trial in spring 2014 to test the USAblight DSS to generate preliminary data but the trial was not funded through any previous block grants.

Project Approach

Per objective 1, we established field trials to evaluate the USAblight DSS in replicated field studies at the Southwest Florida REC, Immokalee (2016 and 2017) and also in cooperative studies (Glades Crop Care and UF) with tomato and potato growers (2017). The tomato trial was planted at SWFREC (Roberts) in

Immokalee in January 2016 for DSS testing but no late blight occurred in the southwest region near Immokalee during the time of these trials. Lack of disease pressure meant that no data was collected. For the same reason, no data was collected from scouted potato and tomato fields by Glades Crop Care in 2016. The tomato trial was planted at SWFREC (Roberts) February 2017 for DSS testing but late blight was minimally established in the plots. No data was obtained for trial at SWFREC.

For the objectives of working with potato and tomato growers, we coordinated with potato and tomato growers to assess the DSS compared to their spray schedule. Plantings of potato and tomato were identified in Immokalee and for potato in Hastings (Dufault). At each designated field site, the grower made their applications of fungicides according to their schedule. We monitored the DSS for each location and communicated with the grower (via Glades Crop in Immokalee) the information regarding recommended sprays from the DSS. We compared the grower's spray schedule, outbreaks of late blight, and the DSS recommended sprays.

Two sites were selected in Southwest Florida. Both were in commercial fields and both sites had a history of late blight. Weather monitoring stations were placed and at each site and weather data was downloaded weekly (Glades Crop Care). For each site, the DSS was ran from the website using the potato cultivar and susceptible tomato cultivar (Roberts). The standard spray program for the tomato and potato crops was obtained. Activities for this objective were scouting and data collection in potato and tomato fields; severity and incidence of late blight disease data; weather station data collection and reporting; and validation. The grower standard was determined to be that blocks were treated with fungicides twice weekly. These applications included protectant fungicides, mancozeb and chlorothalonil, as well as late blight specific fungicides, which were added in response to findings of new disease activity by Glades Crop Care. The tally of applications initiated with the first transfer of weather data to Roberts, SWFREC on 20 Dec 2016 and continued through 23 Feb 2017 for the tomatoes through 23 Mar 2017 for the potatoes in Farm 2. These were the dates when the crops were defoliated at the end of harvesting (tomatoes) or at the start of tuber curing (potatoes). Using these parameters, an estimated a total of 27 and 19 applications in the grower standard for potatoes and tomatoes, respectively. The recommendations from the DSS for the same fields would have resulted in 12 and 10 applications, respectively. The tally in the grower standard tomato program may be high by an application or two due to the usual reduction in applications during the approximately 2-3 weeks of harvesting. Regardless, these results indicate a potential reduction in fungicide use in controlling late blight of 44% and 53% in the potato and tomato crops, respectively.

Activities performed were: scouting and data collection in potato and tomato fields; severity and incidence of late blight disease data; weather station data collection and reporting; and validation (Glades Crop Care).

Per objective 2. Extension outreach to assist growers in becoming aware and implementing the USAblight DSS to manage late blight in tomato and potato crops in all areas of Florida where late blight occurs. Coordinated educational efforts will include presentations at grower meetings, field days,

popular journal articles, extension publications, and newsletters. Survey data indicated high interest in the DSS by participants. For the third objective, Roberts conducted an extension presentation to growers on late blight at SWFREC and participated in a spring field day at SWFREC with the intent of showcasing the tomato trial. However, due to lack of disease pressure, the plots were not exhibited during the field day. Dufault conducted extension outreach with potato growers at field days in Hastings.

Goals and Outcomes Achieved

All of the activities were completed as outlined in our goals for this project. We were unable to accomplish our first goal of evaluation of the DSS at SWFREC due to lack of disease pressure in both years of testings. We accomplished our second goal and worked at two tomato field locations in Southwest Florida and one poato field in Southwest Florida (Roberts and Glades Crop Care) and one potato field in Hastings(Dufault) (for a total of two potato growers). Growers currently apply fungicides on a calendar basis. By using the DSS, the success in demonstrating the nearly 50% reduction of fungicide applications in both crops should be highly significant to all tomato and potato growers. A challenge to implementing the DSS with tomato and potato growers has been the low disease pressure in the years covering the grant. Although the disease has occurred, it has not been at the epidemic levels of previous years and this has led to growers to focus on other concerns. Therefore, our attempts to gain grower adoption and usage are continuing. Despite the ending of this current grant, we will continue to conduct extension programming and promote grower adoption.

Beneficiaries

The specialty crop beneficiaries of the project were tomato and potato producers who have been affected by late blight in their crops. This research demonstrates the usefulness of a decision support system to time applications of fungicides using weather data. We demonstrated that a 50% reduction in fungicide applications using the DSS has the potential to save at least $150 per acre in costs per season. A farm of 1000 A would have a savings of $150,000.

Lessons Learned

The lack of disease pressure and lack of data collection spring 2016 and 2017 negatively impacted field validation of the DSS. This was offset by using field sites in tomato and potato to demonstrate what the DSS would have advised growers to do in their own fields. We will use this information to continue to work with growers interested in implementing this system in the fields.

Total Funding Expended: $149,948.13

Percentage of Project Completed: 100

Project (28): Add value to muscadine grapes via production of high-quality seed oil & flour

Contact Person

Meri Nantz

(352) 294-3725

mnantz@ufl.edu

Project Partners

Food Science and Human Nutrition Department

Institute of Food and Agricultural Sciences

University of Florida

Project Summary

Muscadine is the grape of the south due to its excellent adaptation to the climate. Florida grows 1,000 acres of muscadine, which generates an annual economic impact worth $895 million (FGGA 2011). Nearly all the muscadine grapes in Florida are processed into juice and wine, producing over 500 tons of muscadine pomace every year. About 60% of this pomace, or 300 tons, is muscadine seeds. Muscadine seeds contain 13-15% as edible oil which is highly valued in culinary arts. In addition, seed flour obtained after oil extraction is an antioxidant-rich food ingredient. It is estimated that 38 tons of muscadine oil and 250 tons of defatted seed flour can be produced in Florida alone with a $2 million economic impact annually. Currently, pomace is discarded as a waste and has no value. There is a pressing need to increase the value of Florida muscadine grapes by utilizing grape seeds in the pomace and converting them into high-quality seed oil and flour. The objective of this project was to develop efficient extraction and refining methods that produce these two products. The Florida Viticulture Policy Act declared muscadine grapes as an underdeveloped and underutilized agricultural commodity.

Project Approach

Muscadine grape seeds of two cultivars (Noble and Carlos) were obtained from Lakeridge Winery and Vineyard in Florida and Muscadine Products Corporation in Georgia. These seeds were air-dried or freeze dried. Seed oils were extracted using a Soxhlet extractor by hexane, mechanical press at different moisture and temperature, or enzyme-assisted aqueous extraction using different enzymes. Oils were

refined using degumming, neutralization, and bleaching. Crude and refined oil was evaluated fore peroxide value, free fatty acid content, fatty acid composition and vitamin E content.

Crude oil was obtained with yields as high as 11.1 and 13.9 % (w:w, dry basis) from the Noble and Carlos seeds, respectively. Screw pressing yields were as high as 7.08 and 8.62 % (w:w, dry basis) for Noble and Carlos, respectively. Screw pressing is quicker and more viable for small scale operations. Peroxide values were shown to be affected by drying methods, with freeze drying producing the lowest values with Soxhlet extraction using hexane. Peroxide values of freeze dried seeds were 2.33 and 6.33 mEq/kg oil for Noble and Carlos seed oil, respectively. Air dried seeds in the oven had peroxide values of 17.0 and 35.0 mEq/kg oil for Noble and Carlos, respectively. The oil with these peroxide values were obtained using a Soxhlet extraction using hexane and were significantly lower than all other oils obtained from different temperatures of mechanical press and different enzyme-assisted aqueous extraction methods. This indicates that drying caused some oxidation of the oil within the seeds. Other indications of some oxidation within the seeds were that the oil possessed 2.59 and 0.99 % free fatty acids expressed as oleic acid from Noble and Carlos oil extracted using hexane, respectively. Both Noble and Carlos seed oils consisted primarily of the polyunsaturated fatty acid Linoleic acid (18:2), consisting of about 72-74% of the fatty acids. Noble seed oil from Soxhlet extraction using hexane was shown to be higher in the bioactive forms of tocopherol and tocotrienol than oils of other extractions, with concentrations of 10.8, 21.3, 39.8, and 15.7 mg/100g of alpha-tocopherol, alpha-tocotrienol, gamma-tocopherol, and gamma-tocotrienol, respectively. Carlos seed oil did not show similar levels of tocopherol and tocotrienol with protease extracted oil having the highest concentrations with 6.52, 18.9, 25.4, and 19.3 mg/100g oil for alpha-tocopherol, alpha-tocotrienol, gamma-tocopherol, and gamma-tocotrienol, respectively. Refining of the crude oil to within Codex and FDA standards, removed oxidative products like peroxides and free fatty acids within the oil. Soxhlet extracted oil using hexane was the best at retaining tocopherol and tocotrienol through the refining process of degumming, bleaching and neutralizing. Oil obtained from Soxhlet extraction using hexane had a retention of 47 and 70% of total tocopherol and tocotrienol for refined Noble and Carlos seed oil, respectively.

Defatted seeds were obtained for Soxhlet extraction using hexane and pressed cake was obtained from expression using a continuous screw press. Pressed cake contains residual oil, so it was placed in a RRH-A1000 high speed multi-function grinder and grinded to a fine power. Ash in flour was determined by a previously described by AOAC official method 923.03. Protein in flour was determined using the Kjeldahl method with modifications to the one described by AOAC official method 920.87. Phenolics were determined using Folin-Ciocalteu method. DPPH assay was used to analyzed antioxidant capacity. Phytochemicals were analyzed on HPLC.

The resulting defatted seed flour composed of about 16-17 % protein (w:w) and 44-51% neutral detergent fiber (w:w), and 60-72 % total carbohydrates (w:w). More importantly, muscadine seed flour extracted using Soxhlet with hexane had the highest total phenolic concentration and antioxidant capacity for both Noble and Carlos oils. Total phenol concentration was 32.8 and 22.4 mg gallic acid/mL and 162 and 151 µmol Trolox/ 100g and antioxidant capacity was 162 and 151 µmol Trolox/100g. Noble

seed flour contained delphinidin 3,5-diglucoside, malvidin 3,5-diglucoside, cyanidin 3,5-diglucoside, petunidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, peonidin 3,5-diglucoside, myricetin and kaempferol. Only Myricetin was found in Carlos seed flour.

Project was presented at the Florida Wine and Grape Growers Association on Jan 14, 2017. There were about 50 participants in the meeting. Twenty-eight participants returned survey. All of them indicated that the presentation increased their knowledge about value-added muscadine products, especially muscadine seed oil and flour. Nine out of 28 (32%) were interested to produce muscadine seed oil in their vineyards in the future. Ten out of 28 (36%) were not interested because they only run vineyard not a winery. The rest (32%) were not sure. Two expressed strong interest to buy the products as consumers.

Part of the project research was presented at the American Society of Nutrition Annual meeting as a poster on April 26, 2017 in Chicago. The poster was about phytochemicals in muscadine seeds, defatted seed flour, and their health benefits. About 30 participants in the meeting discussed research with Dr. Gu and expressed their interests in the research and muscadine products. The abstract of the poster was distributed to all 12,000 participants of the meeting. It was published on FASEB Journal April 2017 31:lb397.

Research findings were presented at the Annual meeting of Institute of Food Technologists in Chicago, July 2018. A research paper was published on European Journal of Lipid Science and Technology (DOI: 10.1002/ejlt.201800166).

Goals and Outcomes Achieved

Goal 1: Develop and efficient extraction technology.

By observing extraction yields, oxidative products, fatty acid compositions, and vitamin E concentrations, we were able to determine that using the hexane is both the most efficient form of extraction but also gave us the oil with the least amount of peroxides and higher concentrations of vitamin E. This held true for both Noble and Carlos seed.

Goal 2: Develop an effective refining technology.

Using boiling water, 20% sodium hydroxide, and 10% (w:w) bleaching earth clay, we were able to refine the oils from every extraction method to comply with Codex and FDA standards. We were also able to determine that the refining of the oil extracted using hexane was the most advantageous to preserve the antioxidant vitamin E within the oil.

Goal 3: Evaluate product quality.

Oil was analyzed for vitamin E quality, peroxide value, and free fatty acid value before and after refining. Oxidation of the oil was removed to codex standards at the cost of some vitamin E. The resulting defatted seed flour composed of about 16-17 % protein (w:w) and 44-51% neutral detergent fiber (w:w), and 60-72 % total carbohydrates (w:w). Muscadine seed flour extracted using Soxhlet with hexane had the highest total phenolic concentration and antioxidant capacity for both Noble and Carlos oils. Total phenol concentration was 32.8 and 22.4 mg gallic acid/mL and 162 and 151 µmol Trolox/ 100g and antioxidant capacity was 162 and 151 µmol Trolox/100 g, respectively.

Goal 4: Dissemination of information

We presented our research once at state level, twice at national level. We published a research paper on an international journal (European Journal of Lipid Science and Technology (DOI: 10.1002/ejlt.201800166)). By doing these, we have reached audience in Florida, United States, and globally.

Beneficiaries

24 farm wineries in state of Florida

80-100 members in the Florida Wine and Grape

Growers Association

Muscadine grape growers and wine makers

Consumers who are interested in muscadine seed oils

Lessons Learned

All project goals were met. This research demonstrated that muscadine seed oils can be extracted and refined to meet the Codex standards for edible oils. However,

limitations on receiving the seeds and drying them caused more oxidation in the oil

than if we were able to receive the seeds immediately. Proper steam treatment or

drying are needed in the future to reduce enzymatic hydrolysis and oxidation in the oil prior to extraction. This would drastically decrease the amount of free fatty acids and peroxides within the muscadine seed oil and seed flour.

Total Funding Expended: $100,344.50

Percentage of Project Completed: 100

Project (29): Improved Specialty Pepper Cultivars for Florida's Fresh Produce Market

Contact Person

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Project Partners

University of Florida, Gainesville

Project Summary

Bell pepper is an economically important specialty crop of ~ $468 million farm value and is one of the top 20 most-consumed raw vegetables in the United States. In Florida, bell peppers are cultivated in about 17,500 acres with a total farm value of $183 million (USDA, 2007). While peppers are consumed in a multitude of ways, mini sweet color peppers have emerged as a novel specialty product, often for raw consumption. One serving of raw bell peppers provides a significant per cent of Daily Value of required nutrients including 190% of vitamin C, 4% of vitamin A, 4% of iron and 6% of potassium (Federal Register, 2006). Among plant sources of dietary iron, peppers rank at the very top of the list (USDA, 2014 Nutritional database). Though pepper is already a health promoting vegetable, our premise is that it is possible through focused selective breeding to further increase the nutritional value of this crop at the same time to increase its marketability by improving consumer-preferred traits.

Colored peppers are grown in hydroponic greenhouses and fetch higher prices for the growers than green bell peppers (Jovicich et al., 2009). Currently Sweet Mini Peppers are widely sold in Florida's produce market. Pero Family Farms Food Company LLC, Delray Beach, FL, Bailey Farms South LLC, Bonita Springs, FL, The Leamington, Ontario and Imagination Farms LLC, Indianapolis, IND are some of the major players in the production of sweet minis. However, specialty crop industry finds it a challenge to market mini-vegetable produce because of lack of uniformity in these products (David, 2010). Mini peppers have not become as popular as cherry tomatoes yet. In the vegetable industry, there is a need for new and interesting varieties that are high yielding, tasty and nutritionally valuable. So it is imperative to develop vegetable varieties that are uniform, high quality with high levels of health promoting nutrients so that they could be marketed with a special label to indicate their superiority. Consumers prefer vegetables with high levels of health promoting chemicals and antioxidants. A case in point is the recent success of high lycopene tomato cultivar "TastiLee" marketed in Florida with a special

label. One market study found that consumers with kids were more likely to buy specialty peppers than shoppers without kids in the home (The Packer, 2010). This suggests that if mini sweet peppers are improved for their nutrient profile and nutrient density, this could be promoted to improve children's nutritional needs. Pro-vitamin A carotenoid (all-trans-beta-carotene) is the best substrate for our body's production of retinoids that are important for vision health (Grolier et al., 1997). Peppers contain a variety of carotenoids including three carotenoids contributing to pro-vitamin A activity (beta-carotene, alpha-carotene and beta-cryptoxanthin). The red carotenoids of peppers (capsorubin and capsanthin) are excellent antioxidants. Accumulation of carotenoids in the ripe fruits is responsible for variations in their colors from white, yellow, yellowish orange, bright orange and red.

Our work and research by others have indicated that ripe pepper fruits have high levels of total carotenoids (up to 1 mg per g fresh wt) and about 16% of this to be beta-carotene (Wall et al., 2001; LonKan et al., 2008; Wahyuni et al., 2011). Wide variation in total carotenoid content and the proportion of individual carotenoids in Capsicum germplasm indicates that there is great potential to increase pro-vitamin A carotenoids in peppers via plant breeding.

Anthocyanins are phenolic compounds that occur widely in the red to blue colored parts of most plants. Some of the pepper cultivars that have black, brown or chocolate color fruits have anthocyanins (in the form of their glycosides) that are known as excellent antioxidants (e.g. delphinidin-3-p-coumarosyl-rutinoside-5-glucoside, Lightbourn et al., 2008). Anthocyanin pigments in fruits have been known to prevent diseases induced by oxidative stress such as cardiovascular diseases, cancer and inflammation. Our proposed research project will address the development of pepper varieties specifically improved for their nutritional value and consumer-preferred fruit quality traits.

Importance and Timeliness of the Project

Since 2010, the PD is directing an effort to develop pepper lines with improved traits in a novel integrated research and education effort (Building Better Peppers Initiative, http://hos.ufl.edu/faculty/brathinasabapathi/building-better-peppers). We have built several pre-breeding pepper lines - both pungent and sweet - enriched for flavor, taste, carotenoids and anthocyanins with novel shapes and sizes using traditional plant breeding tools. Currently there are no programs in academic institutions in Florida breeding peppers for fruit quality or other traits. Funding from this project allowed us to field test and thoroughly evaluate four of the most improved open pollinated lines. We have four lines developed now at the end of the funded project and they will be released for cultivation as new varieties for production in Florida within one year from now. This is a crucial juncture in our efforts to breed new and improved pepper varieties for Florida now and into the future. Our efforts are unique and novel and can be expected to result in a positive change in a segment of the specialty crop industry.

Objectives of the Project

Our overall goal is to build novel and value-added pepper varieties for cultivation in Florida. Our aim was to evaluate four lines of peppers developed through breeding efforts since 2010. The four lines have novel fruit shapes, flesh fruit walls and have scored well in overall taste and flavor.

Project Approach

Activities performed during the grant period:

We have developed and evaluated four pepper lines with consumer-preferred fruit quality traits using traditional plant breeding methods. Briefly, a Jalepeno pepper was crossed to a sweet heirloom pepper. From this cross' 200 F2 progeny were grown in a greenhouse. A single F2 plant with elongated fruit shape and good growth characteristics was selected (RJ107). We derived progeny plants from this selected plant using single seed descent method over four more generations (F3, F4, F5 and F6). We collected data on yield per plant, immature fruit color, mature fruit color, overall taste, pericarp thickness and fruit length and width. At the F6 generation, we obtained stable lines that had multiple desirable fruit quality traits. These lines were grown at Citra, Florida using black plastic mulch and drip irrigation during the following seasons: Spring 2017, Fall 2017 and Spring 2018. The fruit traits evaluated were as follows: fruit color, pericarp thickness, flavor, total soluble solids, capsaicin content, fruit shape and fruit yield. The lines we developed from this research had light green fruit when unripe, brilliant red fruit when ripe, had fleshy pericarp (1to 2 mm), and yielded on an average 1 to 1.5 lb fruit per plant when grown under standard management conditions. We expect to release four of the advanced lines as new specialty pepper varieties for cultivation in Florida. During the period of this grant several students received training in plant breeding research. These were Dylan Kovach, McKenzie De New, Jessica Webster, Yanlin Wang, and Yue Yang.

Goals and Outcomes Achieved

The major success of our research is that we developed four stable improved specialty pepper lines that are to be released for wider cultivation in Florida. We have presented our research results at six professional society meetings attended by scientists, plant breeders and students. We have disseminated research information using this mode to at least 600 people.

Rathinasabapathi, B (2016) Building Better Peppers: Novel fruit variations for breeding specialty peppers for Florida. 32nd Annual Florida Seed Association and UF-IFAS Seedsmen Seminar, University of Florida Gulf Coast Research & Education Center, Wimauma, FL (Invited presentation, June 8, 2016).

Rathinasabapathi, B and Sargent, S.A. (2016) Building Better Peppers: Characterization of a variegated pepper line and its utility in breeding novel fruit types. Annual meeting, American Society of Horticultural Science, Poster Presentation, Atlanta, GA, Aug 8-11, 2016). Abstract published in HortScience 51(9), S277.

Kovach, D.C., Zhao, X., Rathinasabapathi, B (2017) Influence of Capsicum baccatum 'Barra do Ribeiro' rootstock on bell pepper (Capsicum annuum 'Hunter') growth, flowering, yield and fruit quality. 130th Annual meeting, Florida State Horticultural Society, Tampa, FL (June, 5, 2017; Oral presentation).

Yue Y, Chang Y, Rathinasabapathi, B (2018) Pollen effects on fruit shape traits in Capsicum annuum L. Southern Region, American Society for Horticultural Science, 82nd Annual Meeting, Jacksonville, FL (oral presentation).

Rathinasabapathi, B (2018) Improving peppers for fruit quality traits: Milestones in a project designed to facilitate active learning in crop breeding. 82nd Annual meeting, Southern Region, American Society for Horticultural Science, Jacksonville, FL (oral presentation).

Wang, Y., Yue, Y., De New, M., Rathinasabapathi, B (2018) Stem and canopy architecture differences and their relationship to productivity in Capsicum annuum lines. Annual Meeting, Florida State Horticultural Society, Fort Lauderdale, FL.

Target 1: 50 web hits per month, 20 email and phone interviews per year, 1 field day and 1 survey of stakeholders per year

Progress toward target: The output from the project were published in FSHS proceedings and presented at professional meetings. Approximately 50 unique readers per month are estimated.

We did not host a field day. However, results were presented at the Seedsmen Seminar at UF Gulf Coast Research and Education Center, Wimauma and about 50 stakeholders were present at that meeting. Some of the results were presented at the Southern Region, American Society for Horticultural Science, Jacksonville, FL as a poster and as an oral presentation. There were about 40 stakeholders at that meeting.

Target 2: 50 web hits per month, 20 email and phone interviews, and 1 survey per year.

Progress toward target: We have conducted surveys to evaluate pepper fruit quality. The results from this survey are part of a publication in preparation.

Target 3: Disseminate knowledge to at least 50 vegetable growers, seed company professionals, etc. and 100 general public members

Progress toward target: At least 600 people have been reached by one of the project's dissemination activities, including both growers and members of the general public.

Beneficiaries

The scientific community is benefited by our novel research results especially our findings about the genetics of fruit quality traits in peppers. We have one manuscript in preparation to be submitted to the Journal of the American society for Horticultural Science. Our results when presented at conferences benefited at least 600 vegetable growers, seed industry professionals and students. The potential future impact of our research completed by the support from FDAC is that the new varieties of peppers released to the public will enhance the options provided to consumers. We expect at least 2000 people will be benefited within one year of release of the new varieties.

Lessons Learned

We did not find any unexpected outcomes during implementation. However, we recorded some unexpected scientific data about genetic variation in peppers which turned out to be an interesting finding.

We learned that it is best to plan our field trials better to escape the fall frost in North Central Florida.

We also found that pepper breeding required most effort at certain points of the season (at the beginning to set up the field trial) and after several months later during fruit harvest and data collection. We needed to hire temporary workers (students as part-time work force) to fill the needs of the project.

Total Funding Expended: $87,426.07

Percentage of Project Completed: 100

FINAL PERFORMANCE REPORT

FINAL PROJECT REPORT TEMPLATE

Final Performance Reports must illustrate the completion of each project within the grant agreement. Each project shall be outlined as separate project profiles. You will report on projects in the same order they were submitted in the approved State Plan and subsequent amendments.

FDACS CONTRACT NUMBER:

022902

PROJECT TITLE

Reducing labor costs in leafy brassica crops with integrated weed management

PROJECT IMPACT AND FINDINGS

Include a summary of the project results of 250 words or less, suitable for dissemination to the public. A statement of results provides a brief description of your project, its success, and/or lessons learned. A statement of results should:

1. *Include the name of the organization that led and executed the project (State department of agriculture or subrecipient);*
2. *Capture the project's purpose and activities completed;*
3. *Outline the outcomes and indicators achieved, results/important findings and lessons learned; and*
4. *Highlight the target population reached and products developed (including intended use and audience).*

The University of Florida used integrated weed management to reduce the weed density in leafy brassica crops to reduce labor requirements. Leafy brassica crops have very few herbicides registered so growers rely on hand weeding. The summer fallow period is an opportunity to use cover crops and herbicides to reduce the weed density in the field. Previous research has shown a reduction in weed density requires less time to hand weed. This project included sunnhemp, cowpea, and sorghum Sudangrass with or without s-melachlor and a weedy fallow treatment with or without glyphosate. The sunnhemp and cowpea did the greatest job of shading the nutsedge, however, the increased nitrogen from these crops caused a quick growth of the nutsedge tubers. Among the cover crops, sorghum sudangrass with s-metolachlor had the greatest nutsedge control in the bok choy. The best nutsedge control was from weed fallow that included glyphosate. Increased nutsedge populations caused had weeding to be 23% slower. All treatments had poor wild radish control and had the greatest impact on late season weed control. The poor wild radish control was because the wild radish only emerges when temperatures are lower so the weed were not growing during the fallow period treatments. This research can be used by 321 growers that have leafy brassica crops. The use of integrated weed management during the summer fallow period that includes cover crops and herbicides can be used by all the growers with fall and winter cash crops.

BENEFICIARIES

Number of project beneficiaries: 321

ACTIVITIES PERFORMED

OBJECTIVES

Provide the approved project's objectives.

#	Objective	Completed?	
		Yes	No*
1	Inclusion of cover crops during the fallow period to reduce the weed seed bank.	X	
2	Reduction of hand weed events with properly timed preemergence herbicides.	X	
3			
4			

**If no is selected for any of the listed objectives, you must expand upon this in the challenges and lessons learned sections.*

ACCOMPLISHMENTS

List your accomplishments for the project's period of performance, including the impact they had on the project's beneficiaries, and indicate how these accomplishments assist in the fulfillment of your project's objective(s), outcome(s), and/or indicator(s).

Accomplishment/Impact	Relevance to Objective, Outcome, and/or Indicator
Reduced weed density with the use of Sorghum sudangrass with s-metolachlor	The reduction of nutsedge helped reduce labor costs by 23%. (Objective 1).
Two applications of glyphosate during the fallow period had the lowest nutsedge population in the bok choy crop.	This treatment does not provide organic biomass or nitrogen to the crop system, but would be a valuable tool to growers that have an excessive population of nutsedge in their field. (Objective 1).
The application of pendimethalin after the 4th true leaf had the greatest grass control, however, some broadleaf weeds and nutsedge were not controlled. S-metolachlor provided the better wide spectrum weed control.	Growers can select the appropriate herbicide based on their historical weed spectrum. In fields, with large seeded broadleaf weeds and nutsedge, they should select s-metolachlor. They can rotate the herbicide mode of action and reduce herbicide resistance. (Objective 2)

CHALLENGES

If you experienced any challenges during the project's period of performance, provide a listing of them below. Also, provide the corrective actions you took to address these issues. If you did not attain the approved outcome(s) and indicator(s), provide an explanation in the Corrective Actions column.

Challenge	Corrective Actions
Weather related.	Delayed planting or data collection.

LESSONS LEARNED

Provide recommendations or advice that others may use to improve their performance in implementing similar projects.

The plot size that we selected 36 ft. x 50 ft. was appropriate for this study. However, the plot size limits the amount of split plot work that could be completed. We discussed completing additional objectives to research the wild radish control in the bok choy within the objective 1 study, but this would have resulted in a subplot that was too small and would have affected the original objective.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Describe your plans for continuing the project (sustainability; capacity building) and/or disseminating the project results.

The results of the study have been shared at the Weed Science Society of America national meeting and Southern Weed Science Society regional meeting. The final data has been prepared for a manuscript that is being submitted to the journal Weed Science. The data is also being shared at the Cole Crop Field Day in Hastings, FL each year.

A proposal will be submitted to another agency to improve the system. The wild radish later in the bok choy season is a concern and improving the system is needed.

OUTCOME(S) AND INDICATOR(S)/SUB-INDICATOR(S)

Provide the results of the project outcome(s) and indicator(s) as approved in your State Plan and project proposal. The results of the outcome(s) and indicator(s) will be used to evaluate the performance of the SCBGP on a national level.

OUTCOME MEASURE(S)

Select the Outcome Measure(s) that were approved for your project.

- ☐ **Outcome 1**: Enhance the competitiveness of specialty crops through increased sales
- ☐ **Outcome 2**: Enhance the competitiveness of specialty crops through increased consumption
- ☐ **Outcome 3**: Enhance the competitiveness of specialty crops through increased access
- ☒ **Outcome 4**: Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources
- ☐ **Outcome 5**: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems
- ☐ **Outcome 6**: Enhance the competitiveness of specialty crops through increasing the number of viable technologies to improve food safety
- ☐ **Outcome 7**: Enhance the competitiveness of specialty crops through increased understanding of the ecology of threats to food safety from microbial and chemical sources
- ☐ **Outcome 8**: Enhance the competitiveness of specialty crops through enhancing or improving the economy as a result of specialty crop development

OUTCOME INDICATOR(S)

Provide the indicator approved for your project and the related quantifiable result. If you have multiple outcomes and/or indicators, repeat this for each outcome/indicator.

DATA COLLECTION

Explain what data was collected, how it was collected, the evaluation methods used, and how the data was analyzed to derive the quantifiable indicator.

We counted two 1 ft^2 quadrats in each plot at 4 week intervals during the fallow period and the bok choy. The weeds were separated by species and the biomass of the cover crop and the weeds were collected then dryed in ovens until a consistent weight was obtained. This biomass was used for the amount of plant material from the cover crop and weeds were being introduced to the soil at the end of the season.

We also measured the light penetration at the soil surface and half-way on the cover crop. The light penetration was important to correlate to weed emergence.

For labor timing, a person was assigned to each plot, then we recorded the start and stop time of each plot. The people were randomized to different plots because some people were more careful around the crop or did a better job of removing the weeds.

For objective 2, the crop injury was visually assessed, the plant heights were recorded, and the weeds were counted at a weekly basis for the first 4 weeks, then biweekly until harvest.

All the data were analyzed using general linear methods in statistical software.

CONTACT PERSON

Contact Person for the Project Peter Dittmar

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FEDERAL PROJECT EXPENDITURES TO DATE

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel		
Fringe Benefits		
Travel		
Equipment		
Supplies		
Contractual		
Other		
Direct Costs Sub-Total		
Indirect Costs		
Total Federal Costs		

PROGRAM INCOME

Source/Nature (i.e., registration fees)	Amount Approved in Budget	Actual Amount Earned
None		
Total Program Income Earned		

ADDITIONAL INFORMATION

Provide additional information available (i.e., publications, websites, photographs) that is not applicable to any of the prior sections.

Be sure to include any documents, publications, or other attachments referenced throughout the report. If the attachments are large, the State Department of Agriculture should consider combining them as an appendix to the full report and submitting the appendix as a separate file.

Project (31): Driving Demand – Increasing Awareness and Marketability of Florida Peaches

Contact Person

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Project Partners

University of Florida

Florida Specialty Crop Foundation

Project Summary

For several decades growers have experimented with growing peaches in Florida, but it was not until more recent years that varieties of peaches suitable for Florida's climate have been developed (Harrison et al., 2008). Low-chill varieties allow for peaches to be harvested in Florida before any other state can enter the U.S. commercial peach market, allowing a unique market window from late March through May (Olmstead et al., 2014). Florida's peach varieties are highly susceptible to the state's variable weather patterns and require significant amounts of care throughout the growing and harvest seasons (Olmstead et al., 2014). In 2006, producers estimated that there were only 60 acres of peaches growing in the state (M. Olmstead, pers. comm.). By 2012, the Census of Agriculture recorded 185 Florida agricultural operations growing 776 harvestable acres of peaches and today growers estimate that the total acres of peaches in Florida has grown to approximately 2,000 acres (M. Olmstead, pers. comm.). Despite the growth in acreage, Florida peaches have yet to gain popularity among Florida consumers. The popularity of peaches from Georgia and California have overshadowed the entrance of the Florida peach into the marketplace, and a need exists to increase awareness and preference for Florida peaches so that the industry is able to remain viable and continue to grow.

The Florida peach industry is estimated to be valued at more than $10 million dollars and annually produces 7.2 million pounds of fruit (M. Olmstead, pers. comm.). In 2014, Florida peaches were exported to more than 37 states (M. Olmstead, pers. comm.). Sources indicate that an opportunity exists to increase Florida's peach acreage to 10,000 acres in order to fulfill market demand for domestic peaches (Harrison et al., 2008). However, successful expansion of the Florida peach industry requires increased consumer and retailer awareness of the industry, as well as an understanding of these audience's preferences for and barriers to buying Florida peaches. According to interviews with Florida peach growers and marketers, Florida peaches are picked tree-ripe and are described as being sweeter than other peaches, but they are smaller by a quarter of an inch than other commercial peaches. The

Florida peach's small size and unique marketing window present challenges to the marketing of Florida peaches.

The small size has led to a lack of acceptance among consumers and retailers. Additionally, the short harvest season requires that the industry has good communication and marketing in place to effectively distribute and sell the product for sufficient returns in this short amount of time. For the Florida peach industry to remain viable and growing, it is essential that the product is effectively marketed and promoted and that consumer and retailer awareness of the product increases. To work toward this need, this grant project examined consumers' perceptions of and barriers to purchasing Florida-grown peaches and the barriers to marketing and communication of Florida-grown peaches. Following this examination, a marketing plan for Florida peach growers and the Florida Peach Industry was developed.

This project addressed the following FDACS specialty crop funding priorities:

• Immediate benefit to the public/industry

• Expand child and adult knowledge of nutritional benefits, improve access, and increase consumption for Florida specialty crops.

• Increase sales and marketability and drive demand for commercially-grown specialty crops.

This project was important and timely as the need to increase consumer and retailer awareness of Florida peaches, indicates the opportunity to create effective messaging and marketing strategies to promote Florida peaches. Through effective promotion of peaches, consumers and retailers are likely to become more aware of Florida peaches, thus increasing preference and demand for the product. The Florida peach industry is at risk of becoming unviable without improved marketing and promotion to drive increase demand for and awareness of the product.

This project was not a continuation of a previous grant, but it was informed by a 2014 funded specialty crop block grant awarded $157,559. The 2014 grant focused on identifying producers and consumers' barriers associated with Florida blueberries and included the development of a marketing plan for Florida blueberries.

Project Approach

The activities performed and any significant results/accomplishments/conclusions/recommendations for each objective of the grant project are outlined below.

Objective 1: To identify consumers' perceptions of and barriers to purchasing Florida-grown peaches

Activities

- The survey was distributed in May of 2016. The survey was completed by a total of 2,061 respondents. Of these respondents, 1,020 were national peach purchasers, 522 were non-purchasers from throughout the nation, and 519 purchasers from Florida.

- The report of the consumer findings was finalized.

- The report was shared with an advisory group of Florida peach growers representing approximately 85% of the Florida peach industry as well as representatives from the Fresh from Florida team.

- The report was posted on the project webpage at http://www.piecenter.com/issues/food/local-food/ and can be found under the tab titled "Driving demand: Increasing awareness and marketability of Florida peaches."

Significant results/accomplishments/conclusions/recommendations

- Respondents most commonly ate peaches as a snack but did not regularly pack them in lunches.

- When asked what states respondents associated with peaches and what states they wanted peaches to come from Georgia, Florida, and South Carolina were the top answers for all three groups.

- A majority of respondents (72% National, 72% Florida, 67% Non-Purchasers) indicated that self-selection by the pound was the way they preferred to buy peaches.

- Nationally, 41% of respondents reported they have handpicked peaches. While 49% reported they have seen a peach orchard in person. Only 18% reported having personally grown their own peaches.

- In Florida, less than a third (32%) of respondents reported they have handpicked peaches. While 39% reported they have seen a peach orchard in person. Only 9% reported having personally grown their own peaches.

- Few respondents (23% National, 35% in Florida, and 10% of Non-purchasers) claimed to know when Florida's peach growing season took place.

- Those who reported knowing when Florida's peach season was, were asked to indicate which months they believed Florida peaches were in season. Nationally, 57% said May, 51% said June, and 48% said July. In Florida, 69% said May, 59% said June, and 45% said July. 49% of Non-Purchasers selected May, 41% chose April, and 31% said August.

- Peaches being a source of fiber was the nutritional benefits question answered correctly the most by all groups, 77% National, 78% Florida, and 69% of Non-Purchasers.

- The statement about peaches Vitamin C content was responded to incorrectly the most by purchasers; 28% National, 26% Florida. Non-purchasers correctly responded to "A peach contains fewer calories than an apple of the same size" the least (25%).

Objective 2: To identify barriers to marketing and communication of Florida-grown peaches through a survey of growers and in-depth interviews of shippers.

Activities

- An advisory council of growers was established.

- Preliminary survey data was collected at the Spring, 2016 Peach field day from growers. Many of the individuals attending the meeting represented the same farm or they were interested in growing peaches and had not yet planted any. Therefore, we only received 7 unique surveys.

- Due to complications with industry contacts (see the problems and delays section for more info) a survey of growers was not feasible.

- Instead 5 in-depth interviews were completed with growers and shippers, accounting for 85% of the commercial peach acreage grown in Florida.

- Information from the in-depth interviews with peach growers and marketers was analyzed and incorporated into the marketing plan

Significant results/accomplishments/conclusions/recommendations

- All seven growers responding to the preliminary grower survey, reported growing another commodity in addition to peaches.

- The preliminary grower survey revealed that the growers were facing marketing difficulties due to price, market window, locating a buyer, and weather.

- The preliminary grower survey identified the following marketing opportunities: Great tasting product, earlier crop, tourism, and increasing consumer awareness.

- The seven growers responding to the preliminary survey had been growing peaches in Florida for an average of 4.71 years.

- Among the seven growers responding to the preliminary survey, some had as few as 2 acres of peaches and others as many as 440 acres.

- These conclusions were supported by the in-depth interviews.

Objective 3: To develop a marketing plan for Florida peach growers and the Florida Peach Industry

Activities

- The marketing plan was finalized and posted on the project website at http://www.piecenter.com/issues/food/local-food/ and can be found under the tab titled “Driving demand: Increasing awareness and marketability of Florida peaches.”

- A webinar discussing the consumer survey results, grower interview results, and the marketing plan was held. The webinar included industry and extension representatives and served as an in-service training for extension faculty. The webinar was recorded and is available at http://www.piecenter.com/2017/01/26/march-23-1030-1130am-the-florida-peach-industry-consumer-research-and-marketing-plan-in-collaboration-with-the-fresh-from-florida-campaign/

Significant results/accomplishments/conclusions/recommendations

The tactics identified in the marketing plan for the Florida peach industry include

- Establish a Florida peach marketing arm or dedicated association
- Continue partnership with UF/IFAS
- Reapply for FDACS grants
- Educate through marketing
- Engage on social media
- Utilize consumer-preferred education tactics
- Consider distribution through the school lunch program
- Demonstrate unconventional ways to use peaches
- Provide a marketing toolkit for growers with U-picks

Objective 4: To develop and disseminate outreach materials for industry and consumers.

Activities

- A blog post focusing on the consumer survey results was completed and can be found at http://www.piecenter.com/2017/03/28/kara-harders-perceptions-of-the-florida-peach-industry/
- A student thesis on the consumer survey results was completed and is titled “People's Perceptions of Peaches: An Exploration of Consumers Perceived Voluntary Label Value.” The thesis is not available online.
- A student thesis on the marketing plan was completed and is titled “Strategic Marketing Plans for Florida Specialty Crops: A Case Study Approach.” The thesis is not available online.
- Three outreach materials were finalized, printed, and shared with an advisory group of peach growers representing 85% of the Florida peach industry as well as represenatives from FDACS. All of the materials can be found on the project webpage at http://www.piecenter.com/issues/food/local-food/ under the “Driving demand: Increasing awareness and marketability of Florida peaches” tab.

- Two EDIS documents were submitted for publication and published. They can be found at http://edis.ifas.ufl.edu/wc288 and http://edis.ifas.ufl.edu/wc287

- The project results insert was designed and finalized and published in the Fall, 2017 edition of Peach News. The Peach News is a trade-publication that is distributed to peach growers and businesses in Florida, Georgia, and South Carolina. The readership of Peach News is estimated to be 11,000. We also requested 1,000 reprints of the results insert and these were shared peach growers and extension agents for dissemination in early 2018.

- The project has received a lot of media coverage among industry, state, and local media sources. Coverage has included the following

o Linden, T. (2018, March). Florida Fruit & Vegetable Association promoting peaches. The Produce News. Retrieved from http://www.producenews.com/category-list/23665-florida-fruit-vegetable-association-promoting-peaches

o Fry, K. (2017, January). What do you know about Florida peaches? VSCNews. Retrieved from http://vscnews.com/1121-2/

o UF/IFAS. (2017, March). Florida peach industry webinar. Morning AgClips. Retrieved from https://www.morningagclips.com/florida-peach-industry-webinar/

o Buck, B. (2017, March). Florida peaches pack a punch as a succulent snack. PerishableNews.com. Retrieved from http://www.perishablenews.com/index.php?article=0059056

o Hines, D. (2017, March). Florida peaches pack a punch as a succulent snack. Today's Seniors Networks Senior's Dietary. Retrieved from http://todaysseniorsnetworkseniordietary.blogspot.com/2017/03/florida-peaches-pack-punch-as-succulent.html

o Buck, B. (2017, March). Florida peaches pack a punch as a succulent snack. Lab Manager. Retrieved from http://www.labmanager.com/news/2017/03/florida-peaches-pack-a-punch-as-a-succulent-snack#.WOfoD461tBx

o Buck, B. (2017, March). Florida peach pack a punch. Morning Ag Clips. Retrieved from https://www.morningagclips.com/florida-peaches-pack-a-punch/?doing_wp_cron=1491593299.3544609546661376953125

o Buck, B. (2017, March). Florida peaches pack a punch as a succulent snack. VSCNews. Retrieved from http://vscnews.com/1989-2/

o Buck, B. (2017, March). Florida peaches pack a punch as a succulent snack. IFAS News. Retrieved from https://news.ifas.ufl.edu/2017/03/florida-peaches-pack-a-punch-as-a-succulent-snack/

o Delgado, L. (2017, March). Florida peaches may have their moment in sun. Orlando Sentinel. Retrieved from http://www.orlandosentinel.com/entertainment/restaurants/foodie/os-et-florida-peaches-20170322-story.html

o Delgado, L. (2017, March). Florida peaches may have their moment in sun. The Daily Meal. Retrieved from http://www.thedailymeal.com/eat/florida-peaches-may-have-their-moment-sun

o Delgado, L. (2017, March). Upgrade peaches from a simple snack. Orlando Sentinel. Retrieved from http://www.orlandosentinel.com/entertainment/restaurants/foodie/os-et-florida-peach-recipes-20170329-story.html

- The evaluation survey was distributed in early 2018. Some of the results are detailed below. Other results correspond correctly with the performance measures and are reported in that section.

Significant results/accomplishments/conclusions/recommendations

Some of the results of the evaluation survey are detailed below. Other results are presented with the corresponding performance measure.

- 26% reported buying fresh peaches (when in season) once a week or more
- 2% reported buying fresh peaches (when not in season) once a week or more
- 42% reported buying fresh peaches when they catch their eye at the store
- 41% reported buying fresh peaches when they are on sale
- 15% reported buying fresh peaches when they see an advertisement for them
- 25% reported eating peaches whole with the peel on once a week or more
- 27% reported eating peaches as a snack once a week or more

Significant contributions and role of project partners

- The PI and research staff worked to speak with industry representatives and do research to understand how the industry and markets operate in Florida. In addition, the researchers collected data from producers, consumers, and marketers/distributors.

- The Florida Specialty Crop Foundation has been instrumental in helping manage the grant, providing feedback on grant components, and building relationships with key stakeholders in the peach industry.

Goals and Outcomes Achieved

Goal 1: Increase consumer knowledge of the availability and nutritional benefits of Florida peaches.

- Performance Measure - Percent of consumers reporting increased knowledge regarding the availability and nutritional benefits of Florida peaches.

- Benchmark - The benchmark was a baseline measure of the number of consumers aware of the availability and nutritional benefits of Florida peaches collected at the beginning of the consumer survey before participants were exposed to information about the Florida peach season.

- Target - 45% of consumers will increase their knowledge of the availability or nutritional benefits of Florida peaches.

- Result/Accomplishment

- Availability – In the initial consumer survey, 1,524 respondents were asked to select the months that Florida peaches were in season. These same questions were asked in an evaluation conducted at the end of the project. In the evaluation, 254 consumers received the seasonality outreach flyer before answering the questions. Another 266 consumers did not receive the flyer before answering the questions. The percentage selecting each month is below. Responses of March, April, and May were considered correct.

- Perceived Florida Peach Season Frequency

☐ Initial Consumer Survey –

- January = 27
- February = 32
- March = 55
- April = 121
- May = 325
- June = 543
- July = 611
- August = 552
- September = 274
- October = 66
- November = 23
- December = 18

☐ Flyer Group –

- January = 12
- February = 22
- March = 181
- April = 197
- May = 215
- June= 92
- July = 64

- August = 48
- September = 18
- October = 11
- November = 7
- December = 12

☐ Control Group –

- January = 18
- February = 20
- March = 36
- April = 102
- May = 146
- June = 158
- July = 140
- August = 112
- September = 64
- October = 37
- November = 20
- December = 18

- Percentage of correct responses

☐ Flyer Group – 67.5% correct responses

☐ Control Group – 32.6% correct responses

- The results suggest that the Florida peach season outreach flyer increased knowledge by 34.9%. While an increase in knowledge was achieved, the target was not met.

- Nutritional benefits - There were a series of eight knowledge questions asked about the nutritional benefits of peaches in the initial consumer survey in Objective one. These same knowledge questions were also asked in the evaluation conducted at the end of the project. In the evaluation, 266 consumers received the health benefits outreach flyer before answering the questions. Another 266 consumers did not receive the flyer before answering the questions. The mean knowledge score for each survey is reported. In addition, the percentage of correct responses for the group receiving the flyer and the control group are reported.

- Mean knowledge scores

☐ Initial Consumer Survey – M = 3.43 SD = 1.93

☐ Flyer Group – M = 4.68 SD = 1.36

☐ Control Group – M = 3.86 SD = 1.57

- Percentage of correct responses

☐ Flyer Group – 58.5% correct responses

☐ Control Group – 48.3% correct responses

- The results suggest that the health benefits outreach flyer increased 10.2% of respondents' knowledge. While an increase in knowledge was achieved, the target was not met.

Goal 2 - Identify barriers to marketing and communication of Florida-grown peaches among growers and shippers.

- Performance Measure - Barriers to marketing and communication about Florida peaches in two audiences: growers, and shippers

- Benchmark – There was no initial knowledge of barriers for these groups, so the benchmark was 0.

- Target - Identification of at least one major and one minor barrier to marketing and communication about Florida peaches for each of the two audiences: growers and shippers.

- Result/Accomplishment -The interviews with the growers and shippers revealed the following barriers to marketing and communication

☐ Major barriers – Low retailer knowledge, Low consumer knowledge, Increasing volumes from other states

☐ Minor barriers – Damaged reputation of Florida peaches, consumer price sensitivity

Goal 3 - Encourage adoption of the developed marketing plan among Florida peach growers.

- Performance Measure - Percentage of growers and shippers who learn about the marketing plan express intent to adopt a component of the marketing plan within six months.

- Benchmark - The benchmark was 0% as no growers or shippers were aware of the marketing plan before its development.

- Target - 65% of growers and shippers who attend the Florida Peach Growers meeting will intend to adopt at least one component of the marketing plan within the six months following the meeting.

- Result/Accomplishment – When the marketing plan was presented to the advisory group of peach growers and shippers representing 85% of the Florida peach industry all (100%) took the marketing plan and/or requested an electronic copy of it. The feedback given from the growers and

shippers was very positive and they said things like “great work” and “great piece of work.” Additionally, 60% took extra copies of the outreach materials and/or requested electronic version of the outreach materials. These growers and shippers expressed intent to share the outreach materials during sales calls, at u-picks, on social media, and on their websites. The percentage that expressed vocal intent to utilize the materials fell short of the target by 5%. However, the requests for electronic versions of the marketing plan and outreach materials suggest that others planned to share the materials with others in their business. Additionally, actual use of the outreach materials has been seen on one prominent grower’s website as well as the new facebook page for the Florida peach industry, Florida Peaches.

Beneficiaries

Florida Peach Growers – Florida’s peach growers benefited from this project. Specifically, the 10-15 peach growers making up 85% of commercial peach acreage in Florida directly benefited. These growers were presented the results and given copies of the marketing plan and outreach materials at a face-to-face meeting to discuss the Florida peach industry. They were also sent the materials electronically following the meeting. Benefits to these growers included understanding consumers’ awareness and preference for Florida peaches, having a comprehensive marketing plan to guide their operation and industry, and having outreach materials (to increase awareness of Florida peaches) to share with retailers, consumers, and marketers. Implementation of the marketing plan and use of the outreach materials should also increase consumer awareness of Florida peach season and drive purchases of Florida peaches. This impact is unable to be assessed until after the 2018 Florida peach season. Assuming there is an impact, all Florida peach growers whether small acreage or commercial will benefit. Additionally, small acreage growers and other peach industry representatives (producers, shippers, retailers) would have received the project results if they subscribe to the Peach News.

Florida Extension Agents – Extension agents in Florida’s 67 counties benefited from this project by receiving the project materials to be used in programming. They were notified of the materials through an online in-service training. Additionally, each extension office was mailed a packet of outreach materials and the insert that ran in the Peach News to use in their programming. Extension agents will help to disseminate the project information in their work with grower and consumer clientele.

Consumers and Retailers – Consumers and retailers would have received the products of this project through dissemination by the peach growers themselves or extension agents. Consumers could benefit from the project by increase knowledge and access to Florida peaches. Retailers could benefit from new contracts, increased sales of Florida peaches, and awareness of product availability.

The economic impact of the Florida peach industry is more than $10 million dollars. Fresh peach consumption once topped out at 13 pounds per capita per year (Agricultural Marketing Resource Center, 2013), but now U.S. consumers only consume 3.7 pounds of fresh peaches per capita per year (Economic Research Service, 2012). The results of this project should allow for consumption and resulting economic contribution to be sustained and possibly even increase as a result of effective marketing and increased consumer awareness.

Lessons Learned

Problems or Delays

- The grant proposal outlined a mix mode survey of peach growers under objective 2. However, researchers were unable to gather a reliable list of peach producers and their contact information in order to distribute a survey. A mailing list was available, but the list was more than 3 years old and included many growers who were no longer growing peaches and did not include any new growers. The out-of-date list was not feasible for survey contacts. In addition, a list serve was made available to the researchers, but researchers were unable to access the information about who was included on the list serve. Due to these complications, the researchers opted to collect data via in-depth interviews from growers in the state accounting for more than 85% of Florida's commercial peach acreage.

- The grant proposal indicated that goal 1 would be measured in the consumer survey. However, the short nature of the online survey did not make it ideal to test knowledge gain. In addition, the researchers concluded that a knowledge gain assessment would be more beneficial once the outreach materials were created to see if those materials impact knowledge. Therefore, goal 1 was achieved with additional consumer testing in the evaluation.

- The Extension specialist specializing in stone fruits left the University of Florida. This person was regularly meeting with and organizing formal professional development meetings and field days with Florida peach growers.

- Due to the semester timing and student availability, both graduate students worked on this project Fall of 2016 and continued working on the project in Spring of 2017. The overlap did not impact the budgeted expenses associated with the students or the timeline of the project.

Project Goals Not Achieved

- Goal one was not achieved. Both measures of increased knowledge fell below 45%. However, an increase in knowledge was achieved. The knowledge increase target of 45% was likely high for the short amount of time respondents were exposed to the outreach materials.

Lessons Learned

- While this project has provided marketing recommendations and resources to the industry, that we hope will be successful in helping to advance the industry, we are unable to assess the impact of our efforts in the time allotted. A longer timeline is needed to be able to conduct the research, disseminate results, and then assess the impact of those results.

- Disseminating information to the industry in a small face to face setting is very effective. In the blueberry project that preceded this peach project we tried to disseminate the information to a large group of producers at a large blueberry event. We weren't able to interact with those producers, answer their questions, or talk about how they plan to use the information, or what other resources they need. We improved this process in this peach project because the small group setting allowed us to have those conversations and disseminate the information in a more intimate and less distractive environment.

- Providing hard copy and electronic files to the growers proved to benefical. They could follow along in the hard copies while we presented results. They also could take the outreach materials and

start using them right away without having to find the time or means to print them on their own. The electronic files allowed them to have the option to share the resources with others and to print additional outreach materials or to use the materials on their websites or social media.

Overall Progress and Funding Expended

The project is 100% complete, with $93,498.61 expended to date.

Project (32): Addressing Critical Needs for the Hydroponic and Protected Ag Industries

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Project Partners

Florida Specialty Crop Foundation, NFREC-Suwannee Valley and the GCREC-Balm.

Beneficiaries Advisory Committee consisting of Farmers (Scott DiMare, DiMare, Ruskin; Emil Belibasis, Beli Farms, Wellborn; Walter Ross, Farmhouse Tomatoes, Lake Worth; Fred Bacon Jr, Bacon's Select Produce, Jacksonville) and Industry Representatives (William Betha, Enza Zaden Seeds, Myakka City; Anna Coco, Double Thumb, Lakeland; Tim Carpenter, Vertigro, Summerfield; Glenn Kauffman, Paramount Seeds, Palm City; Frank Giglia, Signature Supply, Lakeland; Rod Heyerdahl, Johnny's Selected Seed, Winslow, Maine).

Project Summary

HOCHMUTH:

This project's objective was to provide new information to support the successful expansion of the protected agriculture and hydroponics industry which is one of the most rapidly growing segments of the commercial specialty crops industry. Florida has significant potential for increased hydroponic crop production due to the mild climate, long growing season and the proximity to large markets. With additional use of protected agriculture structures such as high tunnels, open shade structures, and greenhouses, crops can be protected from extremes in weather and the growing season for crop production extended. Protected agriculture and hydroponics has been a largely underserved segment of the Florida agriculture industry that has been rapidly expanding and needs new research based information to sustain steady growth. Thru University of Florida survey results in 2013 (Http://edis.ifas.ufl.edu/hs1240), the highest priority areas for information needs as voiced by growers were 1: new production information on the greenhouse environment including managing light and shading; 2: irrigation and nutrient management in soilless culture; 3: new and more adapted crop varieties and/or new crops that can expand the marketing opportunities and extend the growing season; 4: improved pest control using new IPM technologies, and 5: information on the economics of crop and enterprise management to assist in risk management decisions. We created project objectives from

these five researchable areas listed above that deal with information gaps that have been identified by growers to members of this project team over the year prior to this grant funding.

The project objectives are: 1) Evaluate leafy vegetable crops and varieties adapted for Florida. 2) Evaluate commercially available predators for suppression of key pests of tomato, pepper and cucurbits under high tunnel and screen house growing conditions. 3) Determine factors that will optimize protected agriculture environment and improve water and nutrient management. 4) Develop protected agriculture enterprise budgets. 5) Provide extension training and outreach.

Project Approach

HOCHMUTH:

As stated in the submission of this project grant, the Work Plan lists specific objectives to be achieved as a full commitment from each of the research partners. Of the five stated Objectives listed, "Objective 1 – Leafy vegetable variety evaluation and selection", "Objective 4 - Tasks for development of protected agriculture enterprise budgets, and "Objective 5 – Provide Extension training and outreach" represent the bulk of the activity for the staff and faculty at NFREC-Suwannee Valley.

Objective 1:

Leafy green vegetable crops were selected for trials in specific hydroponic systems under specific protected agriculture structures. Each leafy green vegetable was assigned a growing season, during which it was evaluated. An industry standard was included in each cultivar trial for comparison with new varieties within each trial. Faculty and staff at SVAEC initiated cultivar trials for the following leafy vegetables: 1) conducted summer trials to evaluate 3 leaf lettuce cultivars (2016), 5 romaine lettuce cultivars (2017) for adaptability to extreme heat in a greenhouse using NFT system and a nutrient solution chiller. 2) conducted an outdoor/open shade house vs high tunnel cultivar trial for Asian-style greens (2016). 3) conducted an outdoor/open shade house vs high tunnel cultivar trial for spinach (2016). 4) conducted fall cultivar trial for spinach (2016) and Asian-style greens under high tunnel (2017). 5) conducted fall arugula cultivar trial using vertical production system (Vertigro) under high tunnel (2017). 6) conducted spring/summer cultivar trial using open shade structure for kale (2016). 7) initiated late summer/fall red kale cultivar trial using open shade structure vs full sun (2017). 8) initiated late summer thru fall Swiss chard cultivar trial using open shade structure (2017) and 9) initiated summer arugula trials using NFT system in a vented greenhouse (2016).

For all trials, yield data were taken and recorded as well as quality ratings for characteristics such as: tip burn, chlorosis, bolting, pest susceptibility, and other parameters as needed depending on crop and variety. Yield and crop performance were compared between standard varieties and new varieties within each trial using SAS PROC GLIMMIX (SAS/STAT 14.2) conducted by Dr. Edzard van Santen, Director of the IFAS Statistical Consulting Unit, University of Florida.

Objective 2 (Co-PI Smith):

Evaluate commercially available predators for suppression of key pests of tomato, pepper and cucurbits under high tunnel and screen house conditions.

Task 1. Evaluate life cycle, fecundity, predation of silverleaf whitefly and dispersal capacity of Dicyphus hesperus on screen house and high tunnel tomato. The daily and lifelong consumption of whitefly eggs and nymphs by D. hesperus was evaluated. The difference in predation capacity of mated and unmated female D. hesperus was compared, as was the difference between D. hesperus directly shipped by the supplier and D. hesperus established on banker plants. Life cycle, survival and longevity of D. hesperus was evaluated on banker plants and on tomato with and with a protein supplement (Ephestia kuehniella eggs). Long term population growth of D hesperus on banker plants was evaluated in a commercial greenhouse and research greenhouse at GCREC. Impacts of conventional and biopesticides on survival of D. hesperus were evaluated. Multiple release rates of D. hesperus evaluated on greenhouse tomato.

Task 2. Evaluate life cycle, fecundity, predation of broad mite and dispersal capacity of Amblyseius swirskii on pepper.

Inundative releases of A. swirskii were evaluated for control of broad mite infesting pepper at a large commercial greenhouse in Wimauma. The role of banker plants and plant connectivity for dispersal of A. swirskii was evaluated. Survival of A. swirskii upon arrival from the producer was evaluated, as was toxicity of fenpyroximate, a common acaricide.

Task 3. Evaluate life cycle, fecundity, predation of thrips and dispersal capacity of Amblyseius californicus on cucumber.

Management of thrips species on greenhouse cucumber was evaluated at a commercial greenhouse in Wimauma.

Objective 3 (Co-PI Agehara):

This objective involved evaluations of 1) environment control by various protected agriculture systems (e.g. high tunnel, retractable-roof greenhouse, shading net), 2) water and nutrient management practices under commercial protected agriculture operations, and 3) effectiveness of water-absorbing and surfactant polymers in improving water and nutrient management.

Task 1. Monitoring environments under various protected agriculture systems

We installed weather stations to monitor the growing environments (light intensity, temperature, humidity) at three commercial farms (BRV Farm, Red Barn Hydroponic, Five-Star Family Growers). These farms had different protected structures with various environmental manipulation systems, such as ventilation, closed or retractable roof, plastic films, and shading nets. We monitored the environment data to evaluate the key environmental factors and to determine the optimal growing condition for leafy greens, tomato, and blueberry production.

Task 2. Evaluation of water and nutrient management under commercial protected agriculture operations

At the same commercial sites described above for Task 1, we installed sensors to monitor soil moisture and electrical conductivity (EC). We monitored changes in soil moisture and EC during the growing season to evaluate the efficiency of water and nutrient management practices used by the commercial growers and to determine the optimal management practice. In addition, we conducted two nutrient management trials at UF/IFAS Gulf Coast Research and Education Center (GCREC). We tested four different nitrogen rates for strawberry grown in a high tunnel to determine the optimal nitrogen fertilization rate. We installed a vertical stacking container system for strawberry and leafy greens, and monitored EC in each stacked pot to characterize the fertilizer distribution in the vertical growing system.

Task 3. Evaluation of water-absorbing and surfactant polymers

We tested one water surfactant product (Aquatrols), one water-absorbing polymer (Horta-Sorb), and one product with the dual effect (StockoSorb) in a high tunnel strawberry production system at UF/IFAS GCREC. We installed soil sensors to monitor soil moisture and EC throughout the growing season to determine the best approach for improving water and nutrient management and their effects on strawberry growth and production.

Objective 4:

Enterprise budget examples and budgeting literature were reviewed. Hydroponic vegetable budget templates were developed that can be used by growers to estimate costs and returns, and help inform decisions about production systems and practices, crop choices, and product pricing. The templates were created as interactive spreadsheets, in which growers can enter data for their operation and budget summary information and profitability analyses are calculated automatically.

Information was collected on greenhouse tomato production practices, costs, and potential returns. The approach included review of published production guidelines, research reports, and market price data, as well as interviews with university specialists, research staff, input suppliers, and growers. Using this information, a sample greenhouse tomato enterprise budget was prepared to represent Florida tomato production in a 30-foot by 96-foot fan and pad greenhouse. Sample budgets for other crops and systems are being developed using the same approach. These budgets will serve as a reference for growers, consultants, and lenders.

A workshop entitled Economics of Hydroponic Vegetable Production was planned and delivered on February 13, 2018. Workshop participants learned how to use the budget templates and were shown the sample budgets.

Objective 5: An Extension agent in-service training was held at the NFREC-Suwannee Valley. Twice as many attendees registered than initially expected/funded by this grant (36 participants, instead of the 20 expected). This 2-day training was modeled after our "How to Start a Successful Hydroponic Business" which addresses the needs of specialty crop growers. Additional funding, above the amount allotted from this grant, was provided by Nick T. Place, Ph. D. Dean and Director, Florida Cooperative Extension Service providing the opportunity for all 36 agents to attend. A pretest/posttest assessment showed a gain in knowledge for the agents of 34% (from 39% correct answer score prior to class to 73% after class).

A list of outreach and trainings sessions in which we have offered and shared information from the leafy greens portion of the grant which were offered one time included: "Mind Your Business; Hydroponics for profit", "Integrated Pest Management (IPM) for Hydroponics and Protected Agriculture", "Leafy Greens Field Day", "Greenhouse and Outdoor Hydroponics for Teachers", "Hydroponic In-Service Training for Agents"; those sessions offered twice included: "Hydroponic Growing for the Homeowner", "Microgreens 101", "Nutrient and Water Management for Protected Agriculture Systems", "Transplants Workshop", and those offered four times included our signature two day training event, "Starting a Successful Hydroponics Business". All sessions combined reached 470 agents, teachers, prospective and current growers in these face-to-face classes.

While leafy greens cultivar trials were underway during the two-year grant period of 2016 - 2017, the staff at NFREC- Suwannee Valley described the grant and shared preliminary and final observations from the leafy greens trials for several additional events and tour groups not specified in the grant plan of work. We described data from these trials to participants of our Twilight Field Day held on May 2, 2017 (over 150 in attendance), UF/IFAS Administrative Staff tour, the Associate Dean's tour, as well as groups visiting from other nations (Honduras, Haiti) during the trials. In total, over the two-year grant period, over 630 people have participated in educational events during 2016 thru 2017 at which this grant-supported information has been presented.

April 2016 was the official publication date of our baseline UF/IFAS Electronic Data Information Source (EDIS) document for leafy green vegetables in the state: "Leafy Greens in Hydroponics and Protected Culture for Florida" (http://edis.ifas.ufl.edu/hs1279). This document is currently in process of being updated with cultivar information and data gained during our leafy greens trials. In addition, our paper written on 'Yield, Market quality, and Bitterness of Lettuce Cultivars Grown Hydroponically in a North Florida Greenhouse during Summer' was completed and presented at the Florida State Horticultural Society Conference and will be published in the proceedings of the 2017 meeting. Four reports were also completed sharing the results of four of our leafy greens trials: 1) 'Evaluation of Five Cultivars of

Asian Greens Under an Open Shade Structure and High Tunnel Production Systems During the 2017 Spring Season in North Florida', 2) 'Evaluation of Eight Kale Cultivars Grown Under an Open Shaded Structure in North Florida', 3) 'Evaluation of Five Spinach Cultivars in Soilless Culture Under High Tunnel versus Open Shade Structures' 4) 'Performance of Romaine Lettuce Cultivars Grown Hydroponically in a North Florida Greenhouse During the Summer". All four reports were presented at the first Florida Protected Agriculture Conference Dec 5, 2017 in Balm, FL. In addition, an educational poster titled "Protected Ag and Hydroponic Leafy Green Vegetable Variety Trials in Florida" was developed from our leafy greens trial data and displayed during the educational poster session at the Southeast Regional Fruit & Vegetable Conference in Savannah, Ga in 2016 and 2017.

All of the educational information, publications, budgets, etc. produced as a result of this grant are posted on the UF/IFAS small farms web site (http://smallfarms.ifas.ufl.edu/).

Goals and Outcomes Achieved

HOCHMUTH:

Objective 1: Target - Conduct six cultivar trials typical Florida protected ag systems and identify at least the top four performing cultivars of lettuce, spinach and kale. At NFREC-Suwannee Valley, eight cultivar trials were initiated using five different hydroponic systems within three typical Florida protected agriculture systems. Four reports (Lettuce, Kale, Spinach and Asian Greens) have been completed which contain the growing methods and materials used in each trial with the results and top performing cultivars named. Outcome of our summer NFT lettuce trials included lettuce variety 'Salanova' from Johnny's Selected Seeds, which is was one of the top performers in our trials which 85 growers in Florida have adopted.

SMITH

Objective 2

Dicyphus hesperus females consumed on average 10 whitefly eggs per day and 83 per lifetime, and 93 nymphs per day and 835 per life time. There was not a significant difference in the number consumed by mated and unmated females. The predator lived on average for 10 days when provided only with whitefly eggs, and for 25 days when provided with whitefly nymphs. Dicyphus hesperus was unable to complete its development on tomato without prey, but could complete its development in the absence of prey on mullein (Verbascum thapsus) as a banker plant. Nymphal development time ranged from 22 to 26 days, with the shortest development time on mullein with E. kuehniella eggs and the longest development on mullein without a supplemental protein source. Development on tomato with E.

kuehniella eggs was intermediary. Dicyphus hesperus populations increased five fold over 14 weeks on tomato when E. kuehniella eggs were provided, 2.5 fold on mullein when E. kuenhiella eggs were provided, and remained constant on mullein (did not increase) if E. kuehniella eggs were not provided. Dicyphus hesperus was was compatible with some biopesticides, but was negatively impacted by Beauveria bassiana (entomopathogen) products.

Amblyseius swirskii provided effective control of broad mites on heavily infected pepper when released at the the rate of 100 + mites per plant. Dispersal was more influenced by plant connectivity than the presence of prey: mites moved 1 meter or more along teh row over 24 hrs even in the absence of prey.

Objective 3:

Outcomes:

A grower workshop on nutrient management in hydroponic systems was held at NFREC-Suwannee Valley on January 31, 2018. 25 participants attended the class. After the training, 92% indicated they intend to adopt practices learned during the training. Of those attending, 56% indicated they intended to start a hydroponic operation. The pre and post test scores showed an increase in knowledge gain from 44.5% to 80.0% as a result of participating in the day-long class.

Objective 3 (Co-PI Agehara):

• For tomato grown in the 50% coconut coir and 50% perlite mix, optimum soil moisture is 23 to 30%, and the optimum growth medium EC is 0.2-0.8 mS/cm.

• For blueberry production, shading factor should not be more than 20%.

• Advantages of growing blackberry under high tunnels include improved earliness, decreased fruit damage by rain, and increases in average berry weight, yield, and TSS.

• Major environmental changes by the retractable roof system compared to the ambient environment include increased air temperature by 2 - 2.5 F, reduced humidity by 1.4 to 2.5%, and reduced light intensity by 80 to 137 W/m2.

• In a vertical, hydroponic production system, some crops perform better at higher positions, while other crops perform better at lower positions. This variable growth is due mainly to different optimal growing conditions among different crops. For example, butter lettuce with very tender leaves prefer relatively low light intensity, so this crop performs better at lower positions with partial shading. By contrast, strawberry plants tend to grow better in higher pots, causing the medium moisture and EC to decrease faster compared to lower pots.

• The optimum nitrogen rate for high tunnel strawberry production is 2 lb/acre/d for the first 6-9 weeks and 0.75-1 lb/acre/d for the rest of the growing season.

• A grower workshop on nutrient management in hydroponic systems was held at NFREC-Suwannee Valley on January 31, 2018. 25 participants attended the class. After the training, 92% indicated they intend to adopt practices learned during the training. Of those attending, 56% indicated they intended to start a hydroponic operation. The pre and post test scores showed an increase in knowledge gain from 44.5% to 80.0% as a result of participating in the day-long class.

Objective 4: Target - Develop and publish one enterprise budget template that can be used for several specialty crops grown in protected agriculture and update greenhouse tomato budget.

Outcomes:

• A 2017 Hydroponic Budget Template (6-worksheet Excel file) and a 6-page instruction document were developed and published online on the NFREC-Suwannee Valley website: http://svaec.ifas.ufl.edu/featured-3-menus/extension/agricultural-economics/north-florida-enterprise-budgets/. This template was designed to allow growers to prepare enterprise budgets for up to 5 crops in a single greenhouse.

• A 2018 Hydroponic Budget Template (10-worksheet Excel file), Crop Comparison Tool (1-worksheet Excel file), and Hydroponic Fertilizer Calculator (2-worksheet Excel file). The 2018 Template is set up for one crop in one greenhouse, but provides additional analysis for decision making. The crop comparison tool is set up to enable quick comparisons of variable costs, fixed costs, contribution margins, and returns per square foot and square foot week across multiple crops. The fertilizer calculator is an optimization program designed to calculate the cost minimizing combination of different hydroponic fertilizers that will meet crop nutrient requirements. These tools were demonstrated and shared with participants at a workshop in February 2018.

• A Florida greenhouse tomato budget has been updated and will be published online on the NFREC-Suwannee Valley website: http://svaec.ifas.ufl.edu/featured-3-menus/extension/agricultural-economics/north-florida-enterprise-budgets/. An abstract title has been submitted to the Florida State Horticultural Society for an oral presentation at their 2018 annual conference. The title is Economic Analysis of Small-Scale Greenhouse Production in Florida.

Objective 5: Target -Sharing results of project with specialty crop growers and other interested specialty crop stakeholders. Extension agent in-service training was held with almost twice the allotted number of attendees. A pretest/posttest assessment showed a gain in knowledge for the agents of 33% (from 39% prior to class to 73% after class). The first Florida Protected Ag and Hydroponic Conference was held at the Gulf Coast Research and Education Center in Balm, FL with just over 100 attendees in which the results of our leafy greens trials were presented and reports included in conference materials that all attendees received. Information gained during the grant period was passed on to attendees of 20 programs given at the NFREC – Suwannee Valley during the grant period reaching over 600 people. The educational poster titled “Protected Ag and Hydroponic Leafy Green Vegetable Variety Trials in Florida” was developed from our leafy greens trial data and displayed during the educational poster session at the Southeast Regional Fruit & Vegetable Conference in Savannah, Ga in 2016 and 2017. This conference is attended by 3,000- 4,000 people annually. Further sharing of the results will be accomplished by

posting the information from this grant on the UF/IFAs small farms web site (http://smallfarms.ifas.ufl.edu/).

Outcomes:

• A new workshop entitled Economics of Hydroponic Vegetable Production was delivered in February 2018. Twelve prospective and beginning growers attended the workshop. On a scale of 1=no knowledge gained to 5=very much knowledge gained, the mean score reported for knowledge gained of workshop topics was 4.4. On a scale of 1=not likely to 5=very likely, the mean score reported for likelihood to use these business practices in the future was 4.8.

Beneficiaries

HOCHMUTH:

1- Current commercial specialty crop growers (3 acres +) in Florida using protected agriculture techniques and hydroponics including the 240 growers who responded to the 2013 UF survey. Currently, there are very few (less than a dozen) commercial protected ag vegetable operations over 3 acres in Florida. Three of these operations were engaged as stakeholder advisory committee members and two others were represented at the Protected ag conference as panel members. Others were made aware of the educational programs via direct mailing announcements.

2- The over 200 small to mid-sized hydroponic operations less than 3 acres most with less than ½ acre in size who sell mostly local or direct to consumer. This group represents the largest single known target group of stakeholders related to this grant. Of the 100 attendees at the statewide conference 30% were commercial growers and 25% perspective growers, from program surveys we feel this is representative of the attendees throughout the majority of programs offered. Altogether 570 attendees (programs and conference) there were approx. 170 commercial growers and approx. 140 perspective growers reached in this stakeholder category. The 100 attendees at the statewide conference met to discuss the merits of forming a commodity association to represent their needs. As a result, the initial plans are being developed to form a Florida Protected Ag Association in 2018. Members from the industry (growers and allied industry) have volunteered to form the board of directors of this association and move forward with the formal development. All attendees of the face-to-face trainings (470) and the statewide conference (100) received curriculum notebooks at each event.

3- Prospective growers – workshops targeting prospective growers were well attended reaching 470 attendees over the two-year period statewide, with at least 55% of attendees in the grower/prospective grower category. The UF/IFAS County Extension Agent in-service training was a strategical deliverable to create a better trained force of Extension agents statewide. These county faculty are often the first contact of a prospective grower. The agent IST will help improve the future educational experiences of prospective growers. The formation of a new association, Florida Protected Ag Association, will also help prospective growers find a conduit to information.

4- Consumers, local food groups, Farm to School, Farm to Institution, and community farmers' markets. We included educational information via our statewide conference by having Beth Spratt, Child Nutrition Specialist on the topic of Farm-to-school from FDACS. The Extension Agent IST also included several agents (6-8) who work specifically with Farm-to-school audiences.

Lessons Learned

HOCHMUTH:

This project provided the opportunity for our team to evaluate several crops, cropping systems, and various protected ag structures. We learned the importance of having a variety of structures to successfully grow specialty crops year-round. High tunnels were superior to open shade structures for yield and quality of spinach. In addition, high tunnels reduce insect and disease pressure significantly, thereby reducing the need for certain pesticide applications. After several summer lettuce trials, we learned we can grow high quality lettuce in NFT systems as long as we choose the correct varieties and use a nutrient solution chiller to keep the solution temperatures no higher than the low 80s (degrees F). Protected ag can extend the marketing season for specialty crop farmers in Florida. This helps them access and retain new markets. Farm-to-School and Farm-to-Institution sales are new untapped opportunities for this audience.

Many lettuce variety and production trials were conducted during this project. During the grant period, we learned we could, in fact, grow lettuce year-round with the proper varieties and production systems, such as NFT with a nutrient solution chiller. Leaf lettuce cultivar trials during the summer of 2016 resulted in almost no marketable heads, mainly due to bolting and tip burn. We also learned lettuces have a high potassium demand in the summer and frequent nutrient adjustments are necessary. The fall season of 2017 proved to be quite a challenge after hurricane Irma damaged many protected ag businesses in Florida, including minor damage at the two UF Centers in this trial.

Arugula trials were initiated in the summer of 2016 using NFT system. This trial was to provide full sized leaf arugula, however, we learned that arugula is not well adapted to high temperatures in a greenhouse in the summer in Florida when growing in an NFT production system. An arugula trial was also conducted in the Vertigro system in a high tunnel. The soilless media, coco fiber/perlite mix, in the vertical towers was staying too wet leading to plants with iron chlorosis within the lower pots in the vertical towers. Arugula plants in the top pot were fine but chlorosis appeared greater in the lower pots as they retained more water. Arugula appears to be a difficult crop to grow in certain systems and seasons, as it is sensitive to pH shifts and heat (in NFT) as well as excessive moisture in the growing media and extreme cool temperatures. Further research is warranted with growing arugula year-round in protected ag.

We learned that when we develop cropping systems for year-round production of specialty crops, we will likely encounter pest populations not normally seen when growing only during certain seasons. Two pests in particular that were more problematic than expected were broad mites and armyworms (under open shade). Both of these pests were more challenging during the summer and early fall season. We learned that early detection is critical in managing these two pests. Research conducted at the UF/IFAS

GCREC and the written fact sheets will help growers in the future and will help Extension teach these principles of integrated pest management.

From the updated tomato budget and other budgets under development we learned that achieving a positive net return from small-scale greenhouse vegetable production is challenging. Accessing higher value markets and finding production efficiencies are critical for success.

Co-PI Agehara

On Sep 10 and 11, 2017, Hurricane Irma severely damaged high tunnels at UF/GCREC, where nutrient management trials were being set up. Because the structural damage on the tunnels was too great to be repaired, we were not be able to conduct nutrient management trials in high tunnels in 2017. Instead, we installed a vertical stacking container system in a net house at GCREC. We conducted nutrient management trials for strawberry and leafy greens using the vertical growing system.

Co-PI Smith

Commercially available predatory mites continue to be effective tools for protected ag growers for control of key pests of horticultural crops, namely whiteflies, thrips and mites. Inundative releases are required, so questions regarding the disperive ability of mites and other predators are not the most relevant. The biocontrol agents must be released at high numbers and frequently to work. Our evaluations of Dicyphus hesperus, which is an insect and not a mite, have confirmed that it can contribute to management of whiteflies under Florida's subtropical conditions, but cannot necessarily control high populations.

Total Funding Expended: $133,742.29

Percentage of Project Completed: 100

Project (33): Disease management strategies for Florida's emerging pomegranate industry

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Project Partners

Florida Pomegranate Association; Georgia Pomegranate Association; Florida Department of Agriculture & Consumer Services and Florida Specialty Crop Foundation

Project Summary

Driven by consumer demand for fruit and juice with high nutritional value, and grower desire for an alternative fruit crop, there is an increased interest in commercial pomegranate production in Florida. Currently, California is the only state with large commercial acreage and is the primary supplier of pomegranate in the U.S. A previous SCPBG project found that Florida's climatic conditions favor pomegranate production; however, fruit yields and fruit quality are severely compromised by disease. A survey of pomegranate orchards in Florida identified various diseases, including several fungal fruit rots that could cause nearly complete fruit loss in orchards. Differences in susceptibility to fruit rots were also observed among pomegranate varieties. The previous SCPBG project identified disease management and varietal improvement as key needs for developing an economically viable pomegranate industry in Florida. The lack of epidemiological information restricts the development of effective disease management strategies and even with proper cultural and pesticide controls, an economically viable pomegranate industry in Florida may be limited without developing better adapted, resistant pomegranate varieties. The overall goal of this SCPBG project is to understand the epidemiology of fruit rot pathogens affecting pomegranate, develop integrated approaches to manage diseases, and continue efforts to screen available pomegranate germplasm and breeding lines for resistance to fruit rots.

Project Approach

Objective 1a. Understand the pattern of latent infection by identified pathogens on flower buds and conditions favoring disease development.

We sampled flower buds, flowers, and fruit at different stages of development throughout three growing seasons (2015 to 2017). In 2015, buds were collected from late January through March from three locations in Florida and were divided into six different stages from an unopened flower bud to fruit initials. Three orchards in Central Florida were also monitored every other week from late February to August and samples were collected to monitor pathogen prevalence. Several pathogens were recovered in the absence of any symptoms during bud stages to include Colletotrichum spp., a novel Diaporthales species and two Botryosphaeriacea. In 2016, almost 600 asymptomatic flower buds (at several stages) were collected in multiple locations in Florida. Our results from 2016 showed that less than 20% of the flower buds were not harboring any fungal species, while 80% of the flower buds harbored several fungal species such as, Alternaria sp. a novel Diaporthales species, Aspergillus sp., Botryosphaeriacea, Colletotrichum spp., and Pestalatiopsis sp. Again, in 2017, over one hundred asymptomatic flower buds were collected from two sites in Plant City, FL (var. 'Angel Red') and Parrish, FL (vars. 'Christina', 'Wonderful', 'Azadi', 'Vikusnyi', 'Alsirinnar' and 'Sakerdze'). Fungal isolates were recovered from over 95% of the asymptomatic flower buds sampled. More than 50% of the asymptomatic flower buds collected from April to May were infested with a Chaetomium sp. The second most common fungus on the flower buds was Pestalatiopsis sp. The third was Botryosphaeria sp. Colletotrichum and the same novel Diaporthales species was also recovered from flower buds. These results showed that most pathogenic fungi are already present in almost 100% of the asymptomatic flower buds early in the season, which suggests the need to apply fungicides preventatively at prior and during flower bloom for disease management.

Objective 1b. Quantify seasonal dispersal patterns of important pathogen(s).

Spore traps were used to sample and collect spores during two growing seasons. However, several attempts to detect pathogens were unsuccessful. While the presence of certain pathogens could be confirmed, DNA quality was not adequate to perform quantitative real time PCR or to obtain consistent results from environmental samples. So, this sub-objective was abandoned, and efforts refocused on the recovery of pathogens from flower blooms based on results from Objective 1a and conducting preventative at-bloom fungicide trials at additional grower locations.

Objective 2a. Determine fungicide sensitivities of pathogen populations.

The sensitivity of Neofusicoccum parvum, Lasiodiplodia theobromae, and two Colletotrichum spp. to fungicides pyraclostrobin, tebuconazole, thiophanate methyl, pyrimethanil, mancozeb, boscalid, and copper sulfate was determined based on in vitro, mycelial growth assays. Two Colletotrichum spp. and N. parvum were sensitive to pyraclostrobin with an average EC50 value of 0.66 µg/ml. Initial assays

found L. theobromae were insensitive to pyraclostrobin (average EC50 > 100 µg/ml), but the addition of SHAM significantly ($P < 0.001$) increased sensitivity to an average EC50 value of 0.19 µg/ml. All species, except Colletotrichum spp. (EC50 for thiophanate methyl, pyrimethanil and mancozeb > 50 µg/ml), were sensitive to tebuconazole, thiophanate methyl, pyrimethanil, and mancozeb. None of the tested species were sensitive to boscalid or copper compound (EC50 > 100). This study provides a preliminary assessment for the effectiveness of different fungicides to the major fungal pathogens of pomegranate in Florida.

Objective 2b. Test and demonstrate pesticide programs for managing diseases.

In 2015, field trials conducted at two different locations in Central Florida evaluated Cabrio, Switch, Scala, Penncozeb, and Luna Experience for disease control on pomegranate. Fungicides were applied beginning in Early-March when > 5 % flower buds were visible and reapplied every two weeks until the end of growing season in August. Significant differences were observed between the treatments at both locations ($P < 0.001$). Cabrio, Luna Experience, and Penncozeb reduced the disease severity by 87, 84, and 67 % respectively; while Switch and Scala reduced by 53 and 39% respectively compared to untreated control.

Findings from our spring surveys determined that pathogens, such as Colletotrichum spp., could frequently be recovered from flower buds (objective 1a) suggesting further improvement in disease control by properly timing fungicide application with flowering. Thus, in 2016, we tested efficacy and timing of application of Luna Experience, Merivon and a rotational program including Merivon, Luna Experience and Penncozeb at three FL sites, Dover, Parrish and Plant City. Disease severity ranged from 7.5 to 45.8% in Dover, 4.5 to 62.5% in Plant City, and 4.5 to 30.8% in Parrish based on the final rating in late August. Based on AUDPC, all treatments that included at least one application at-bloom statistically reduced disease severity compared to post-bloom applications and the non-treated control across all trial sites, regardless of application rate. Two at-bloom applications of Merivon, at a low 7 fl oz/A rate reduced the final disease severity compared to the non-treated control by 55.8%, 42.4% and 89.2% in Dover, Parrish and Plant City, respectively. Two at-bloom applications of Luna Experience at the high 17 oz/A rate reduced the final disease severity compared to the non-treated control by 52.3%, 64.8% and 62% in Dover, Parrish and Plant City, respectively. The rotational program applied at-bloom reduced the final disease severity compared to the non-treated control by 64.2%, 50.3% and 87.5% in Dover, Parrish and Plant City, respectively. At all trial sites, two at-bloom applications were statistically equivalent to a seasonal program, consisting of four applications every six weeks beginning at bloom, of either Merivon or Luna Experience. The integrated use of cultural practices and effective fungicides offers growers a viable means to manage seasonal outbreaks of anthracnose on pomegranate.

In 2017, several biopesticides and conventional fungicides (systemic and contact) were evaluated for efficacy against leaf spot and fruit rot on pomegranate. Fungicide timing and rotational programs that used conventional contact fungicides were also evaluated in separate trials. Replicated trials were performed in Plant City, FL (var. ‘Angel Red’) and Parrish, FL (vars. ‘Christina’ and ‘Wonderful’). The

rotational programs included (i) Merivon, Captan and Topsin; (ii) Merivon, Captan and Merivon; and (iii) Merivon, Topsin and Merivon. Treatments for timing trials included Serenade (Bacillus subtilis), Tenet WP (Trichoderma spp.), Merivon (fluxapyroxad + pyraclostrobin), Topsin (thiophanate-methyl), Penncozeb (mancozeb), Captan and a non-treated control. Studies conducted in 2016 demonstrated the efficacy of the fungicides to manage fruit rot when applied twice at bloom. Our results showed that all fungicides tested had a significant effect at reducing foliar disease severity in comparison to the non-treated control. Based on AUDPC, two at-bloom applications of Merivon and Topsin (systemic conventional fungicides) and Captan and Penncozeb (contact conventional fungicides) were significantly more effective than Tenet and Serenade (biopesticides), except at the Parrish site where Captan was statistically equivalent to Serenade. Systemic and contact fungicides were statistically equivalent when applied twice at-bloom. Two Merivon applications at-bloom followed by three additional applications of either contact fungicide had a significant effect based on AUDPC compared to the non-treated control or a season-long program of neem oil applications (beginning at bloom). Based on the AUDPC, all three rotational programs that included contact fungicides significantly reduced the levels of leaf spot and fruit rot at both sites. The rotational programs were equivalent to either Merivon, Pencozeb, Captan, or Topsin applied alone at-bloom. Regardless of the benefit of preventative fungicide applications for managing foliar disease and the initial incidence of fruit rot during the season, fruit losses at the end of the season still exceeded 60% to even 100% across many trials. However, these losses coincide with heavy periods of seasonal summer rains and may be directly related to physiological stress (extended periods of saturated soils) that lead to fruit cracking than to disease alone.

Objective 2c. Evaluate pomegranate germplasm and breeding lines for disease resistance.

Forty-four pomegranate varieties were evaluated in 2016 for susceptibility to leaf spots under natural disease pressure in the pomegranate orchard at the Gulf Coast Research and Education Center. Disease severity, as an average percentage of diseased leaves, increased rapidly from 31.6% on July 29 to 67.1% on September 9 across all varieties. Most of the varieties were highly susceptible to leaf spots, with disease severity ranging from 50% to 91%. Two varieties, Arakta and Cedar Key Sunset, exhibited a good level of resistance to leaf spots with disease severity below 12% across all evaluation periods. Another six varieties exhibited a moderate level of resistance to leaf spots.

In 2017, leaf spots began to appear in mid-June; about one to two weeks after the initial summer rain season began. By the end of September, more than 70% of the leaves on pomegranate trees showed disease symptoms, and some of the trees were almost completely defoliated. Arakta and Cedar Key Sunset still exhibited high levels of resistance to leaf spots with disease severity less than 8%.

A collection of 44 pomegranate varieties were evaluated for growth characteristics during the 2016-2017 overwintering period. Three varieties remained evergreen, one variety was semi-evergreen, and the remaining forty varieties were essentially deciduous. Leaf spots were present on overwintering green leaves and are likely an important source of inoculum for the 2017 growing season.

A total of 872 breeding lines derived from nine different breeding populations were evaluated for susceptibility to leaf spots; 87 of the breeding lines showed moderate levels of resistance to leaf spots, and two lines showed good levels of resistance to leaf spots. We also evaluated nearly 2,000 breeding lines for their overwinter growth habit and found out that the evergreen habit is dominant over the deciduous habit. When one of the parents is evergreen, progeny are evergreen to semi-evergreen. Evergreen varieties seem to be more common in pomegranate varieties originating from India than those pomegranate varieties originating from the Middle East.

Objective 2d. Address the survival of pathogens in leaf litters and fruit residues.

A survey of pathogens recovered from leaves that remained on trees during the overwintering period was completed, as well as pomegranate debris, such as leaf litter, mummified fruits and dead branches. Our results showed a high success in the recovery of pathogenic pathogens from symptomatic tissues, with high levels of recovery for Pilidiella granati, Colletotrichum spp. as well as the novel Diaporthales species. The presence of these pathogens indicates that crop debris is a likely source of inoculum for subsequent seasons.

Objective 2e. Identify host range of Colletotrichum spp.

Multilocus sequence analyses showed that isolates from pomegranate grouped within C. acutatum and C. gloeosporioides species complexes. Representative isolates from the two species complexes were selected to test their aggressiveness on pomegranate fruits and cuttings (spray inoculation). The potential host range was also evaluated by inoculating these isolates onto strawberry, blueberry, citrus and mango. Results from fruit inoculation showed that all C. acutatum isolates were more aggressive than the ones from C. gloeosporioides on pomegranate fruits. However, C. gloeosporioides isolates were overall more aggressive than C. acutatum when inoculated on the leaves. Isolates from pomegranate were able to infect blueberry, strawberry, citrus and mango, and vice-versa, which can complicate disease management and breeding efforts. Thus, the presence of other susceptible fruit crops in the vicinity of pomegranate orchards can pose a potential source of inoculum to pomegranate fruits.

Objective 3a and 3b. Extension meetings and other industry venues and Field tours.

Much of the research described was conducted at three grower field sites in Dover, Plant City and Parrish, Florida; in addition to studies conducted at GCREC in Balm, Florida. Grower meetings were held in Plant City (2 meetings), Zolfo Springs (2 meetings), Gainesville (1 meeting) and at GCREC in Balm (4

meetings) in conjunction with the Florida Pomegranate Association. Meetings covered topics such as pruning pomegranate trees for production and disease control; how to recognize and diagnose pomegranate diseases, and implementing integrated disease management strategies. Research findings were disseminated at all meetings, which were highly attended by growers and other industry stakeholders. In addition, field tours of the GCREC pomegranate orchard were provided during the Florida Pomegranate Association (FPA) growers meetings, FPA business meetings, the FL AgExpo meetings, and other meetings, which gave participants an opportunity to visit pomegranate varieties and breeding materials in the field and to learn about disease management strategies.

Objective 3c. We have submitted "Diseases of Pomegranate (Punica granatum) in Florida" to EDIS.

We have three manuscripts in preparation for the field data 2016 and 2017, as well as the characterization of Colletotrichum isolates across the Southeast.

Goals and Outcomes Achieved

Goal 1: Identify the most vulnerable flower and fruit stages and relate the information to the timing and amount of spore production of economically important pathogens.

Target: Proper timing of protective fungicide applications will be determined thus reducing infection and fruit losses throughout the production season. The information will also be used for developing future disease forecasting models to further refine the timing of fungicide applications.

Outcomes achieved: We collected more than 700 flower and fruit samples at six different developmental stages throughout three growing seasons and tested for the frequency and incidence of multiple fungal species, such as, Alternaria sp. Diaporthales, Aspergillus sp., two fungi from the Botryosphaeriacea family, Colletotrichum spp., Chaetomium sp., Pestalatiopsis sp, an unknow fungus of the order Diaporthales. Fungal isolates were recovered from 80% and 95% of the asymptomatic flower buds sampled in 2016 and 2017, respectively. These results showed that most pathogenic fungi are already present in the asymptomatic flower buds early in the season, which suggests the need for pre-bloom and bloom fungicide applications for disease management. We deployed three spore traps at 2 locations in Parrish and Plant City in Florida to collect aerial spores and quantify spores via conventional laboratory methods and quantitative PCR techniques. However, results were inconclusive and inconsistent, likely due to the inability to consistently extract high quality DNA from these dirty environmental samples.

Goal 2: Develop integrated statewide recommendations to improve disease management.

Target: Based on testing, we will partner with other states and fungicide companies to nominate at least three promising fungicides for registration through the IR-4 program. We will determine the importance of infected plant debris and other host plants as a source of inoculum and will identify specific sources of resistance/tolerance to each pathogen.

Outcomes achieved: We determined pathogen sensitivities (N. parvum, L. theobromae, and two Colletotrichum sp.) to the fungicides pyraclostrobin, tebuconazole, thiophanate methyl, pyrimethanil, mancozeb, boscalid, and copper sulfate in vitro. We also evaluated a series of fungicides (Cabrio, Switch, Scala, Penncozeb, Luna Experience, Merivon, Serenade, Tenet WP, Topsin, and Captan) alone or in a rotational program (Merivon, Luna Experience and Penncozeb, or Merivon in rotation with either biopesticides or conventional fungicides) in field trials during three growing seasons at different locations in FL. We determined the importance of infected plant debris by recovering important pomegranate fungal pathogens from leaf litter, fruit residues and dead twigs. Fruit crops such as, strawberry, blueberry, mango and citrus were identified as a potential source of Colletototrichum spp.

Eight pomegranate varieties were identified having moderate to high levels of resistance to leaf spots under Florida's subtropical climatic conditions. Two breeding lines with good levels of leaf spot resistance were identified. These varieties and breeding lines will be valuable as an important tool for management of leaf spot disease in pomegranate orchards. Working with Georgia, Alabama, South Carolina the fungicides Merivon (pyraclostrobin + fluxapyroxad), Luna Experience (fluopyram and tebuconazole) and Penncozeb (mancozeb) were nominated for residue testing at the 2015 Food Use Workshop IR-4. Residue testing was conducted and completed for Merivon in 2016 and labelling by BASF is in progress.

Goal 3: Dissemination of research findings and recommendations to pomegranate growers and other specialty crop stakeholders.

Target: We will disseminate information on proper cultural practices, sanitation, fungicide application, and varietal selection towards integrated disease management to pomegranate growers. This will also increase involvement and interest of new and potential growers beside pomegranate industry.

Outcomes achieved: In collaboration with the Florida Pomegranate Association, 10 grower meetings were conducted to disseminate research findings and give training in pomegranate management, disease recognition and diagnosis, and integrated disease management strategies. In addition, field tours of the GCREC pomegranate orchard were provided during FL AgExpo meetings in 2016 and 2017. An EDIS article titled "Diseases of Pomegranate (Punica granatum) in Florida" was submitted to EDIS and two manuscripts to the International Symposium on Pomegranate and Minor Mediterranean Fruits.

Beneficiaries

An estimated 500 growers and other interested parties have benefited from this research. In 2016, field trials were conducted at 3 grower field sites, Dover, Plant City and Parrish; growers and other industry representatives were given an opportunity to visit the sites. A field tour of the GCREC pomegranate orchard was provided during the annual Florida Pomegranate Association meeting in October, which gave participants an opportunity to visit the pomegranate varieties and breeding materials in the field.

In 2017, research was conducted at two grower field sites, Plant City and Parrish (12 beneficiaries). Two meetings of the Florida Pomegranate Growers Association were held in Plant City (estimated 35 and 40 attendees). One meeting had hands-on instruction for growers on pruning pomegranate trees; the second taught growers how to diagnose several diseases on the fruits and leaves and how to implement integrated management strategies to reduce foliar disease in the orchards. Research findings were disseminated through annual and summer meetings of the Florida Pomegranate Association. All meetings were highly attended by growers and other industry stakeholders. In addition, a field tours of the GCREC pomegranate orchard was provided during the AgExpo meeting in November of 2016 (50 attendees) and 2017 (45 attendees), which gave participants an opportunity to visit pomegranate varieties and breeding lines at GCREC and to learn about the efficacy of several fungicides tested in our research trials and varietal differences in susceptibility to leaf spot diseases.

Lessons Learned

In September 2016, Dr. Achala Nepal departed UF for a permanent faculty position with Oregon State University. A new Post-Doctoral Scholar, Dr. Katia Xavier was hired, and just started with UF on December 12, 2016. Dr. Nepal's departure delayed some of our studies to assess pathogen survival in plant debris over the fall. In addition, it took longer to receive the spore traps purchased to examine seasonal dispersal patterns per objective 1 in 2015. We were still able to collect spore dispersal data for the 2016 and 2017 field trial. These slight setbacks forced us to ask for an extension to the project. In addition, our efforts to quantify spores collected with spore traps was complicated by technical difficulties of separating and identifying spores from dust, pollen and insect debris present in the traps. Another technical difficulty we had was to consistently extract high-quality DNA for Real-time PCR. While these technical difficulties limited our progress to assess seasonal fluctuations with the spore traps, our efforts to further evaluate survival of pathogens on trees and tree litter will likely be more relevant to the project, since splash dispersal is likely more important for the long-term management of these pathogens in pomegranate orchards. In 2017 and early 2018, the frequent recovery of Colletotrichum spp., P. granati and the novel Diaporthales from the crop debris confirmed the importance of orchard sanitation to Pomegranate production in Florida. Multilocus sequence analyses determined that Colletotrichum isolates from pomegranate grouped within the C. acutatum and C. gloeosporioides species complexes, and were also cross-pathogenic with other fruit crop isolates.

Trials demonstrated that pomegranate varieties vary in susceptibility to leaf spot diseases and that it is feasible to identify varieties with useful levels of resistance to leaf spot diseases. The identified resistance can be transmitted from pomegranate varieties to progeny to develop new breeding

materials. These results suggest an opportunity to develop new pomegranate varieties with genetic resistance to major diseases faced by Florida pomegranate growers.

Total Funding Expended: $187,787.45

Percentage of Project Completed: 100

FL Specialty Crop Grant Recipient**: Florida Agricultural and Mechanical University/Center for Viticulture and Small Fruit Research**

Final Performance Report

Project (34): **Florida *Eutypa* Dieback Vine Diagnostic and Prevention Program**

Contract Number: **0022900**

Specialty Crop Block Grant Funding Year: **2016**

Project Summary

Diseases can be transmitted from the mother vine to planting stock, if the materials are not properly screened for pathogen infections. Our southern grapes including muscadines, although considered tolerant to some economically important diseases (Pierce's disease and anthracnose) are no exception in regards of disease transmitted with the planting stock like *Eutypa*, viruses and crown gall. Between them, *Eutypa* dieback is the most broadly spread and detrimental to the industry. Dieback symptoms (pathogen: *Eutypa lata*, *E. leptoplaca*, and other fungi in the Diatrypaceae family) are not generally visible in vines younger than 5 to 6 years old although vines may still be infected. The fungus survives in diseased wood and produces perithecia in old, infected host tissue under conditions of high moisture. Ascopores are discharged from perithecia soon after rainfall. Infection occurs through pruning wounds, which remain susceptible much longer early in the dormant season than later in the dormant season (UC, Davis, CA: Pest Management Guidelines http://www.ipm.ucdavis.edu/PMG/r302100611.html).

The rapid expansion of wine industry in Florida and southeastern U.S. already predispose the viticulture enterprise to several disease problems. This has resulted in a growing number of muscadine vineyards being affected with *Eutypa*, after the third year of growth. The disease is usually spread by contaminated planting materials without knowledge of the growers. This situation, in addition to the naturally very high disease pressure (PD's and fungal diseases) if allowed to continue will have serious repercussions for the grape and wine industry in the region.

The ability to detect and diagnose disease such as *Eutypa* dieback early and sensitively is the cornerstone for effective prevention strategy. Our objectives were to scout for *Eutypa* dieback disease in muscadine grapes from Florida vineyards. Symptoms can appear on cordons, dead spurs, and arms leading vine death due to impaired water translocation through the vascular tissues (Figure 1 and 2).

Fig 1. Muscadine var. 'Noble' showing symptoms of *Eutypa* dieback disease

Fig. 2. Grapevine samples originated from vineyard at the Center for Viticulture and Small Fruit and Research, Florida A&M University. (A) Muscadine grape var. 'Supreme' (foreground) showing stunted growth and distorted leaves. (B) Cross-sectional cut showing wedge-shaped discoloration associated with *Eutypa* dieback.

Project Approach

Our project was focused on the collection of scientific data and developing molecular-based tools for early detection of *Eutypa* Dieback disease.

We targeted the developing of a rapid highly specific early monitoring program for Eutypa dieback in muscadine grapes using molecular diagnostics (ELISA and PCR based tests). Our approach aimed to facilitate the detection of pathogen infections before symptoms are revealed by:

1/ Developing a rapid highly specific early monitoring program for Eutypa dieback in muscadine grapes using molecular diagnostics;

2/ Conducting research on diagnostic for Eutypa in muscadine and Florida hybrid bunch grape varieties;

3/ Training growers, extension personnel and developing community of practice (CoP) for disease prevention and treatment of Florida grapes.

Early monitoring of fungal diseases in grapevine is crucial for the survival and sustainability of the growth of the viticulture in Florida. Diseases can be transmitted from the mother vine to planting materials, if not properly screened for pathogen infections. PCR-based tests were employed for this purpose due to their specificity and simplicity. The success of the program rested on our designation as one of the five National Clean Plant Centers for Grape, which serves the very challenging region for grape growing in the southern U.S. Furthermore, our experience and specialization in working with the American native grapes necessitated us to develop *Eutypa* diagnostic and prevention program in Florida. As a result, it improved our ability to carry out disease diagnostic on "non *vinifera*" grapes.

Goals and Outcomes Achieved

1. To develop a rapid highly specific early monitoring program for *Eutypa* dieback in muscadine grapes using molecular diagnostics;

We developed a molecular-based test to conduct early detection of *Eutypa* dieback, which is one of the economically important diseases in Florida vineyards. Also, we used microscopic analysis to identify and characterize the fungus causing *Eutypa* dieback disease in southern grapes. Microscopic analysis determined that fungal colonies growing on potato dextrose agar had acervuli that developed from a yellow mass of hyphae and originated from big masses of black spores. In addition, each colony showed a slight pigmentation with gray concentric rings, typical of *Pestalotiopsis* species. Under light microscope we observed bi-setulate, 5-celled conidia (3 colored median and 2 hyaline end cells) with three olivaceous-brown median cells (distoseptate) from the genus *Pestalotiopsis* from grapevine samples (Figure 3).Our results have been presented at the 2016 FWGGA conference.

2. To conduct research on diagnostic for *Eutypa* in muscadine and Florida hybrid bunch grape varieties;

A total of 85 plants were initially tested for fungal species causing *Eutypa* dieback. The study identified five different fungal species (*Botryosphaeria* sp., *Colletotrichum* sp, *Greeneria* sp, *Pestalotiopsis* sp., and *Phomopsis* sp.) (see Figure 3a and 3b). All suspected vines were marked and discarded.

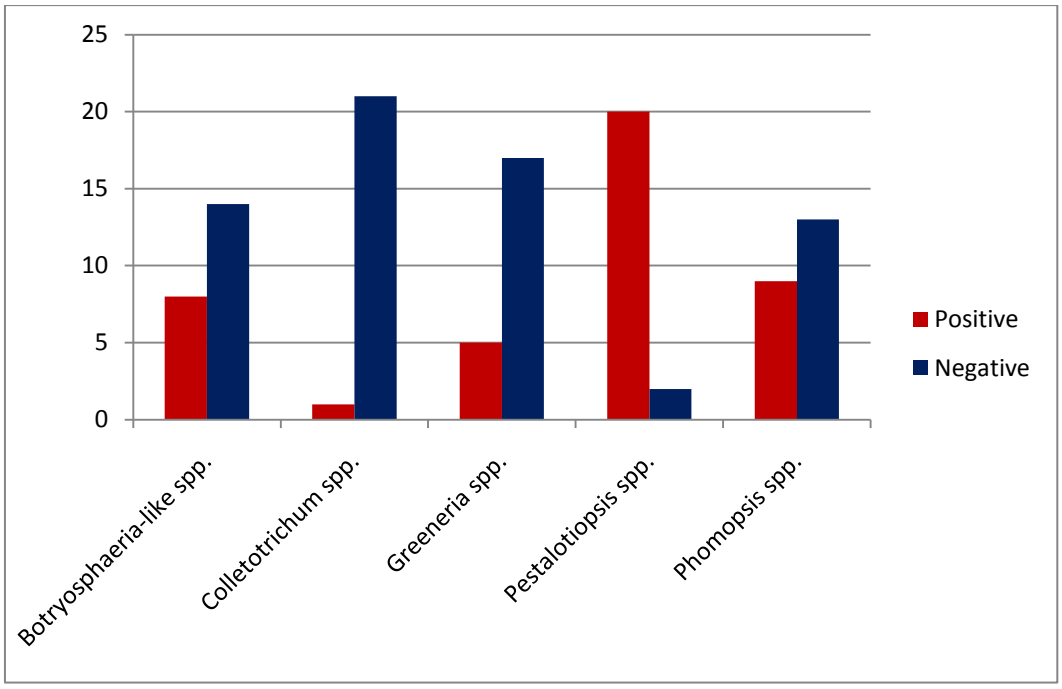

Fig. 3a. Fungi detected from vine samples collected from muscadine grapes

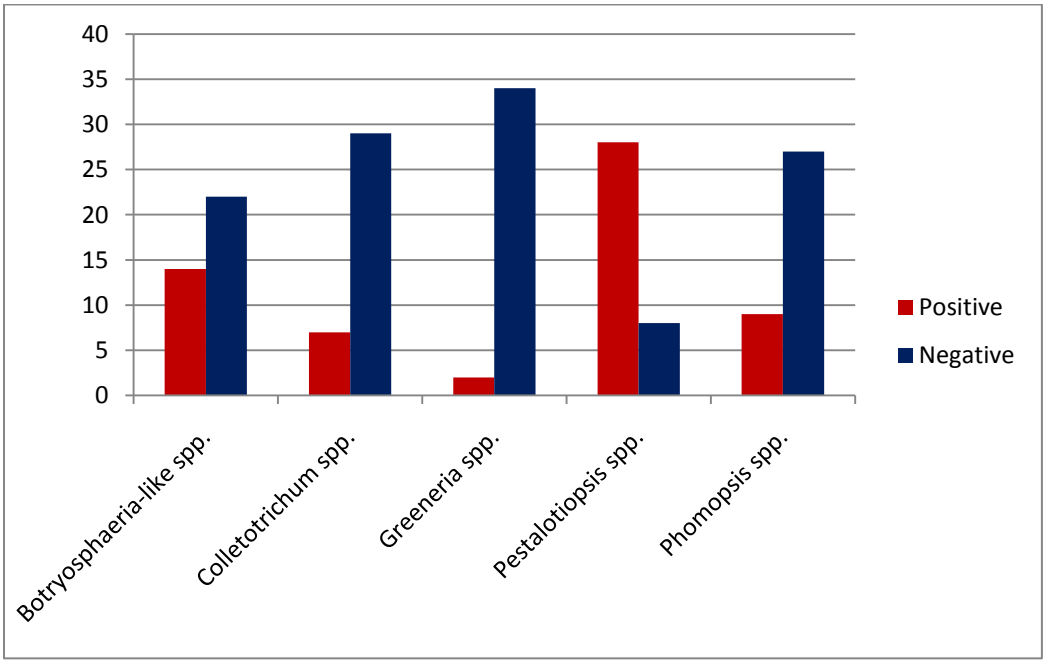

Fig. 3b. Fungi detected from tested vine samples collected from American native hybrid grapes

Also, we developed a rapid diagnostic of *Eutypa* dieback from muscadine and American native hybrids grapes using molecular-based tools. Through the project, we identified a set of primers (ITS1-F and ITS4-R) that targeted the internal transcribed spacer (ITS) region between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA genes. These set primers have been used to test grapevine samples collected from Florida vineyards to determine the extent of

Eutypa dieback infestation in the region. Fungal strains were isolated from infected vines that showed wedge-shaped discoloration on the xylem tissues (Figure 4).

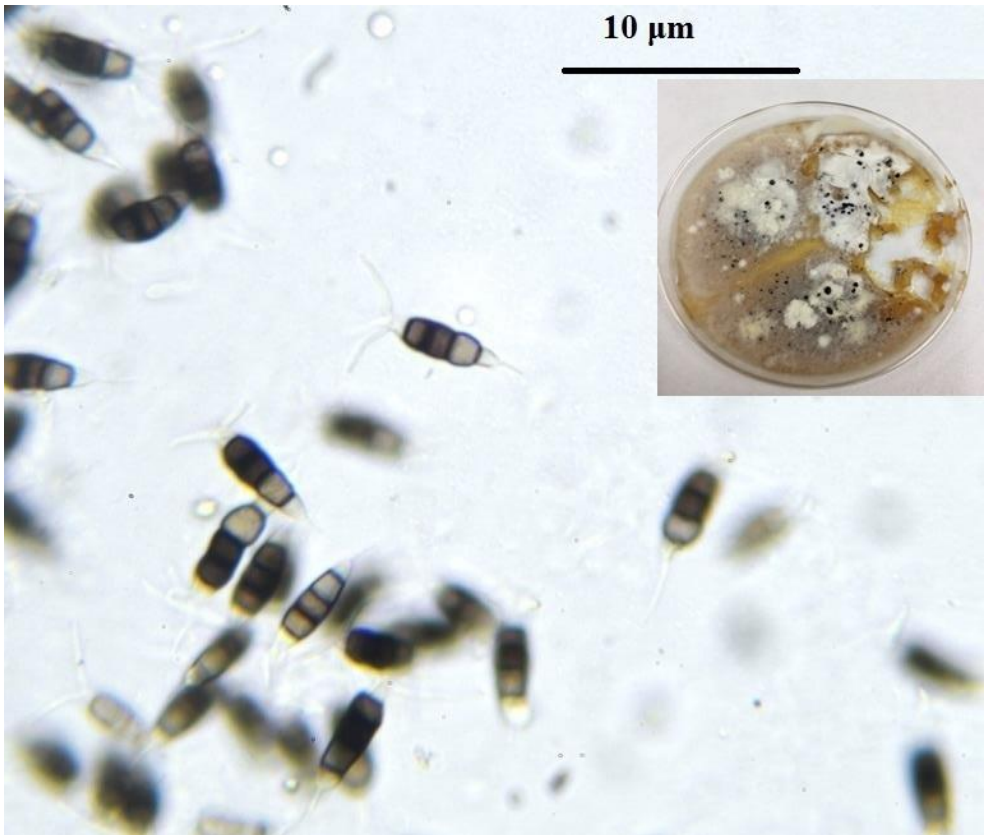

Fig 3. *Pestalotiopsis* sp. identified using microscopic analysis. Inset is typical appearance of *Pestalotiopsis* sp. growing on potato dextrose agar.

Our results determined that *P. microspora* and *P. clavispora* were associated with *Eutypa* dieback disease in muscadine and American native hybrid grapes.

3. To develop community of practice (CoP) for disease prevention and produce clean planting stock of southern grapes.
We developed a fact sheet (see appendix), which was distributed to growers through the Florida Wine and Grape Growers Association meetings as well as to the industry by vineyard site visits, workshops, field days, lectures and demonstrations. In addition, we shared with the industry previously developed extension material on "best practices for vineyard management". We have used training workshops conducted for dissemination of the study outcomes and regularly educating grape growers on the importance of using clean planting materials in their vineyards.

Beneficiaries

Our program provides assistance on a regular basis for grape growers and nursery producers from Florida, Georgia, Alabama, Louisiana, North Carolina and Texas.
We are the only specialized research facility for muscadine and American native hybrid varieties

in Florida and Southern U.S. and one of five National Clean Plant Centers with the mandate to propagate and distributed clean plant materials for muscadines and American native grapes. We estimate approximately 50 of the 380 recorded grape growers in Florida will benefit.

Lessons Learned

Southern grapes, including muscadine and American native grapes, although considered Pierce's disease (PD) tolerant are susceptible to economically important diseases transmitted with the planting stock such as *Eutypa* dieback disease. The disease is broadly spread and can be debilitating to the viticulture industry, with yield losses up to 83% reported in older vineyards for other regions. Disease symptoms are not generally visible in vines younger than 5 to 6 years old although vines may still be infected. These fungi can survive in diseased wood and can be transmitted with non-certified planting materials.

Our region, however, is characterized with one of the fast growing non-*vinifera* viticulture industry and is one of the nations' biggest wine markets with capability to generate economic impact close to 1 billion. The rapid expansion of wine industry in Florida and southeastern U.S., which surpassed the 1 million/year production, predisposes the viticulture industry to debilitating diseases such as *Eutypa* dieback. Several vineyards can be affected with economically important diseases. The situation, if not addressed on time, can negatively impact grape and wine industry in the region.

This research generated important knowledge about the fungal species that may be associated with *Eutypa* dieback disease. We will continue our cooperation with FDACS and the Florida Wine Grape Growers Association (FGGA) to address growers' needs for healthy and improved quality planting stock, and we will continue assisting growers in the state considering nursery business.

Due to the detrimental impact of the disease, there is no doubt it remains a high priority for research and development in the state of Florida and the region. It is our certainty that research and development of new innovative tools as well as targeted extension programs will contribute to *Eutypa* disease control and will lead to sustainable growth and competitiveness of the viticulture industry in the region.

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FLORIDA A&M UNIVERSITY
FLORIDA AGRICULTURAL AND MECHANICAL UNIVERSITY

Appendix

Additional Information

Financial Report – will be submitted, as a separate attachment.

Category	Budget	Expended	Available Funds
SALARY $ Benefits	$24,356	$24,356	$0.00
EXPENSE			
Material & Supplies	$4,408	$4,408	$0.00
Contractual	$790	$790	$0.00
Other	$1,498	$1,498	$0.00
Travel	$3,948	$3,948	$0.00
EQUIPMENT			

I. Community of practice: Extension Factsheet on *Eutypa* dieback disease

FACT SHEET

FAMU COLLEGE OF AGRICULTURE & FOOD SCIENCES
FLORIDA AGRICULTURAL AND MECHANICAL UNIVERSITY

Eutypa Dieback

What is the cause and what are the symptoms?

Eutypa dieback is caused by a complex of several fungal pathogens which include *Eutypa lata, E. leptoplaca* and other fungi, such as members of the Diatrypaceae family. Dieback symptoms are not generally visible in vines younger than 5 to 6 years old, although they may be still be infected. The disease cause cankers on trunks and cordons (arms) of infected vines. Typically cankers are found in areas surrounding old pruning cuts. They may appear as flattened areas on the trunk and are usually concealed by dead bark (Figure 1). Cankered wood becomes distorted and twisted after a number of years. A cross-section cut through the cankered area, reveals a wedged-shaped brown discoloration, bordered by white healthy wood (Figure 3). The wedge-shaped dead wood alone is not conclusive diagnostic evidence of the disease. Other factors, including freeze injury, can cause identical wedge-shaped regions of dead wood.

Characteristic leaf symptoms develop on shoots arising from infected portions of the vine. As the new shoots begin to grow in the spring, they appear stunted and have short internodes. The first leaves that unfold are small, cupped, pale green to yellow (Figure 2). The leaf symptoms are most obvious early in the growing season when healthy shoots are 12 to 24 inches. Leaf symptoms, however, can easily be confused with herbicide damage. Symptoms become more pronounced each year until the affected vine dies. It is common to find one side of a vine dead or with disease symptoms and the other side apparently healthy.

After the bark weathers away from the canker area, the fungus produces hard and thin layered stroma with black fruiting bodies (perithecia) on the surface of dead wood. The fruiting bodies resemble a honey comb and contain abundant spores (ascospores) which spread the disease.

How serious is the disease?

Eutypa dieback contributes to vineyard decline by reducing growth and yield. The disease threatens the sustainability of premium vineyards, especially those 8 years or older, and is becoming an increasing problem in the southeast region of the United States.

Figure 1. Canker disease on grapevine trunk

Figure 2. Pale green leaves typical of Eutypa dieback on grapevine

Figure 3. A cross section of wood with wedge-shaped discoloration on grapevine cordon

The disease reduces growth and yield. For example, economic losses of at least US$260 million per year have been attributed to Eutypa dieback disease in grapevine growing areas of California. The fungus infects vines through pruning wounds, colonizes wood and causes a characteristic discoloration of wedge-shaped dead tissue. It is thought to produce toxic metabolites which are translocated to the foliage, causing stunting of the shoots, distortion and necrosis of leaves, reduced bunch size and uneven ripening and if left unmanaged eventually kills vines. The severity of foliar symptom expression varies from year to year, and could possibly be influenced by weather conditions.

Where does the pathogen survive and how does it spread?

The fungus survives on dead wood in fruiting bodies (perithecia) which have a darkened, charcoal appearance. These structures release spores throughout the pruning season and following hydration are spread by wind and rain splash. Infection occurs when spores land on fresh pruning wounds, or invade reworking or other wounds (Figure 4). Progress is being made to develop highly specific and sensitive methods for the detection and characterize the complex fungal pathogen family that cause canker disease in grapevines. Furthermore, the developed methods will enhance the detection of pathogen(s) from asymptomatic dormant cuttings collected from the vineyard. To-date thirteen primers have been published for Eutypa, and we are conducting research to optimize a diagnostic method for the detection of fungal pathogen(s) that cause the disease in muscadine and American native grapes.

Are clean plants important to the overall management of the disease?

Ongoing research at the FAMU Viticulture strives to characterize a complex of fungal pathogens causing the Eutypa dieback in muscadine and American native grapes in southeast region of the United States. A better understanding of the complex of fungal pathogens causing grape canker disease is an important goal for clean planting material research. It is important to establish vineyards with clean plants. Thus, contaminated older vines should be managed by cultural methods or replaced with clean plants.

How can the disease be managed and what are the future prospects?

Infected trunks or cordons should be removed early in the growing season when shoot has symptoms of disease. Trunk removal is recommended because wounds are less susceptible to infection during the growing season and reinfection from ascospores is at a minimum. Preventative practices (delayed pruning, double pruning, and applications of pruning-wound protectants) are the most effective management approach for canker diseases in grapevines.

- Delay pruning until late in the dormant season to reduce the risk of infection. Consider double pruning; pre-pruning in early winter by cutting canes to 12 to 18-inches above the final pruning cuts, followed by hand pruning to create spurs in February or later.
- Protect pruning wounds to reduce infection.
- Use post-infection practices (sanitation and vine surgery. Vine surgery involves retraining new cordons or trunks as needed to maintain production.
- Apply fungicides labeled as pruning-wound protectants. Apply alternative materials such as a wound sealant with 5% boric acid in acrylic paint (Tech-Gro B-Lock), and Esca, or an essential oil (Safecoat VitiSeal).

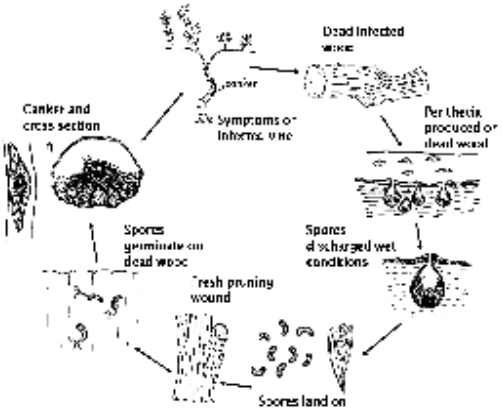

Figure 4. Disease cycle

Authors

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V.M. Tsolova, FAMU Center for Viticulture & Small Research

II. List of publications

a. Henry, M., Obuya, J.O., Ananga, A.O, Ochieng, J.W, Muchovej, J.J. and Tsolova, V.M. (2017). Ribosomal ITS analysis unambiguously identifies fungi causing wood vascular canker (*Eutypa* Dieback) disease in southern grapes (in preparation).

b. Obuya, J.O., Henry, M., Ananga, A.O, Ochieng, J.W, Muchovej, J.J. & Tsolova, V.M. (2017). Ribosomal ITS analysis unambiguously identifies fungi causing wood vascular canker (*Eutypa* Dieback) disease in southern grapes [Abstract submitted to ARD Conference in Atlanta, GA].

Project (35): Management of emerging tospoviruses in south Florida

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Project Partners

USDA-ARS, Fort Pierce, FL; University of Florida, NFREC, Quincy, FL; University of Florida, GCREC, Wimauma, FL; Florida Specialty Crop Foundation, Maitland, FL

Project Summary

Recent damaging outbreaks of two emerging tospoviruses, Tomato chlorotic spot virus (TCSV) and Groundnut ringspot virus (GRSV), have significantly impacted the south Florida tomato industry in the Homestead growing region. Fruit from infected plants are not marketable. And because TCSV-infected fruit can develop symptoms well after harvest due to latent infections, even a low incidence of disease in a field can ultimately result in serious, if not total economic loss because packers are not willing to accept the risk of their fruit developing symptoms after they have been harvested and packed. Current management strategies for these viruses and their insect vectors (thrips) have proven ineffective. Development of effective strategies requires a multi-pronged approach beginning with the evaluation of commercial cultivars and inbred lines possessing virus resistance genes for suitability for production in the Homestead growing region. The Sw-5 gene for resistance to Tomato spotted wilt virus (TSWV), a related tospovirus, was known to also confer resistance to TCSV and GRSV although its field effectiveness needed to be validated. Thrips populations and epidemic development within tomato cultivar/line trials also needed to be characterized to begin to enable us to determine how current management practices can be adjusted to better manage disease. Rapid, field-based diagnostic tests for specific identification of the major tospoviruses affecting south Florida production were developed as part of this proposal, as the need to quickly and accurately identify which disease is present is the first step in any disease management program. Finally, we accelerated development of TCSV-resistant cultivars adapted to south Florida. Collectively, this information is helping us to develop management strategies as part of subsequent funding cycles (including FDACS-SCBG project, contract number 02049) that are better focused on disrupting virus and/or thrips biology.

The project was important and timely because outbreaks of TCSV and/or GRSV have occurred in every season since its introduction in the Homestead growing region, and with each subsequent season disease severity has increased. In addition, these emerging viruses were widely present in southeast and southwest Florida production areas, and it was likely that outbreaks would soon extend beyond south Florida into peninsular Florida. Under current circumstances, an outbreak of TCSV in the greater Florida production regions would be devastating to the tomato industry because we are simply not prepared to effectively manage such an outbreak. The use of insecticides for management of the thrips

vector has proven ineffective in preventing outbreaks, and resistant cultivars, which are the only effective control, have not been fully tested under field conditions and are not widely grown. Horticulturally acceptable virus-resistant tomato cultivars will provide the foundation for the long term solution to this problem. Also, alteration in grower practices, such as adjustments to planting dates for specific varieties or targeted management of alternate host plants and weeds, are possible control strategies that might show possibilities from analysis of the epidemiological data.

This was a new a project but TCSV was discovered during tomato surveys conducted as part of a prior thrips-vectored tospovirus FDACS-SCBG project (contract number 018014) due to similarities in symptoms with GRSV. Results from this current project provided the foundation for management strategy development as part of a subsequent thrips-vectored tospovirus FDACS-SCBG project (contract number 02049) as noted above.

Project Approach

Four activities guided this project:

1) Evaluate tomato cultivars and lines

2) Characterize distribution of thrips and TCSV

3) Develop rapid field-based detection for tospoviruses

4) Accelerate development of TCSV-resistant tomato varieties

Outcomes from each of these four objectives were regularly communicated to growers and industry stakeholders as described below in "Goals and Outcomes Achieved." Summaries for each activity are presented below.

1) Evaluate tomato cultivars and lines for horticultural acceptability and tospovirus resistance:

Two trials were planted by Hutton in Homestead starting in the fall of 2015 to coincide with the winter 2015-2016 tomato season. Unusually heavy rain resulted in a nearly complete loss of the first field trial. However, a few samplings of thrips (for species identification) and one sampling of plants (for tospovirus identification) were made by Hutton, Turechek and Adkins, for Activity 2 below. The second field trial fared better although heavy rain in late December/early January resulted in bacterial spot disease. Plants largely recovered from this disease, but tospovirus pressure remained low throughout the trial. Two harvests of 32 different hybrids were completed in March, 2016, and all fruits were graded for size and marketability. Results from these trials were presented to growers at the 2016 Florida Tomato Institute in Naples.

Results from winter 2015-2016 trials were used to guide tomato line selection for similar variety trials planted by Hutton in Homestead in October 2016 (to coincide with the winter 2016-2017 tomato season) and in October 2017 (to coincide with the winter 2017-2018 tomato season). Results from winter 2015-2016 were also used to guide two large block plantings (for the 2016-2017 tomato season) of two commercial tomato cultivars with tospovirus resistance to evaluate horticultural acceptability in the Homestead production area. Results for these trials was completed by Hutton to coincide with winter 2017-2018 tomato season.

2) Characterize distribution of thrips and TCSV within field trials:

Thrips collected in flower samples during winters of 2015-2016 and 2016-2017 in Homestead were identified by Funderburk. Plant samples from Homestead were determined to be infected with TCSV by Adkins. However, between poor weather and insufficient numbers of thrips and TCSV-infected tomato plants, large scale sampling by Turechek was not practical in our Homestead field trials. Instead, large scale sampling of thrips and TCSV-infected plants was made in Palm Beach County by Turechek and Adkins. Funderburk identified thrips and Adkins identified TCSV collected from these Palm Beach County grower production fields.

3) Develop rapid field-based detection for tospoviruses:

Two independent types of diagnostic tests that will differentiate TCSV from the related tospoviruses, Tomato spotted wilt virus (TSWV) and Groundnut ringspot virus (GRSV) have been developed by Adkins and Turechek. The most promising of these tests is currently being optimized for all combinations of viruses in different hosts.

4) Accelerate development of TCSV-resistant tomato varieties adapted to south Florida:

The UF/IFAS tomato breeding program of Hutton maintains an ongoing breeding project for tospovirus resistance, which is primarily based on the Sw-5 gene. Crosses for developing new hybrids containing this gene were made by Hutton in spring 2016, and these hybrids were evaluated in the winter 2016-2017 Homestead field trials. To offset unfavorable weather and low disease pressure in Homestead, experimental greenhouse inoculations of Hutton's lines were made by Adkins in Fort Pierce.

Goals and Outcomes Achieved

Four goals guided the outcomes of this project:

1) Evaluate tospovirus-resistant tomatoes for Homestead area

2) Determine thrips and TCSV distribution within Homestead trials

3) Develop a test to distinguish tospoviruses present in Florida tomatoes

4) Disseminate research results to stakeholders

Summaries for each goal are presented below.

1) Evaluate tospovirus-resistant tomatoes for Homestead area:

Based on results of multiple field trials in Homestead, Hutton prepared and Funderburk, Turechek and Adkins co-authored an Extension fact sheet "tospovirus-resistant tomato varieties for southern Florida." Results from 2015-2016 winter variety trials led to the planting of two large block (1 acre) plantings of two new tospo resistant hybrids in 2016-2017; production of one of these hybrids expanded in 2017-2018 to eight 25-acre plantings.

2) Determine thrips and TCSV distribution within Homestead trials:

Although thrips and TCSV were identified in Homestead trials by Funderburk and Adkins, respectively, large scale sampling by Turechek was not possible. Instead, thrips and TCSV were sampled from an unexpected outbreak in Palm Beach County by Turechek, Adkins and Funderburk.

3) Develop a test to distinguish tospoviruses present in Florida tomatoes:

Tests developed by Adkins and Turechek are being optimized so that TCSV can be unequivocally distinguished from TSWV and GRSV in Florida tomatoes.

4) Disseminate research results to stakeholders:

Funderburk and Adkins presented an update on management of thrips and viruses at the Thrips Management Workshop for growers in West Palm Beach, FL on January 19, 2016.

Funderburk and Adkins presented an update on management of thrips and viruses at the Thrips Management Workshop for growers in LaBelle, FL on January 20, 2016.

Hutton presented an update on the winter 2015- 2016 field trials on September 7 at the 2016 Florida Tomato Institute.

Hutton, Adkins, Funderburk and Turechek co-authored “Tospo-resistant variety outlook for south Florida” in the 2016 Florida Tomato Institute Proceedings (PRO 532), pp 6-7.

Adkins co-authored first report of TCSV infection of peanut in Haiti in Plant Disease 100:1797.

Adkins presented an update on Florida TCSV research for thrips-tospovirus project meeting at the American Phytopathological Society national meeting, August 2016.

Adkins presented an update on Florida vegetable viruses detected in 2016 and expected in 2017 (including TCSV) at the Florida AgExpo at UF-GCREC on November 2, 2016.

Adkins, Turechek, Hutton and Funderburk co-authored first report of TCSV infection of non-solanaceous weeds and sweet chili pepper in Puerto Rico in Plant Health Progress.

Adkins co-authored invited book chapter including thrips and tospoviruses emergence as crop pests entitled “Role of insect supervectors Bemisia tabaci and Frankliniella occidentalis in the emergence and global spread of plant viruses” for Annual Review of Virology 2:67-93.

Adkins, Funderburk, Turechek and Hutton co-authored a refereed scientific report with collaborators describing three new TCSV hosts (erect spiderling, Asian spiderflower and sweet chili pepper).

Adkins, Turechek, Funderburk and Hutton co-authored a refereed scientific report with collaborators describing sweet basil, purslane and portulaca as new hosts for TCSV.

Hutton, Funderburk, Turechek and Adkins authored Extension fact sheet "tospovirus-resistant tomato varieties for southern Florida."

Funderburk presented management information at a workshop for growers.

Hutton made presentation on tospovirus resistance genes at 32nd Annual Tomato Disease Workshop at UF-GCREC in October 2017, with Funderburk, Turechek and Adkins as co-authors.

Adkins, Turechek, Funderburk and Hutton responded to multiple requests from growers and Extension personnel for tomato cultivar selection, thrips information and management options, and TCSV information and diagnosis.

Beneficiaries

The principal beneficiaries are the Florida tomato growers. According to the "2013 Florida Agriculture by the Numbers", there were 30,000 fresh-market tomato acres planted in 2012. The Florida Tomato Committee website notes that "Florida is the nation's largest producer of fresh tomatoes. With almost every southern county in the state cultivating tomatoes, Florida produces virtually all the fresh-market, field-grown tomatoes in the U.S. from October through June each year, and accounts for about 50% of all fresh tomatoes produced domestically." Additional beneficiaries include Florida pepper, ornamental and lettuce growers since these crops are known TCSV hosts. Based on the number of farmers and farm managers of these commodities in Florida, the number of beneficiaries is 1200. Tomato, pepper, ornamental and lettuce growers benefitted by receiving near real-time updates on TCSV geographic and host range expansion in Florida, and regular updates on tomato cultivar evaluation and TCSV detection protocol development, by project personnel as noted in "Goals and Outcomes Achieved" above.

Lessons Learned

Excessive rain and lack of tospovirus disease in the Homestead area for the winter 2015-2016 tomato production season resulted in less than optimal outcomes for our variety trials and eliminated spatial sampling for thrips and tospoviruses. Low tospovirus disease in our Homestead field trials for the winter 2016-2017 tomato production season again resulted in less than optimal outcomes for our variety trials and eliminated spatial sampling for thrips and tospoviruses. However, sufficient data were collected to develop relevant presentations and fact sheets for growers. In addition, TCSV epidemics in tomato and pepper in Palm Beach County during the winter 2016-2017 production season provided unexpected spatial sampling opportunities for thrips and tospoviruses. Finally, Hurricane Irma and other tropical rains severely damaged much commercial vegetable production in south Florida for the fall 2017 season.

Our major lesson learned was the strength of a diverse project team whose members were located throughout the different Florida tomato production areas. This allowed us to quickly adjust plans and collect data wherever TCSV outbreaks occurred.

Total Funding Expended: $110,900.71

Percentage of Project Completed: 100

Project (36): Urban Farming – Aquaponics

Project Partner: Fresh Ministries, Inc.

Project Summary

The purpose of the project was to establish an educational site to teach local schoolchildren and youth in food desert areas how food is grown and promote the consumption of specialty crops. The area to be served is a core-city food desert in Jacksonville, Florida. The project was important and timely because the Fresh Ministries aquaponics facility is located within Jacksonville's health zone 1 (HZ1). Residents residing in Health Zone 1 (80% African-American) lack access to healthy affordable fresh foods, sustainable jobs, and health, nutrition and diet education. A large percentage of HZ1 residents live in poverty (31% of general population; children under 18: 43% - more than double other health zone rates).

Project Approach

FreshMinistries has introduced hundreds of individuals. particularly youth, to the workings and benefits of aquaponics and other urban farming techniques. These benefits and techniques were incorporated into FreshMinistries' existing Fresh Path and Fresh Futures programs that regularly provide training in the areas of financial literacy, college and job readiness, academic enrichment, health and wellness, family involvement, cultural outings, and leadership and character development. By providing access to a demonstration aquaponics model in which vegetables and culinary herbs were grown, individuals were given a hands-on experience to a system in which they were able to experience multiple cycles of different types of harvests, including but not limited to: basil, various types of lettuces, peppers and beans, sprouts, chard, dill, celery, and many more. Students learned about the techniques used in growing specialty crops in aquaponics and other urban agriculture systems, different ways of preparing the crops harvested, and the nutritional benefits of the system's yield. The demonstration aquaponics facility, housed in the Weaver Center for Community Outreach, also attracts countless questions and follow-up outreach by the thousands of individuals that utilize the Weaver Center each year for a variety of reasons.

FreshMinistries, in partnership with the University of North Florida and the Florida Small Business Development Center, hosted a series of agribusiness workshops at the Weaver Center for Community Outreach. The series, which was made available the public, provided agricultural producers of all sizes, industries, and stages of growth the tools to succeed in different agricultural sectors. The workshops covered a variety of topics including, but not limited to: "Agribusiness planning and startup development," "Financial Management - Financing a farm," Marketing Agricultural Enterprise," 11Agricultural Resources, 11 etc.

FreshMinistries also closely works with Groundwork Jacksonville, an organization dedicated to improving the overall environment of the neighborhood, the social interaction of the residents, and greater prosperity. This is accomplished through the creation of sustainable strategies that improve a community's overall health and quality of life. One such strategy is the creation of a public market in Jacksonville's Urban Core. The Jacksonville Public Market, which uses the Weaver Center for Community Outreach as its base of operations, focuses on providing opportunities for aspiring entrepreneurs and working toward closing the gap in health disparities through access to fresh, healthy food.

While no SCBGP funds were used to build or equip the facility itself, this project effectively demonstrated the production capacity of a small aquaponics facility by enabling the hiring of a production manager and director of FM's educational program. Since completion of the project, Fresh Ministries has begun monthly donations of the produce grown at the facility to the Sulzbacher Center, a facility serving homeless residents of downtown Jacksonville (Health Zone 1). Donations have consisted of lettuces and herbs grown at the facility and the produce of 1,512 plants was donated in August 2018.

Entrepreneurial training has also been a component of the project, with 2 entrepreneurial trainees employed in production through a refugee education program run at the facility.

An urban farmer's cooperative was originally envisioned, but the time needed to execute this undertaking proved greater than expected, and the goal remained out of reach during the project period.

Goals and Outcomes Achieved

1. Conduct educational outreach to youth

The initial proposal targeted a total of 150 participants. The program actually served over 300 inner-city youth between the ages of 14 and 19 in 2016.

2. Number of new urban farmers graduating from entrepreneurial training

While the 2 entrepreneurial trainees have not yet left the program, they continue to assist with the operation of the aquaponics facility, greatly increasing their knowledge of aquaponic production and agribusiness.

Beneficiaries

The beneficiaries of this project included the 300 core-city youth who attended an education program at the facility. Additionally, the results of this project laid the groundwork for a state-funded initiative to begin in July to expand Fresh Ministries' educational programs, which is expected to impact an another 300 elementary and middle-school students, as well as provide employability skills training to young adults from the surrounding neighborhoods.

Lessons Learned

It was found that there is a market to train individuals in Jacksonville's Urban Core in aquaponics' and other forms of urban agriculture. Community interest shown in the demonstration model, coupled with the enthusiasm for 6,000 sq ft facility currently being constructed behind the Weaver Center for Community Outreach, has proven that individuals are interested in the systems and their multitude of benefits.

By way of challenges, an aquaponics-based job training program will be largely dependent upon the existence of aquaponics-related businesses located in the same area as the training program. While there has been a statewide increase in the number of such businesses, there are not yet enough of them in the Jacksonville area to provide reliable opportunities for the graduates of a job training program at this time. This is being taken into account in the planning of future projects.

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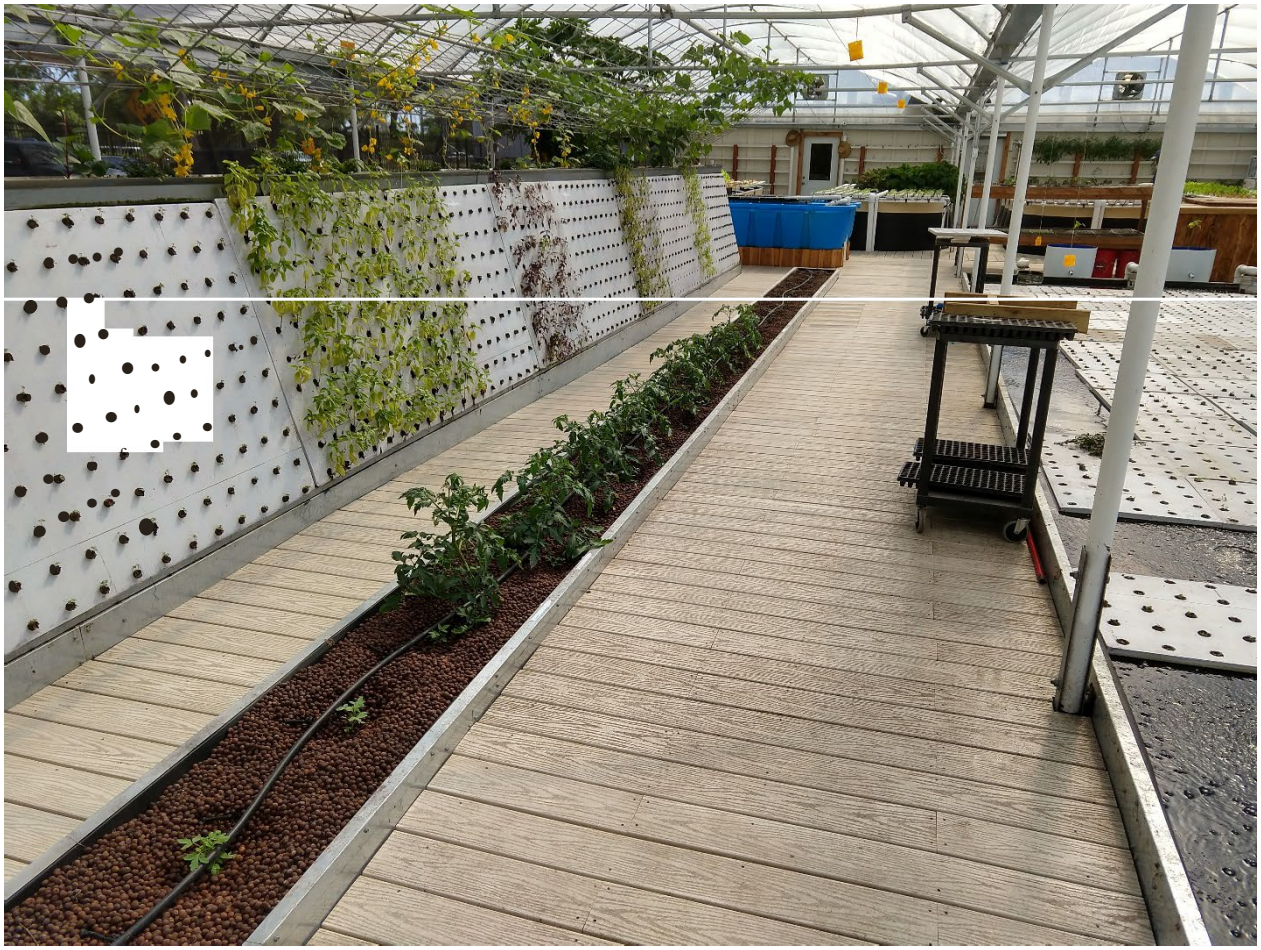
Other Information



Project (37): Advancing integrated practices and host resistance for managing target spot

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Project Partners

University of Florida, Southwest Florida Research & Education Center, 2685 State Road 29 North Immokalee, FL 34142. North Florida Research and Education Center, University of Florida, 155 Research Road, Quincy, FL 32351. Florida Fruit and Vegetable Association, Florida Tomato Committee, Lipman Produce, DiMare Fresh, Pacific Tomato Growers, Dunn Diehl Farms.

Project Summary

Fresh market tomato production in Florida accounted for over half of U.S. production based on yields (57%) and market value (58%) (NASS 2013). Florida's lucrative winter tomato production has suffered in recent years due to increased competition from foreign imports and increased production costs. Target spot, caused by the fungus Corynespora cassiicola, is a common disease of tomato in Florida that affects all aerial portions of the plant (leaves, stems and fruit) leading to dramatic yield reductions. Growers are reliant on the timely application of fungicides for disease management since no resistance is commercially available to the disease. However, growers have observed a rise in the severity and frequency of target spot outbreaks over the last few years, with only limited levels of success managing the disease. There is growing evidence that fungicide resistance is jeopardizing the effectiveness of several classes of fungicides. The long-term goal of this proposal is to develop integrated approaches to manage target spot, to screen tomato germplasm for sources of resistance, and to begin the process of introducing such resistance into commercial backgrounds. This proposal is a direct result of grower demand for additional research to limit production losses to target spot. Outcomes from this proposal will improve our knowledge of fungicide resistance issues throughout the state and environmental factors driving epidemics, leading to better management recommendations. Furthermore, we will take steps to identify sources of resistance and begin a crossing and selection program to incorporate this resistance into cultivated backgrounds; resistant materials that arise from this work will be available to private sector breeders, since the ultimate (albeit long-term) goal will be the development of commercial hybrids with improved resistance to target spot. Information gained from this project will be shared with members of the Florida tomato industry through traditional grower extension meetings

(Tomato Institute, FLAgExpo), extension periodicals (EDIS, newsletters, and popular press articles), and with demonstration trials and workshops at the GCREC, NFREC and SWFREC. In addition, many of the management strategies will likely be applicable to other susceptible, specialty crops produced throughout the state.

Project Approach

Per Objective 1: In 2016, thirty-four Solanum pennellii accessions were initially screened for response to C. cassiicola using a newly-developed seedling assay. Little to no resistance was evident within this set of germplasm. A list of additional accessions to be screened was compiled based on resources at the Tomato Germplasm Resource Center (TGRC) in Davis, CA, but seed shipments from TGRC were delayed for more than six months due to their detection of Potato Spindle Tuber Viroid in seed stocks. Seed of the requested accessions was obtained in spring 2017, and 83 tomato accessions representing nine different Solanum spp. for resistance to C. cassiicola. Accessions displayed a range of foliar and stem symptoms with some highly susceptible accessions exhibiting complete blighting of all foliage, while others only developed small lesions; also, some accessions displayed only foliar symptoms, while others had both foliar and stem lesions. These screens identified approximately 30 accessions with resistance or moderate resistance to C. cassiicola. The remaining accessions were either susceptible or moderately-susceptible. Twelve of the more resistant accessions were subjected to subsequent, repeated assays in fall 2017 to validate initial screening results. Resistance was confirmed in accessions from S. lycopersicum, S. habrochaites, S. cheesmaniae, and S. pimpinellifolium spp., which is advantageous for breeding, since all of these are sexually compatible with cultivated tomato. Each of these twelve accessions was crossed with a UF/IFAS inbred line in fall 2017, and F1s are presently available for further breeding to introduce resistance into cultivated tomato. In spring 2018, the 12 accessions were tested again in seedling disease assays along with 20 additional accessions that demonstrated potential resistance in spring 2017 screens. All 32 of these accessions were more resistant than susceptible controls, and some of these demonstrated consistently high levels of resistance. Germplasm screening results were presented to growers at the 2017 Tomato Institute in Naples; to researchers and industry representatives at the 2017 Tomato Disease Workshop at the GCREC, Wimauma, FL; and to researchers at the 2018 annual conference of the American Society of Horticultural Sciences in Washington D.C.

Per Objective 2a: A statewide survey was conducted from fall 2015 through Spring 2017 with 123 isolates of C. cassiicola collected from tomato production areas. Twenty-nine isolates were chosen to establish sensitivity to quinone outside inihibitor (QoI) and succinate dehydrogenase inhibitor (SDHI) fungicides based on mycelial growth using fungicide-amended media. Based on mycelial growth inhibition on increasing levels of fungicide, an effective concentration that reduced growth by half (EC50) was established for each isolate. The EC50 values for azoxystrobin and fenamadone in the presence of SHAM ranged from 0.1 to >100 ppm and 3 to >100 ppm, respectively; with 5 ppm chosen as the discriminatory dose. Across the SDHI fungicides, EC50 values varied greatly from 0.1 to > 100 ppm;

again, a 5 ppm discriminatory rate was chosen. Screening a larger collection of 94 C. cassiicola isolates based on the 5 ppm discriminatory dose found that over 90% of all isolates were insensitive to QoI fungicides. Insensitivity among SDHI fungicides varied from a high of 79% for boscalid to only 12% for fluopyram. In addition, several isolates exhibiting an intermediate level of insensitivity to several SDHI fungicides were identified. Ten representative isolates that exhibited novel fungicide sensitivity profiles were chosen for subsequent spore germination, plant assays, and sequence-based confirmation of their status. Results confirm the growing problem of fungicide resistance and the importance of establishing appropriate fungicide rotations to preserve respiration inhibitor fungicides. We completed the screening of 123 C. cassiicola isolates to two QoI (azoxystrobin and fenamidone) and five SDHI (boscalid, penthiopyrad, fluxapyroxad, benzovindiflupyr, and fluopyram) fungicides that account for nearly 3/4 of the fungicides available for target spot on tomato (based on available active ingredients). Sequenced cytb, sdhB, sdhC, and sdhD genes of 10 representative C. cassiicola isolates exhibiting diverse patterns of tolerance to the various SDHI and QoI fungicides.

Twenty-nine isolates were chosen to establish sensitivity to phenylpyrrole (PP; fludioxonil), anilino-pyrimidine (AP; pyrimethanil) and demethylation inhibitor (DMI; flutriafol) fungicides based on mycelial growth using fungicide-amended media. Doses of 0.1, 3 and 0.3 PPM were identified as an ideal discriminatory dose for screening the full collection based on the generated EC50 values for fludioxonil, pyrimethanil and flutriafol, respectively. The discriminatory dose will be used to screen the remaining 94 C. cassiicola isolates from our collection.

Objective 2b. Several target spot trials evaluating commercially available fungicides were conducted in fall 2016 at GCREC, NFREC and SWFREC. However, results from field trials vary widely. For example, trials at NFREC found that weekly applications of Mancozeb alone, was effective at reducing target spot. While at GCREC, Mancozeb was also effective at reducing disease severity compared to the non-treated control, but was not sufficient alone to dramatically stop the epidemic or reduce fruit infection. Fall trials at GCREC assessing application rates and timing are still in progress. In 2016, NFREC also found that Zing and Gavel as standalone products and Zing in rotation with Mancozeb also significantly reduced disease severity compared to the control. Bravo Weather Stik in rotation with Aprovia Top, at all concentrations also significantly reduced disease severity compared to the control. However, Bravo Weather Stik in rotation with Fontelis or with A20259 was not significantly different from the control.

Field trials were conducted in spring and fall of 2017 to evaluate several commercially available fungicides, including those that were effective in prior trials (Penncozeb, Bravo Weather Stik, Fontelis, Aprovia Top, Zing!, and Topguard). In the fall of 2017 at NFREC, no significant differences were noted between any of the treatments, and disease incidence was very low. Numerically, Bravo Weather Stik in rotation with Fontelis or Aprovia Top reduced disease compared to the control. Bravo Weather Stik in rotation with A20259 at the lower concentration also provided better protection the control.

Objective 2c. Conditions affecting spore germination of C. cassiicola were examined in vitro. Our results showed only 3 hours of wetness are required for spore germination. C. cassiicola spores can germinate either in the light or dark. In an attempt to better understand environmental factors governing infection and disease development, we determined optimal moisture requirements favorable for disease

development among a subset of 10 C. cassiicola isolates. Tomato seedlings were inoculated with prepared spore suspensions of C. cassiicola and incubated for varying periods (from 6 to 72 hours at 6-hour increments) of leaf wetness. Results showed that the leaf wetness required for disease development was 12, 18 and 24 hours for 2, 2, and 6 isolates, respectively. We also evaluated 10 isolates for aggressiveness on tomato foliage and fruit, establishing differences in their response to two tomato varieties.

Our results indicated an inverse correlation between aggressiveness and leaf wetness. However, isolates exhibited differences in the minimum duration of leaf wetness required to initiate disease. We have also developed a detached tomato leaf assay from which we chose isolate GEV3142 for inoculation. Spore suspensions of C. cassiicola isolate GEV3142 were used to inoculate detached leaves that were incubated at varying temperature ranges from 4 ºC to 35 ºC. The C. cassiicola isolate tested was able to cause disease starting from 10 ºC up to 35 ºC. From 10 ºC to 25 ºC we observed a significant increase in disease severity; the optimal temperature for disease development was 25 ºC. For temperatures higher than 25 ºC disease severity dropped significantly.

Based on colony morphology, differences in 'moisture requirements', and aggressiveness of C. cassiicola, we've chosen to forgo Objective 2d "Address the survival of C. cassiicola in soil and crop residues." C. cassiicola survival in a sand-cornmeal mixture was assessed. Mixtures were inoculated with mycelial plugs of C. cassiicola and allowed to consume cornmeal over several months, then completely dried out. Recovery of isolate GEV3145 on general isolation media was evident four months after drying, although no subsequent plant assays were conducted. Instead, we were presented an opportunity to assess the genetic diversity of our isolates using microsatellite makers developed by Dr. Marin Brewer at the University of Georgia. An improved understanding of genetic diversity is critical for breeding efforts, as well as understanding how our C. cassiicola isolates from tomato relate to those from other crop.

Objective 2d. We have characterized the genetic diversity of C. cassiicola isolates based on microsatellite markers and multilocus sequence analysis. Using 13 microsatellite markers (SSR) we found that the population was structured by host, however, there was genetic variation among isolates from the same host. Preliminary results from SSR analysis in C. cassiicola population from tomato utilizing 8 out of the 13 simple-sequence repeat markers identified high genetic diversity, distinguishing 33 distinct haplotypes out of 123 isolates. Understanding genetic variation among C. cassiicola isolates from multiple hosts and its ability to infect non-host isolates on tomato was critical in screening for host resistance, identifying fungicide resistance, and determining how tomato isolates relate to those from other crops.

Objective 3. In 2016, results from our research on target spot were presented to the tomato growers at Vegetable Extension Meeting at SWFREC and at the Tomato Institute in Naples, FL; several grower groups also visited field trials during meetings and other events at the NFREC, SWFREC, GCREC (during the Florida AgExpo in early November).

In 2017, we drafted an EDIS article to educate the industry about target spot and the causal pathogen, C. cassiicola. We also conducted several demonstration and field trials at NFREC, GCREC and SWFREC that

were on display to the industry. Conducted a hands-on workshop at the Florida AgExpo where participants learned about target spot. Conducted 9 presentations at local, regional and national meetings to growers, tomato industry representatives, extension specialists, and research specialists about target spot research. Dr. Paret published three articles at the Tomato Forum of the Gadsden County Tomato Growers Association and one at the UF/IFAS Crop Management School.

Goals and Outcomes Achieved

Objective 1: Develop tomato breeding lines with resistance to C. cassiicola.

Target: Screen at least an additional 100 PI lines and land accessions for resistance to C. cassiicola; create initial backcrossed lines from identified sources of resistance.

Outcomes achieved: Eighty-three tomato accessions representing nine different Solanum spp. and obtained from the Tomato Germplasm Resource Center (TGRC) in Davis, CA were screened for resistance to C. cassiicola using a newly developed seedling assay. Initial screens identified 29 accessions with resistance or moderate resistance, and 54 accessions that were moderately or highly-susceptible. Subsequent assays confirmed resistance in more than 30 accessions, and high levels of resistance were observed in multiple accessions from S. cheesmaniae (LA1037, LA1036, LA1414, LA1447, LA0746, LA1043, LA1042, LA0749, LA0421, LA1042, LA0524, LA0422, LA0529, LA0521, and LA1427), two accessions from S. galapagense (LA0526 and LA0317), and two accessions from S. pimpinellifolium (LA2093 and LA1617). This is advantageous for breeding, since each of these species is highly compatible with cultivated tomato. To initiate breeding to introgress resistance genes into cultivated tomato, crosses were made between the parental inbred, Fla. 8059, and several of these accessions. F1s and F2s from these crosses are now available for building populations for genetic studies to identify resistance loci; and for continuing breeding efforts to combine resistance with acceptable horticultural qualities.

Objective 2: Develop statewide fungicide programs for managing target spot on tomato.

Target: Update fungicide recommendation in Florida to control target spot and limit the development of fungicide resistance among C. cassiicola populations.

Outcomes achieved: A statewide survey was conducted from fall 2015 through fall 2017 with more than 130 isolates of C. cassiicola collected from tomato production areas. Screened a collection of 123 C. cassiicola isolates to two QoI (azoxystrobin and fenamidone) and five SDHI (boscalid, penthiopyrad, fluxapyroxad, benzovindiflupyr, and fluopyram) fungicides that account for nearly 3/4 of the non-contact fungicides available for target spot on tomato (based on available active ingredients). A set of 29 isolates was chosen to establish sensitivity to phenylpyrrole (PP; fludioxonil), anilino-pyrimidine (AP;

pyrimethanil) and demethylation inhibitor (DMI; flutriafol) fungicides. Doses of 0.1, 3 and 0.3 PPM were identified as an ideal discriminatory dose for screening the full collection based on the generated EC50 values for fludioxonil, pyrimethanil and flutriafol, respectively. Sequenced cytb, sdhB, sdhC, and sdhD genes of 10 representative C. cassiicola isolates exhibiting diverse patterns of tolerance to the various SDHI and QoI fungicides. Field trials were conducted in 2016 and 2017 to evaluate several commercially available fungicides, including those that were effective in prior trials (Penncozeb, Bravo Weather Stik, Fontelis, Aprovia Top, Zing!, and Topguard). However, results from field trials vary widely. For example, trials at NFREC found that weekly applications of Mancozeb alone, was effective at reducing target spot. While at GCREC, Mancozeb was also effective at reducing disease severity compared to the non-treated control, but was not sufficient alone to dramatically stop the epidemic or reduce fruit infection.

Objective 3: Refine our understanding of C. cassiicola survival in soil and crop residue; and the temperature and moisture requirements that favor target spot development.

Target: Improved recommendations for field sanitation, crop-free periods, and crop rotations to destroy C. cassiicola. Improved recommendations for timing preventive fungicide applications based on knowledge of favorable conditions.

Outcomes achieved: A greenhouse study to address the survival of C. cassiicola in soil was initiated. This initial study used porous satchels filled with lab produced C. cassiicola cultures and buried in either a raw or pasteurized field soil. Satchels are being incubated in each field soil (raw or pasteurized) that is either kept dry or periodically moistened to maintain soil moisture to near field capacity.

We tested in vitro conditions to C. cassiicola spore germination and our results showed that it is only required 3 hours of wetness for spore germination. However, C. cassiicola spores can germinate whether in the light or dark. We also evaluated 10 isolates for aggressiveness on tomato foliage and fruit, establishing differences in their response to two tomato varieties as well as response to varying periods of leaf wetness and temperature.

Beneficiaries

Through outreach and research efforts, this research has benefited an estimated 2,500 people, including all tomato grower operations in Florida, to include Lipman Produce, DiMare Fresh, Pacific Tomato Growers, West Coast Tomato Growers, Frank Diehl Farms, Artesian Farms, Gargiulo Produce Inc., and others represented by the Florida Tomato Committee and Florida Fruit and Vegetable Association that represent 32,200 acres of tomato production and accounts for 35% of all tomato production in the US. This project has benefited the tomato growers by developing integrated disease management strategies, based on cultural practices and fungicide applications, reducing losses caused by target spot, improving yields, and reducing post-harvest losses. Our identification of resistance sources will enable the incorporation of host resistance in commercial germplasm, which will contribute in the long term to better disease management options.

Lessons Learned

For Objective 1: Stalled seed requests from the TGRC due to the detection of Potato Spindle Tuber Viroid in seed stocks delayed objective 1. However, TGRC resumed seed shipments in time for seedling assays to be completed and resistant accessions to be identified.

Per objective 2: We originally proposed to expand our fungicide sensitivity evaluation for 50 isolates to include QoI, SDHI, BI, DMI, MBI, PP and AP fungicides. However, due to the variation we observed in collected isolates, we expanded our evaluations to 123 isolates. We focused our initial evaluation efforts to QoI and SDHI fungicides, which represent nearly 75% of non-contact fungicides labeled for target spot on tomato (based on active ingredient). We also expanded our original proposal to include all 5 commercially labeled SDHI fungicides (boscalid, penthiopyrad, fluxapyroxad, benzovindiflupyr, and fluopyram) based on concerns and reports on other pathogens about different levels of cross-resistance among the SDHIs. We developed thresholds for the 7 fungicides and screened all 123 isolates with a discriminatory dose. The pattern of resistance observed among isolates was more complex than expected, which forced us to sequence the SDHI complex.

Total Funding Expended: $162,301.04

Percentage of Project Completed: 100

Project (38): Establishing a Tea Industry in Florida: Cultivar and Disease Assessment

Contact Person

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Project Partners

University of Florida

(No additional partner organizations)

Project Summary

The goal of this project was to assess the viability of tea (Camellia sinensis) production within Florida. Tea is the second most consumed beverage in the world after water. Tea sales in the US alone are valued at over $10 billion wholesale, with imports increasing by 63% between 1990 and 2012 (Tea Association of the USA, 2013). The majority of tea consumed in the US is imported, but small acreages of tea are grown in Florida, Mississippi, Alabama, Louisiana, Georgia, South Carolina, Virginia, California, Oregon, Washington and Hawaii. Limited resources are available to US growers for advice on cultivar selection, cultural techniques, or disease management. Recently research has begun at the University of Hawaii, Mississippi State University and Louisiana State University to address regional assessment of different cultivars, and to monitor and assess potential diseases, laying the groundwork for the establishment of IPM practices and breeding programs to establish new lineages and cultivars. We assessed eight of the most readily available tea varieties to develop recommendations for teas suitable to production in Central Florida, based on field performance, heat tolerance, and disease resistance. Providing this information will allow us to optimize production of tea and improve its chances of success as a new economically important crop for Florida.

With repeated introductions of devastating citrus diseases and pests, farm acreage in Central Florida is increasingly moving away from citrus, and few suitable replacement crops have been identified. Tea may represent an alternative, environmentally friendly crop for Florida farmers. Central Florida has some of the most suitable qualities for tea production within the US. Acidic, loose, sandy soils are considered optimal for tea, a member of the Camellia family, and other camellias (Camellia japonica and C. sasanqua) are already grown widely in the region as ornamental plants. Tea is considered drought tolerant once established and is cold tolerant to approximately -13°C/ 8°F. The Florida growing season is

also among the longest in the US, providing a unique crop window. Our study was be the first to assess the suitability of currently available tea cultivars for Florida production.

Project Approach

Activity #1: Field Site Establishment and Plant Growth

We established field plots with 45 plants each of 8 different tea varieties: four from the collection of Dr. Guihong Bi at Mississippi State University ('Fair Hope,' 'Georgian,' 'Small Leaf,' and 'Big Leaf') and four purchased from Camellia Forest Nursery in Chapel Hill, NC ('Large Leaf,' 'Assamica,' 'China,' and 'Quinquebracteata'). At the time of project establishment, no additional varieties were available in the quantities required for our project. Availability remains a bottleneck for tea research and production, and nursery production should be an area of emphasis for future research and development projects.

Plants were assessed throughout the two year study period for general plant performance (survival, presence of environmental damage, overall plant appearance) and growth (height, canopy size, stem diameter), and during the second year, for yield. Tea plants are typically harvested beginning in the 2nd or 3rd year, and are not expected to reach full yield potential for approximately four years after transplanting to the field. In our trials 'Large Leaf' and 'Big Leaf' (thought to have originated from the same parental lineage) showed the best survival under full-sun growing conditions in central Florida (90% survival, PSREC in Citra, FL). 'China,' 'Fairhope,' and 'Georgian' showed intermediate survival, and 'Small Leaf' and 'Assamica' showed poor survival (<40%). 'Quinquebracteata' did not survive at all (100% mortality). Most mortality was observed during a drought period that occurred in the second year of the study. The plants were drip irrigated, and root zones remained moist throughout the drought; it is hypothesized that mortality was due to intense heat and sunlight on leaves combined with low relative humidity. Shading is used in lowland tea production in many parts of the world, and may help to improve establishment of tea in Florida. During the fall of the second year, a hurricane passed near our site, exposing the plants to tropical storm force winds. We took advantage of the opportunity to assess plant damage, by marking stems and counting leaves before and after the storm; none of the varieties suffered significant defoliation or breakage. Average biomass production (both years) and harvested tea yield (second year) were highest in 'Fairhope,' 'Big Leaf,' 'Georgian,' and 'Large Leaf.' Overall, "Big Leaf'/'Large Leaf' and 'Fairhope' were considered the most promising planting material for central Florida conditions, particularly in full sun. These two varieties are being carried forward into a new research trial examining companion ground cover and shade plants for tea establishment and production.

Tea quality attributes were assessed during the second year on select varieties from the field trials. ‘Assamica’ had the highest caffeine concentration, across all 7 accessions and the positive control (‘Lipton Black Tea’). ‘China’ had the highest total concentration of the polyphenols Epigallocatechin gallate, Epigallocatechin, Catechin, and Catechin-gallate across all 7 accessions and the positive control (‘Lipton Black Tea’); however, measured polyphenols were highly variable. Work is now being done to

further optimize quality assessment protocols, and to examine changes in polyphenols resulting from tea processing, production style, and storage.

Activity #2: Heat Tolerance Testing

Heat tolerance testing was conducted with ‘Fairhope,’ ‘Small Leaf,’ and ‘Big Leaf,’ based on field observations during the first year of the study, at which point these varieties were the top performers. Prior to heat stress, plants were grown at optimal conditions (30°C Day/25°C Night) for 1 week. During heat stress (41°C Day/ 27°C Night), all varieties showed a decrease in photosynthetic efficiency, with ‘Small Leaf’ having the lowest rating. After 4 days of heat stress, growth chamber conditions were returned to normal and plants were monitored for their recovery. All three accessions returned to their previous efficiency ratings, with ‘Fairhope’ and ‘Big Leaf’ reaching normalcy earlier than ‘Small Leaf.’ Ion leakage data was inconclusive and additional tests will examine this approach to evaluating heat stress tolerance in tea. Based on these results, it appears that 'Big Leaf' and 'Fairhope' varieties can be expected to recover well even after unusual heat stress events (41C/106F), provided that they have adequate humidity and/or shading.

Activity #3: Pest and Disease Assessments

Field plants were monitored monthly for pest and disease occurrence. Anthracnose was the most common and detrimental disease problem observed; severity was lowest in 'Large Leaf,' 'Big Leaf,' and 'Fairhope,' and highest in 'Assamica,' 'China,' and 'Georgian.' Laboratory testing was done to confirm the species of the anthracnose pathogen, and to assess relative susceptibility among available tea varieties. All tested varieties were susceptible to infection, but 'Big Leaf' developed the smallest lesion sizes on inoculated leaves, among six varieties tested (four from the field trials, plus two additional varieties, 'Black Sea' and 'Korean'); lesions on 'Big Leaf' were significantly smaller than on 'Small Leaf,' 'Fairhope,' or 'Korean.' Sporadic incidences of mites and aphids were noted; these were managed with as-needed applications of horticultural oil. Predatory insects were commonly encountered in the plots and may have helped to keep these pests from reaching damaging levels during most of the study period.

Activity #4: Product development and dissemination

Results from this work have been presented at meetings of the Florida Society for Horticultural Science, the American Phytopathological Society, and Florida Organic Growers, and via a grower field day held May 1st, 2018, at the Plant Science Research and Education center in Citra, FL, with over 50 participants. Additional manuscripts are still in preparation from this project.

Goals and Outcomes Achieved

Goal: Help Florida growers diversify their crop options by developing recommendations for tea cultivars/varieties suitable for central Florida production.

1.1 Performance measure: Short term (within project): number of people who participate in grower field day; Long term (beyond project): number of Florida growers producing tea.

1.2 Benchmark: Although several growers are experimenting with small plots, there is currently no commercial tea production in Florida, and there have been no previous grower field days or other commercial tea production events or resources.

1.3 Target: By December of 2017, at least 100 Florida growers will have been made aware of tea-growing options (cultivar performance, pest and disease impacts) through a grower field day and other venues.

1.4 Progress: Our short-term target of reaching 100 Florida growers was met within the project time frame. We had 68 registrants for our grower field day, 51 of whom attended the event (the remainder joined online or were included in our mailing list for research updates). We also presented to over 50 growers at a multi-county alternative crop grower meeting (May 9 at Lake County Extension). Approximately 30 growers attended the presentation at the 2017 Florida Organic Growers meeting, and we also reached numerous nursery producers and extension personnel through FSHS presentations and publications (two presentations of approximately 20-30 participants each, plus an undocumented number of readers). This research has been featured in at least four news articles, which generated direct inquiries from growers and tea business owners. We have established a social media presence and a tea research website; our most recent facebook post about continuing tea research reached 509 people and had 120 engagements. Grower partnerships established through this project have led to funding and initiation of a SARE project involving six growers, four of whom are growing tea for the first time as part of the project. We consider the achievement of this short-term target to be indicative of progress towards our long-term goal of fostering increased tea production in Florida.

Beneficiaries

Most of our stated potential impacts are long-term, not expected to be realized within the two year scope of this project (e.g., economic impacts from local tea production). Tea is a perennial crop with a two-year establishment period before initial harvest, and an additional two years before full productivity is reached. The short-term impacts of this work have been in providing well over 100 growers with foundational information to determine whether tea may be a crop worth pursuing. This project is the first to address tea as a potential crop for Florida. We have examined the most readily available sources of tea plants, and found that two lineages, 'Fairhope' and 'Big Leaf' / 'Large Leaf' are particularly promising for Florida production, based on survival, growth, yield, and disease resistance. We have identified the most likely disease and pest problems to be expected in tea production, and laid the groundwork for continued studies in Florida-specific production systems.

Lessons Learned

Our first delay was strategic: we delayed the start of the work to wait for the arrival of a new graduate student, dedicated to this project. Subsequent delays were not. Pathogenicity studies were delayed due to difficulties in maintaining clean plant material; the anthracnose pathogen was already present on plants brought in from both sources, which created challenges in setting appropriate negative controls. We had planned to use rooted cuttings from our original plants for these assays, but cutting production was too slow, and cuttings became infected with Colletotrichum; we had to alter the protocol to use detached leaf assays and time the assays according to growth flushes on our greenhouse plants, which did not occur in accordance with our scheduled timeline. We also had two air conditioning failure events in our main greenhouse, each of which constituted a major heat stress event, killing some of our tea

plants and rendering the rest useless for any research purpose until the plants had time to fully recover. These events delayed both pathogenicity and heat stress testing, as neither could be performed with plants that were already stressed or damaged.

Data from our first polyphenol analysis, performed at the Mass Spectrometry REC, was too variable to be useful; samples showed a pattern of drift indicating a problem with the equipment (decreasing values over time, regardless of sample origin), and the controls run with the samples were insufficient to correct for the magnitude of the drift. Subsequent submissions were run in smaller batches with enhanced controls, and these analyses were scaled down from our originally planned comparisons.

For the field trial, we had originally planned to analyze performance of 10 tea accessions, but only 8 were available in sufficient numbers at the time of study establishment. Poor plant availability is a serious hindrance to both research and production. Only one nursery carries sufficient numbers of any single variety to conduct research, and none carry production-level volumes. Although it did not impact our FDACS project, an unusual freeze event in North Carolina this past winter eliminated 100% of the tea plants stocked by this nursery, cutting availability further. This dependence on a small number of current growers, focused primarily on ornamental camellias, represents a serious bottleneck for current and future work. In the field, we experienced a major weed problem and had to adjust the original protocol to add weed cloth in the plots. The weed cloth was highly successful, but represents a significant production cost. We are currently examining cover crops as a potential sustainable alternative.

Total Funding Expended: $45,959.29

Percentage of Project Completed: 100

Project (39): Reaching New Consumers with Florida's Tropical Fruit Website in Spanish

Contact Person

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Project Partners

Tropical Fruit Growers of South Florida, Inc.

Project Summary

Florida's tropical fruit industry is unique in that it is the only place in the continental United States in which many of these specialty crops (which are well known around the world) are grown. The Tropical Fruit Growers of South Florida's website has proven to be an excellent means of making the availability of these American-grown fruits known to consumers. But the information currently on the website needs to be updated and refreshed, and newer website programs have become available that make for far more efficient updating and editing of website content.

Also, the availability of these tropical fruit crops remains largely unknown to Spanish-speaking consumers who are unable to read the existing website. A major task of this project will be to translate our website into a seperate Spanish website to reach these consumers.

This project is built upon a previous SCBGP project that funded the creation of the current TFGSF website. The website program originally used to create the website has been replaced with a more updated program that will make management more efficient. The original content is been refreshed with new recipes and current fruit uses. A second duplicate website has been created and the translation of the English content into Spanish is complete.

Project Approach

The three main Tasks from the Work Plan include:

Task 1. Transfer of the existing Tropical Fruit Growers of South Florida, Inc., TFGSF, website, www.tropicalfruitgrowers.com, from its current platform to a more user friendly platform, and train the webmaster in the management of the new website.

The webmaster from Creative House of Florida, CHF, created a new design for the TFGSF website in a new website management program. The existing website had been built in the Word Press platform, but that platform had been difficult to manage and had been hacked during the time we used it. The new website was made in the WIX platform and has proven to be a much easier program with which to manage the content. The CHF webmaster met with the TFGSF webmaster to train her in the new WIX program. This training was very quick as this program is very easy to use, and well thought out.

The webmaster from the Creative House of Florida made and presented several designs for the overall look and feel of the website. He was very responsive to suggestions and comments, and was very easy to work with. The new design was chosen and the website conversion began.

Task 2. Review, edit, and update all content on the website site.

The TFGSF webmaster reviewed the material on each of the 42 pages of the existing website. The TFGSF webmaster contacted area growers, the Tropical Research and Education Center' tropical fruit crops specialist, and the Miami-Dade County Cooperative Extension agent to learn of the most recent information about tropical fruit in the Florida industry. All weights, counts, and fruit descriptions were reviewed and, if needed, corrected and updated. Recipes were replaced with new ones. Grower information was updated. Photographs were changed. New web links were inserted into the new website.

This new website program, WIX, is much easier and more intuitive to manage the website content than the previous website program, Word Press. This will be an asset when the as content is updated throughout the year.

Task 3. Have all English content translated into the Spanish language and place on a new, duplicate website for Spanish speakers. This new website address is www.tropicalfruitgrowersespanol.com

As the English pages were updated, the text of each page was emailed to Mr. Ruben Regalado, a retired Miami-Dade County Extension agent. Spanish is his first language and he translated all the website content into Spanish. We had considered using a translation feature on the English site, such as Google Translate, but decided against this after seeing the results of a translated page using Google Translate.

According to several Spanish speakers who read the translated page, the resulting text was not well written and “was not something Spanish speakers would say”.

Once the translated pages were emailed back to the TFGSF webmaster, they were then forwarded to the CHF webmaster who posted them on the Spanish website. By forwarding the pages to the TFGSF webmaster, she could keep track of which pages had been completed.

No other commodities benefited from this project.

Analytical tracking programs have been set up on both sites to monitor usage by the public.

Goals and Outcomes Achieved

Goal #1 - Create a more administratively friendly website and train TFGSF webmaster.

The existing website was converted from the Word Press platform, to a more user friendly platform, WIX. The TFGSF webmaster was trained on the new platform. The TFGSF webmaster has been the webmaster for the TFGSF site since its inception in 2008 with a Specialty Crop block grant. She agreed that the new website platform is easier and intuitive to manage the content of the site. While changing the website over to a new platform the look and feel of the website was updated to a fresher, cleaner look.

This task is complete and our target was met.

Goal #2 - Present consumers with the latest information about Florida tropical fruits.

Much of the content on the existing TFGSF website had not been updated or reviewed since it was created in 2008. The TFGSF webmaster reviewed the existing website content with area growers, and University of Florida Tropical Research and Education Center fruit specialist, and Miami-Dade County Extension agents. The Internet was also searched for new information on tropical fruits. All information

that had changed or needed revision was incorporated into the new website. All 42 pages of the website have been updated and revised.

This task is complete and our target has been met.

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Goal #3 - Increase consumer awareness of Florida tropical fruits to the Spanish speaking population.

Each of the updated English pages of the website were translated into Spanish, and then incorporated into a duplicate Spanish site that was made with this translated content. The new website address is www.tropicalfruitgrowersespanol.com. An analytics tracking program has been set up to monitor and track the usage of the site.

We had projected to have a few months of data of website usage before the grant ended, but we did not include that time table in our proposal. We were delayed during the grant development due to personal issues, and had requested and received a six month extension. We will monitor this new Spanish site as well as the newly updated English website for website usage.

The task is complete, and we will monitor the website usage.

Beneficiaries

We expect that the approximately 2,000 Florida tropical fruit growers as well as the ancillary businesses including packers, pickers, shippers, retailers, will benefit from this updated website by the increase demand from the exposure to the Spanish speaking consumer. According to the current analytics program on our existing website, of the top ten countries who visited the TFGSF website in the last 12 months, none of them were Spanish speaking countries. With our information now posted in Spanish on the Internet, we expect to be able to reach more Spanish speaking consumers within the United States and Canada, and well as consumers in Spanish speaking countries for possible export of Florida tropical fruits. We will monitor the analytics program to follow the progress.

Notification of the updated Tropical Fruit Growers website and the existence of the new Spanish Tropical Fruit Growers website is being sent to area agricultural organizations such as USDA, University of Florida, Miami-Dade, and local media.

Lessons Learned

We were very fortunate in the progress made by the grant. All work was completed with little incident, and all of our goals were met. There were few participants involved in the project which helped to reduce the problems that can occur with many participants. The transfer of the existing website to the

new platform was without incident due to the experienced webmaster that was employed. The translations were well done and the creation of the Spanish site went according to planned.

The only issue that occurred was a delay in the work progress due to the TFGSF webmasters personal issues that slowed work on the project for a few months. As reported, an extension was requested and approved. This did not affect the work being completed according to extended schedule, but we had failed in our proposal to allow for a monitoring period for the websites so that the data of the website usage could have been included in the final report. For those who may try a similar project with their websites, perhaps additional time should be factored in to the proposal so that initial data can be recorded in the final report. As we have been doing since the first TFGSF website was created, we will continue to monitor the website usage with the analytics program.

Overall Progress and Funding Expended

The project is 100% complete, with $8,336.28 expended to date.

Project 40: The Macon Community Garden Project

Contact
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Project Partners

The Community Organizing Assistance and Community Help Foundation, Inc. (COACH Foundation) is a private, not for profit community-based organization founded in 1981. COACH operates in economically depressed areas and helps to provide stable environments for growth. The Foundation partnered with the Macon Community Garden, Florida Agricultural and Mechanical University (FAMU), the City of Tallahassee and Mt. Zion Primitive Baptist Church to improve food security and nutrition within the Macon Community.

Project Summary

Leon County's poverty rate is over 20%, higher than the national and state rate. Within Leon County, the Macon Community is a low income predominately African-American neighborhood with limited access to grocery stores (50%) and a relatively high number of residents with limited transportation (20%) according to recent census data. Health studies show that low income African-Americans suffer from greater incidents of heart disease, obesity, diabetes and other health issues that can be avoided or improved with better nutrition education and a plant based diet. These families often have limited access to fresh produce, because food pantries concentrate on the distribution of packaged and canned foods.

The Macon Community Project is a Local Food System Model consisting of an active community garden site that promotes education to the community's children and adults, demonstrates specialty crop production, diversity, and the benefits of specialty crops that are key to successful preventative health strategies.

*A vital aspect of the Macon Community Project is to bolster specialty crop food consumption and the sustainability of local food systems by providing the community access to specialty crops producers at the Growers' Market at Lake Ella, a local participatory community farmers' market that features fresh organically grown specialty crops direct from local small farmers.

The Macon Community Model worked to provide local food choices that support local food systems, expands community education of nutritional benefits of specialty crops, improved public awareness of local organic methods specialty crop farmers and increases consumption of Florida's specialty crops. The model supports the "buy local Florida specialty crops" campaign which provides outreach to the community and public.

Macon Community is a neighborhood often overlooked because of the more affluent neighborhoods that surround it. This project provided direct support of food to insecure households within the Macon Community in an active and sustainable way. Families were introduced to Florida Specialty Crops

through nutritional education and hands-on participation in the organic gardens located within the neighborhood. By creating a sustainable organic food system, residents were provided a tool to improve their overall well-being though high quality nutritious food. The project also developed a blueprint for bringing together community resources such as churches, community gardeners, university faculty and others to improve nutrition while increasing food security in low income neighborhoods.

The project is timely because it provided direct support of food to insecure households within the Macon Community in an active and sustainable way throughout the year. This project has not been submitted to any other Federal or State grant program for funding.

Project Approach:

In the fall of 2015 and early 2016 the residents, community partners and volunteers built and installed fifty-two raised garden beds. These spaces were planted out each season of the project with Florida Specialty crops and herbs. After requests from residents with limited mobility in 2017, volunteers also built four standing raised beds. This allowed several disabled veterans to participate in the project. These new participants also provided additional maintenance support in the garden.

The garden beds produced over one thousand pounds of vegetables and herbs in 2016 and six hundred and fifty pounds in 2017. Vegetables and herbs were distributed to households in the Macon Community to increase food security. The crops planted included broccoli, cauliflower, cabbage, collards, squash, watermelon, green beans, basil, and oregano.

The Macon Community Garden and Florida A&M University Small Farmers 4-Week-Ends Youth Camp of (2016), focused on educating and providing hands on learning to youth within the Macon Community and surrounding areas. The camp introduced the youth participants and their parents to gardening and healthy eating at the Macon Community Garden, Tallahassee, FL. The main components of the camp consisted of teaching and learning sessions that allow children to have hands-on practice with organic gardening skills including planting crops, weeding, and watering the garden. Simple and healthy food demonstrations were conducted in order to expose youth participants to new healthy recipes they can recreate at home.

The FAMU Statewide Small Farm Program played an integral role in activities associated with increasing awareness of agriculture through a "Buy Local" program included participation in several planning meetings to discuss project development, providing education, training /capacity building to enhance the sustainability of local food systems, the development and implementation of qualitative evaluation tool to gather information from public participating in the specialty crop Growers' Market. The development and implementation of a capacity building Earth Day event, the development and implementation of a "What is Organic Agriculture" learning session at the FAMU Farm FEST. The advertising was done through radio spots supporting "Buy Local" and a billboard near the Lake Ella Farmer's Market.

Nutritional seminars were held to support the educational component of the project. These seminars introduced residents to strategies that improved their diet by incorporating Florida Specialty Crops.

The Macon Community Garden project team and The FAMU Statewide Small Farm Program together created a Blue Print and a Policy & Procedural manual for on-going garden operations along with a blueprint document to assist others in developing similar garden projects. Residents also contributed

recipes to be shared at events. These recipes encourage the use of Florida Specialty Crops prepared in a healthy way.

Goals and Outcomes Achieved:

Goal: Increase seminar participant knowledge of the use of specialty crops in a nutritious diet from a baseline of 20% to 70%.

Measure: Percent of participants exhibiting knowledge of Florida Specialty Crop use in a nutritious diet.

Results: Participants of each nutrition seminar were reluctant to complete surveys on personal activity, but were willing to provide verbal responses regarding the lessons learned in the seminars and discuss among themselves how they would adopt a more nutritious diet.

Goal: Increase the consumption of Florida Specialty Crops by Macon Community participants.

Measure: Percent of community participants consuming Florida Specialty Crops in their weekly diet.

Results: The baseline of community participants who consumed Florida Specialty Crops in their weekly diet was about 20%. Over 50% of the surveyed participants reported they weekly consumed Florida Specialty Crops. While the basic crops provided to each household were common items in their diet, the project also introduced herbs to each participant and how to use them in cooking as part of the effort.

Goal: Increase food security for Macon Community Garden participants from a baseline of ten households to seventy-five households.

Measure: Number of households receiving Florida Specialty Crops to use in a nutritious diet.

Results: During the first year of the project at least 75 low income households were provided fresh vegetables and herbs during the growing seasons to improve their overall food security. These families reported consuming fresh vegetables and herbs daily during the project instead of a few times per week before the project.

Due to crop damage from weather and vandalism, only forty-five households received direct food support in the second year of the project.

Goal: Increase awareness of community supported agriculture through a "Buy Local Program" focused on the Growers Market at Lake Ella whose organic growers produce Florida Specialty Crops.

Measure: Percent of shoppers at Growers Market at Lake Ella have heard or seen advertising campaign and were motivated to attend.

Results: Baseline surveys were completed, as well as short interviews with shoppers showed that the implementation of the marketing campaign, which included radio advertising and a billboard located near the farmers market, was a success. It was reported that more than 90 percent of shoppers at the Lake Ella Market saw the billboard / road signs and attended the market based on the advertising. Participants reported hearing radio advertising supporting the "Buy Local Program". The most important finding was that "word-of-mouth" advertising was the most common driver of new shoppers trying out the Lake Ella Farmer's Market.

Beneficiaries:

Food insecure families within the Macon Community were the direct beneficiaries of the project. These households directly benefited from consuming Florida Specialty crops as part of their weekly diet.

Currently food insecure households have to stretch their food budget, often not having enough food for an entire month. This project provided supplemental food for each household and improved the overall health of these households by ensuring that healthy options for meals and snacks were available.

During the first year of the project at least 75 low income households were provided fresh vegetables and herbs during the growing seasons to improve their overall food security. These families reported consuming fresh vegetables and herbs daily during the project instead of fewer times per week before the project.

Due to crop damage from weather and vandalism only forty-five households received direct food support in the second year of the project.

Adult residents of the Macon Community also had access to nutritional seminars during the project.

Approximately fifty youth in the Macon Community over the age of 16 participated in the 2016 Youth Training program which provided hands-on gardening sessions, and education about the nutritional benefits of eating of local specialty.

Lessons Learned:

Incentives

The first lesson learned is that incentives can increase participation in both program attendance and with completing surveys. Offering incentives such as lunch for youth programs and other incentives for additional garden work can bring additional volunteers to the project. Another lesson learned about survey responses was that participants value anonymity. Removing any identifying questions or collecting the information with a person to person interview instead of a written survey was preferable.

Youth Participation

Increasing the budget for youth education and training would have provided more opportunities for reinforcing healthy eating habits and hands on gardening among young people in the community. The 2016 project was well attended and the community requested more opportunities like this.

Maintenance Staffing

On-going maintenance on the irrigation and basic garden infrastructure was needed. Since many community residents are elderly or very young, it is important to have a person's responsible for maintenance and to have a budget to pay for both the materials and labor needed.

Financial Management

Financial management was one of the key issues throughout the project. Communities with few resources have difficulty prepaying for work and materials and then waiting to be reimbursed. Future projects need to adjust timelines to prepare for the time needed to draw down advances or find a community partner that will provide resources as a stop gap for reimbursements.

The preparation of monthly financial reports would also help ensure everyone on the project is aware of the resources consumed and still available. It will also help when quarterly and semi-annual reports are due.

Crop Security

The reduced food production due to weather was further complicated by some vandalism to several beds, in which crops were uprooted and the bed structures were damaged. Local law enforcement officials recommended the installation of wildlife cameras as a cost effective method of monitoring the area while continuing to pursue funding for fencing and other physical deterrents on the property. Fencing the garden area should also reduce crop theft which was a problem throughout the project. The garden area is on a road that is traveled not only by residents, but is used as a cut through to neighboring areas.