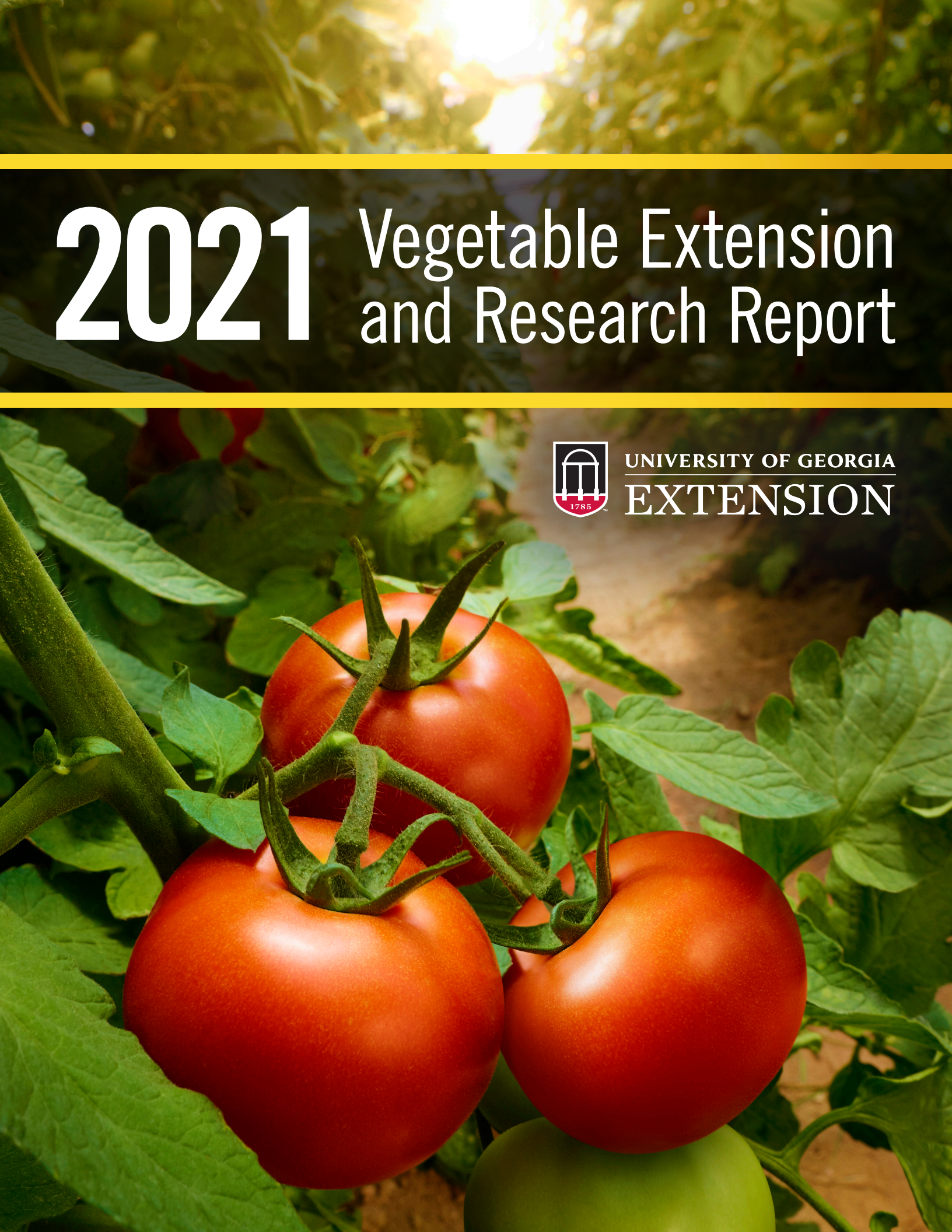
2021 Vegetable Extension and Research Report



UNIVERSITY OF GEORGIA
EXTENSION

Letter from Commodity Commission Chair

The Georgia Commodity Commission for Vegetables is pleased to submit this annual report to the vegetable growers of Georgia outlining the accomplishments made during 2021.

In the year covered in this report, GACCV supported 18 research projects with more than $180,000. These projects focused specifically on benefiting and educating producers that grow the following commodities:

- Beans
- Beets
- Bell Pepper
- Broccoli
- Cantaloupe
- Carrots
- Cabbage
- Cucumbers
- Eggplant
- Greens
- Specialty Pepper
- Sweet Potatoes
- Squash
- Tomatoes

With grower assessment funds, these researchers have evaluated irrigation rates on peppers, studied mulch types in row-middles to reduce disease, continued research in whitefly management in sweet potato and snap beans, evaluated sweet potato varieties, and much more.

The research performed for these projects has provided growers with the opportunity to reduce production costs, increase yields, and improve profitability. If you have an interest in serving on any committees or the commission, please let us know.

We look forward to continuing to serve the vegetable growers of Georgia.

Sincerely,

Dick Minor, *Chair*

Funds from the Georgia Vegetable Commodity Commission were used to support all of the research outlined in this report. Without the continued support of the farmers who contribute to the commission, this research would not be possible. In addition to outlined research, commodity grant funds are used to support activities at the Tifton Vegetable Park and the Plant Pathology Diagnostic Lab at the UGA Tifton campus.

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COMMISSION FOR VEGETABLES

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Efforts in Education

County Extension Agent Continuing Education

In 2021, the Commission provided funding for Georgia county Extension agents to pursue continuing education opportunities.

Faces of Georgia Grown

To help promote Georgia grown products, the Commission provided funding to the Georgia Grown Pavilion at the Georgia National Fair.

Georgia Farm Monitor

The Commission gave $4,000 to the Georgia Farm Monitor in 2021. This TV show is produced by Georgia Farm Bureau and works to tell the story of Georgia farmers.

Southeast Fruit and Vegetable Conference Education Supporter

By supporting the Southeast Regional Fruit and Vegetable Conference Educational Conferences, farmers are presented with the latest in vegetable research. The Commission gave $6,000 to the conference in 2021.

Vegetable Commodity Fund Financials Fiscal Year 2021 (July 1, 2020, to June 30, 2021)	
Item	***Amount***
Assessment Received	$247,396
Bank Account Balance (6/30/21)	$327,226
Liabilities	$99,293
Uncommitted funds to carry forward to fiscal year 2022	$227,933
Items Paid in Fiscal Year 2021	
Bank Charges	$328
Mailing of Referendum Ballots	$175
Sponsorship for SE Regional Fruit and Vegetable Conference	$6,000
Preparation and Printing of Annual Report	$4,007
Administrative Cost to Georgia Department of Agriculture	$6,071
GA Farm Bureau – Farm Monitor Show Sponsor	$4,000
UGA Research Projects	$188,100
Total Expenses	**$208,681**

2021 University of Georgia Vegetable Extension and Research Report

Timothy Coolong, ***Editor***

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2021 University of Georgia Vegetable Extension and Research Report

Using real-time diagnostic tools to enhance fertilization and reduce costs with fertilizer on vegetables

A. da Silva, T. Coolong

Introduction

Nutrient analysis of tissue samples assists growers in monitoring, evaluating, and adjusting fertilizer rates for a more efficient use of nutrients. Samples are commonly analyzed using laboratory techniques, which have a low cost but sometimes delay receiving results. Using real-time diagnostic tools to analyze plant tissues in field conditions is an alternative that may more quickly determine nutrient content in the plant tissues. Real-time diagnostic tools that evaluate levels of nitrate (NO_3-N) in the foliar sap and/or the soil solution are rapid tests that can provide important information about the nutritional state of plants. This allows faster decision-making to determine fertilization rates during crop development. The objective of this study was to evaluate a real-time diagnostic tool for nitrogen content in bell pepper production in Georgia.

Material and methods

Field experiments were conducted at the UGA-Tifton Vegetable Park during spring 2020. Bell pepper plants were grown using conventional practices with black plastic mulch and drip irrigation. Three fertilizer strategies of low fertilization (100 lb of N/acre), recommended fertilization (180 lb of N/acre), and high fertilization (260 lb of N/acre) were set as treatments and replicated four times in a randomized complete block design. Fertilizer was applied at preplant using complete granular fertilizer (5-10-15 at rate of 50 lb of N/acre), while fertilizer during the growing season was applied weekly with fertigation using liquid fertilizer (7-0-7), in which N rates varied according to treatment.

Crop development was monitored weekly and plant samples were collected at four stages of crop development: vegetative growth, flowering, fruiting, and fruit maturity. Two plants from each plot were collected and combined as a composite sample. Composite samples were analyzed for nitrate (NO_3-N) concentrations using the LAQUA Twin meters (real-time diagnostic tool). Subsequently, samples were oven-dried at 65 ºC to constant weight and submitted for nitrate (NO_3-N) analysis at the commercial lab (Waters Agricultural Laboratories in Camilla, GA). This allowed for correlations between data from the real-time diagnostic tool and commercial lab. Bell pepper fruit were harvested at maturity and yield estimated in number of fruit and total yield. All data were statistically analyzed at R-Studio.

Results

During the growing season, the three fertilizer strategies were used to allowed to identify optimum and stress levels (e.g., deficiency and excess of nutrients) in plant tissue (Figure 1).

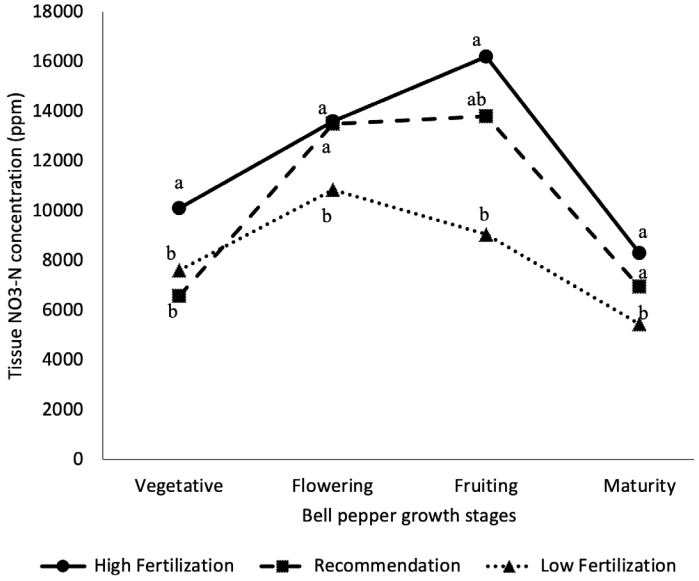

Figure 1. Concentration of NO_3-N in bell pepper plant tissue according to growth stage.

In the vegetative stage of crop development, the high fertilization treatment allowed for a higher concentration of NO_3-N in the bell pepper plant tissue compared to the recommendation and low fertilization treatments. However, the concentration of NO_3-N in bell pepper plants were similar for the high fertilization and recommended treatments at flowering, fruiting, and maturity. As would be expected, the low fertilization treatment had the lowest concentration of NO_3-N in bell pepper plants at flowering, fruiting, and maturity.

The concentration of NO_3-N in bell pepper plants from each fertilization strategy was reflected in yield of bell pepper plants. The high fertilization and current recommendation treatments had a higher number of bell pepper fruit and total yield compared to the low fertilization treatment (Figure 2). Those results indicate that the application of 260 lb of N/acre does not result

in higher yields, compared to 180 lb of N/acre. Consequently, there can be savings with fertilizer application if the current recommendation of applying 180 lb of N/acre is used.

All data collected with the real-time diagnostic tool for NO_3-N were correlated with the concentrations from the commercial lab results for NO_3-N. This correlation was considered statistically significant; however, it did not present a 1:1 ratio, which means results from the real-time diagnostic tool for NO_3-N cannot be directly compared to results from the commercial lab (Figure 3).

Conclusion

Overall, results indicated that the current recommendation of N fertilizer application (i.e., 180 lb. of N/acre) for bell pepper can provide similar yields compared to higher N fertilizer applications (i.e., 260 lb. of N/acre). The evaluated real-time diagnostic tool proved to be a good resource for the growers to access NO_3-N in bell pepper plants; however, it will not show similar results samples sent to a commercial lab. More data sets are required to ensure proper use of this tool and continued studies are required for additional calibration of the evaluated real-time diagnostic tool before providing growers training.

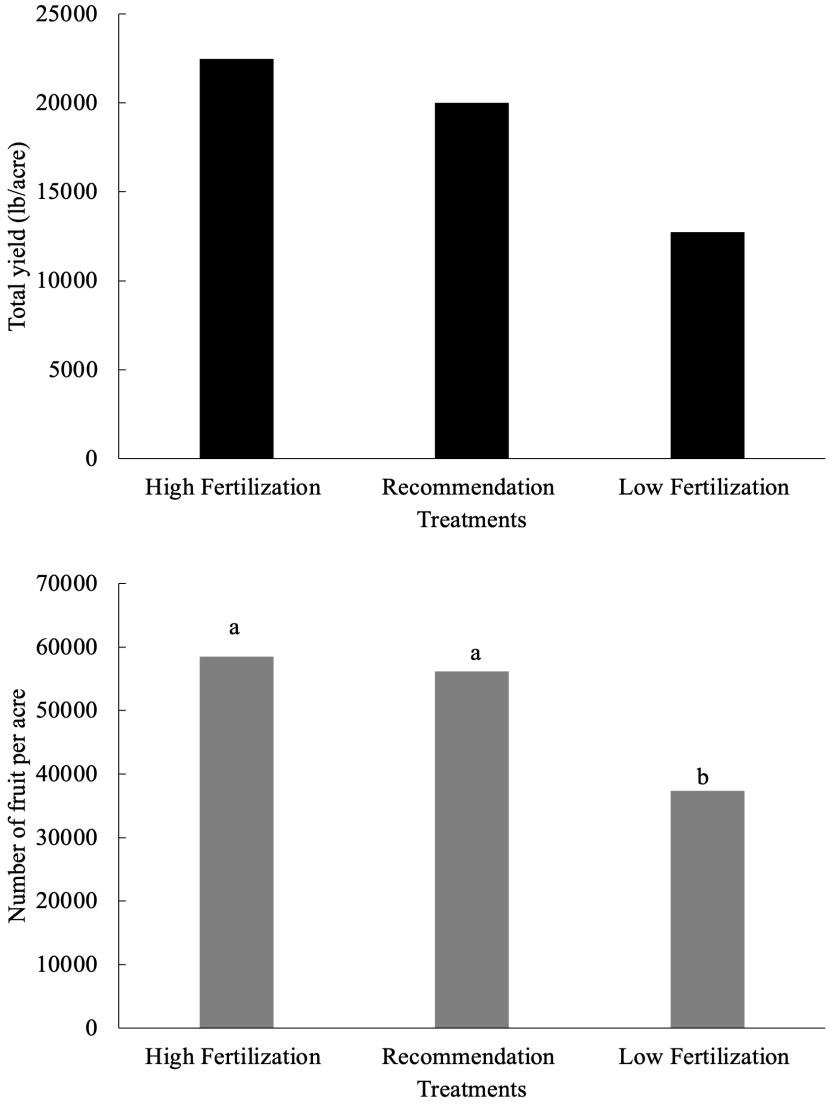

Figure 2. Number of bell pepper fruit per acre and total yield (lb/acre) for fertilization treatments.

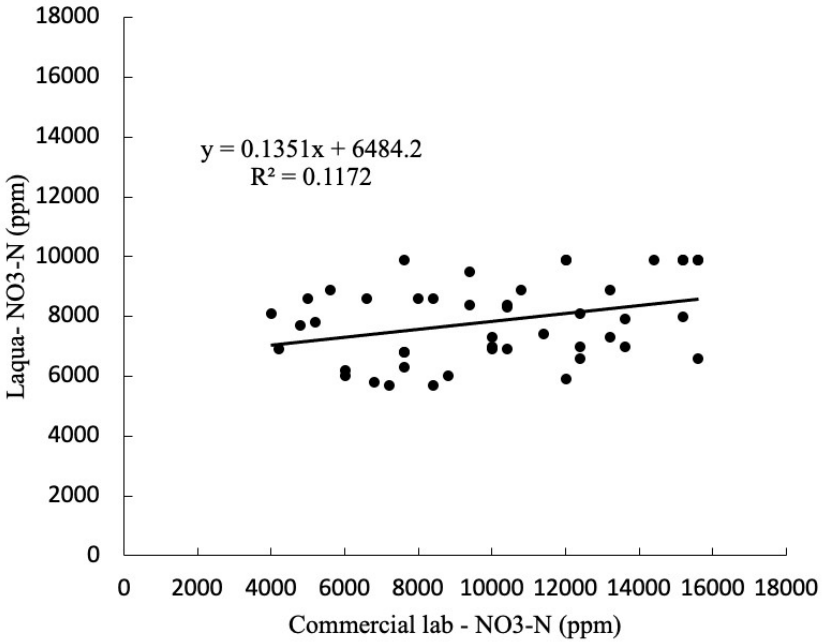

Figure 3. Correlation between NO_3-N measure with the real-time diagnostic tool (Laqua) and at the commercial lab.

Management of tomato yellow leaf curl disease in tomato using colored plastic mulches and shade nets in high tunnels

S. Bag, J. Díaz-Pérez

Introduction

Plastic film mulch has significantly contributed to increases in vegetable production and yield. The current project mainly focuses on determining the effects of plastic film mulches and shade cloth on the incidence of whitefly transmitted tomato yellow leaf curl virus (TYLCV) on plant health and fruit yield in tomato grown organically in high tunnels. Whiteflies are a pest of a wide variety of horticultural and agronomic crops in southern Georgia and have been detrimental to vegetable production for several years. Management of viruses mainly relies on controlling the insect vector though the deployment of broad-spectrum synthetic insecticides, but new strategies are immediately needed for their control because of the rapid evolution in insecticide resistance.

Objectives of the study:

1. Evaluating the role of black and white mulches on the whitefly-transmitted TYLCV, and
2. Determining the incidence of TYLCV in the high tunnels covered with three different shades, no shade, silver, and black.

Material and methods

Tomato cultivars 'Red Snapper', and 'Focus' were planted at the UGA Tifton campus and grown organically in high tunnel structures. Experimental design was randomized block design, with three replications and four treatments (two plastic mulches: white and black; and three shade net treatments: black, silver, and unshaded. Disease incidence was monitored weekly and symptomatic plant tissues were collected from individual plants to detect the virus using molecular tools.

Results

In the fall of 2020, the impact of either black or white mulch inside high tunnels covered with black (Figure 1a), silver (Figure 1b) or no netting (Figure 1c) on tomato yellow leaf curl disease (TYLCD) was evaluated. Plants were monitored weekly for eight weeks for symptom development starting 15 days after transplant (DAT) (Figure 1d-f). The virus was detected in 99.8% of symptomatic samples (n =72) tested by PCR using TYLCV specific primers, giving high confidence to visual scoring of the disease.

There was high pressure from whiteflies, and 100% disease incidence was observed by 42 DAT (Figure 2). In 2020, disease incidence and severity under both black and silver shade nets were more than no shade control. There was no significant difference between mulch types on disease incidence and severity.

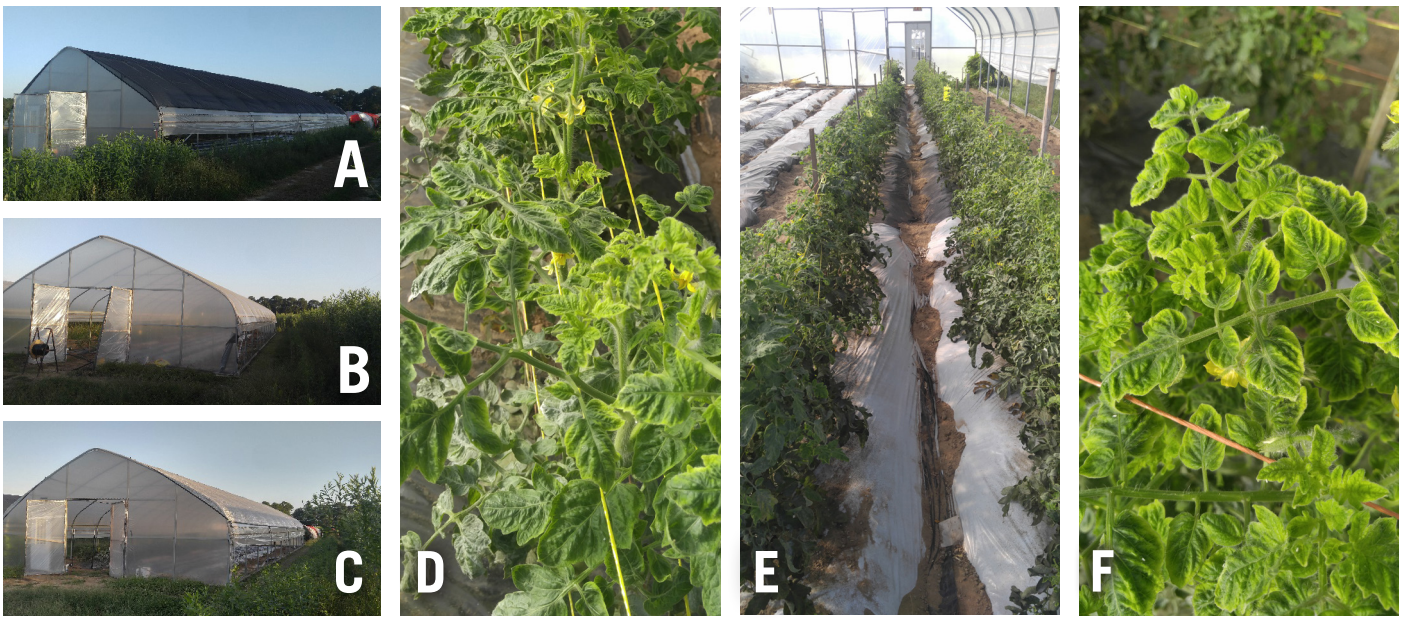

Figure 1: High tunnel with shades (A) black, (B) silver, and (C) no shade. (D-E) Symptomatic plant with white and black mulches and (F) tomato yellow leaf curl disease symptoms on tomato.

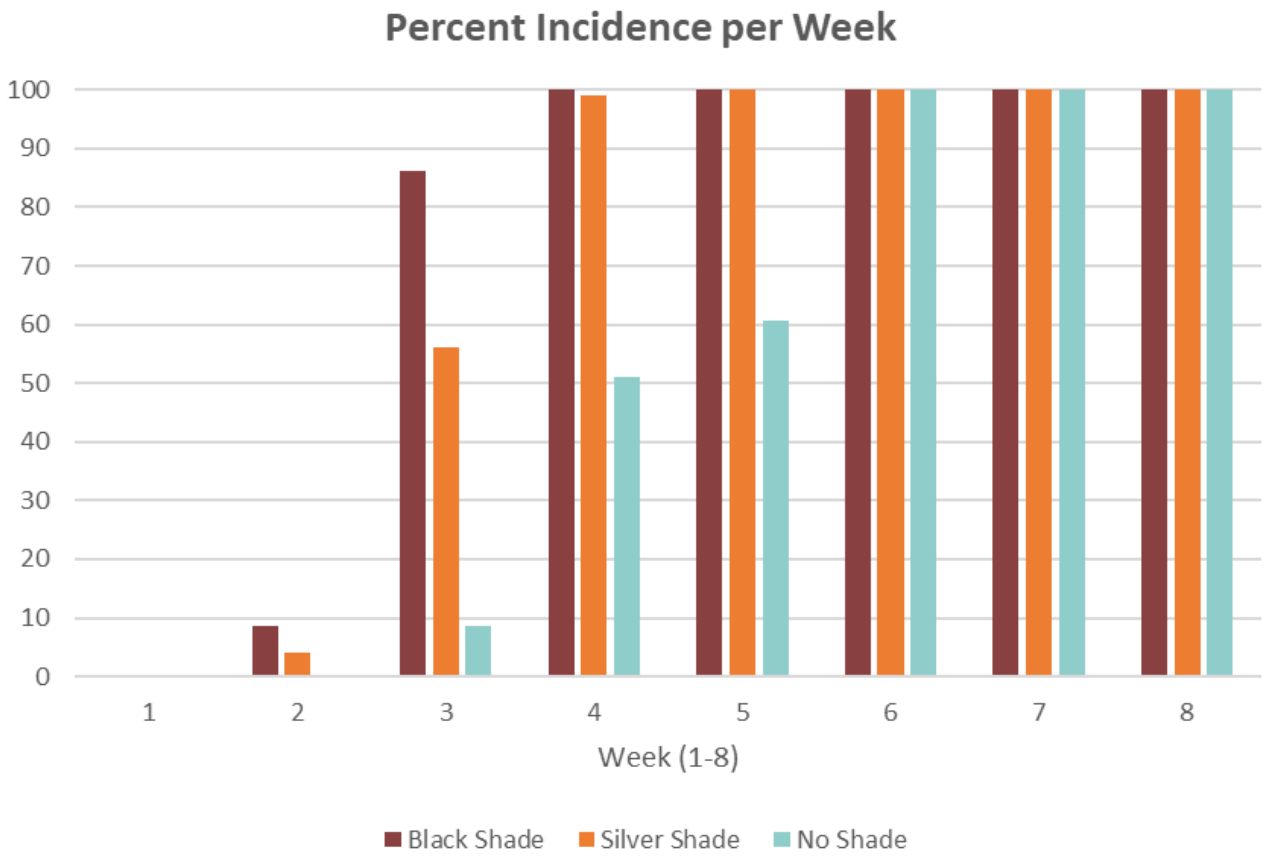

Figure 2. Percentage incidence of tomato yellow leaf curl disease using different shades.

Conclusion

Results indicate that these shade nets and plastic mulch treatments were not effective at reducing TYLCD severity in two resistant tomato varieties under organic cultivation, underscoring the need for further investigation to improve TYLCV disease management. There is a need to screen the commercially available resistant varieties against TYLCV strains prevent in Georgia under organic cultivation.

Residual activity from Roundup and Rely can damage vegetables when applied preplant over plastic mulch

S. Culpepper, T. Randell, J. Vance

Introduction

Georgia vegetable growers often use the same raised beds covered with plastic mulch for three to five cropping cycles, extending over an 18-month period. Glyphosate (Roundup) and glufosinate (Rely) are tools that could assist in removing weeds between crops, facilitating a weed-free planting window for the next crop. To avoid crop contact through foliar splashing from the surface of the mulch, these herbicides can be effectively washed from the mulch with rainfall or overhead irrigation. However, potential crop injury from residual activity through herbicide concentration in old planting holes, or in other openings where natural degradation of the plastic has occurred, is unknown.

Material and methods

Six weed-free plastic mulch studies determined tomato, cucumber, and squash response to preplant applications of Roundup and Rely when transplants were placed 1. in newly punched plant holes, avoiding contact with herbicide treated soil (new hole), 2. in an existing plant hole, contacting soil exposed to herbicides (old hole), and 3. in a newly punched plant hole that was located exactly 6 inches over from old plant holes (each side) exposed to the herbicide (adjacent hole). Within each planting arrangement study, Roundup PowerMax II or Rely was applied at 32, 64, or 128 oz/acre with treatments replicated four times per study. ***Following each herbicide application but prior to transplanting, overhead irrigation of approximately 0.5 inch was implemented to wash the herbicide from the mulch.*** Visual injury, crop biomass, crop growth, and yield were measured; tomato, cucumber, and squash were harvested 13-16, 15, and 30 times, respectively.

IMPORTANT NOTE: Rely is not currently labeled for use in vegetable crops. This research is being conducted in support of a hopeful future label. An update on Rely labeling for Georgia produce growers will be provided at the annual vegetable conference in Savannah on Jan. 7, 2022.
ALL HERBICIDES MUST BE APPLIED FOLLOWING LABELED DIRECTIONS ONLY.

Results

Vegetable response to Roundup: Crop injury was less than 4% from all treatments when plants were placed in new holes or adjacent to old holes; no impact on crop growth, biomass, fruit number, or fruit weights were recorded (*as noted in the methods, the herbicide was washed off the mulch prior to punching holes or transplanting and plants did not contact treated soil*).

When transplants were placed in old holes contacting herbicide treated soil, a negative influence on plant development was observed (Figure 1). Tomato, cucumber, and squash injury reached 23% (squash) to 40% (tomato) at the higher use rate. Reducing rates lessoned the negative impacts on crop injury, growth, and yield, but note that injury was observed for all plants transplanted into the old plant hole.

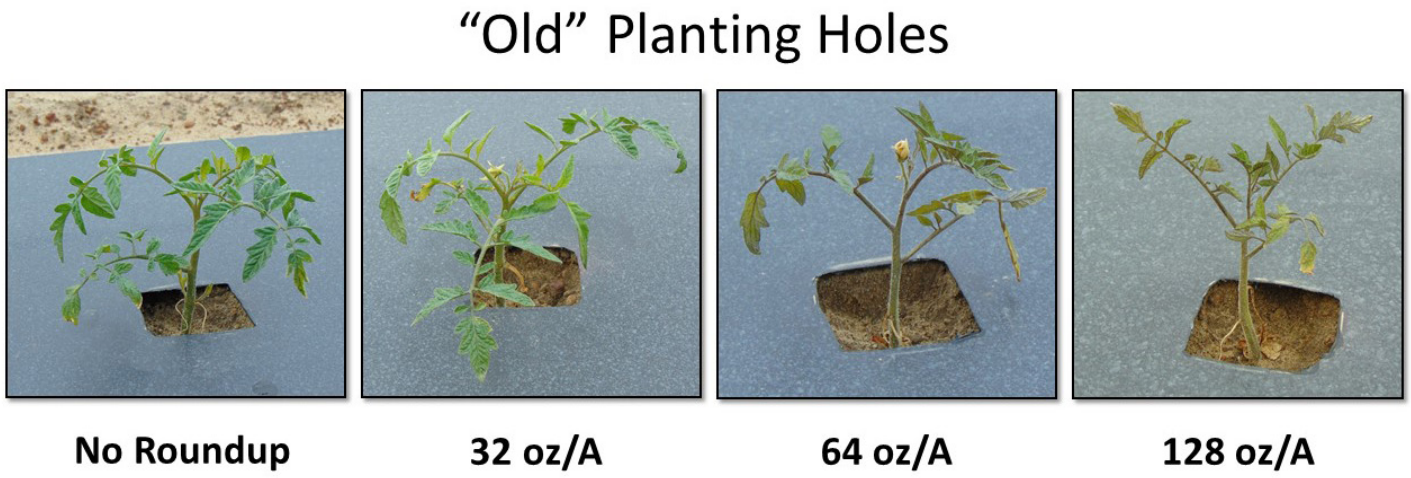

Figure 1. Influence of Roundup rate on tomato injury at 23 days after planting.

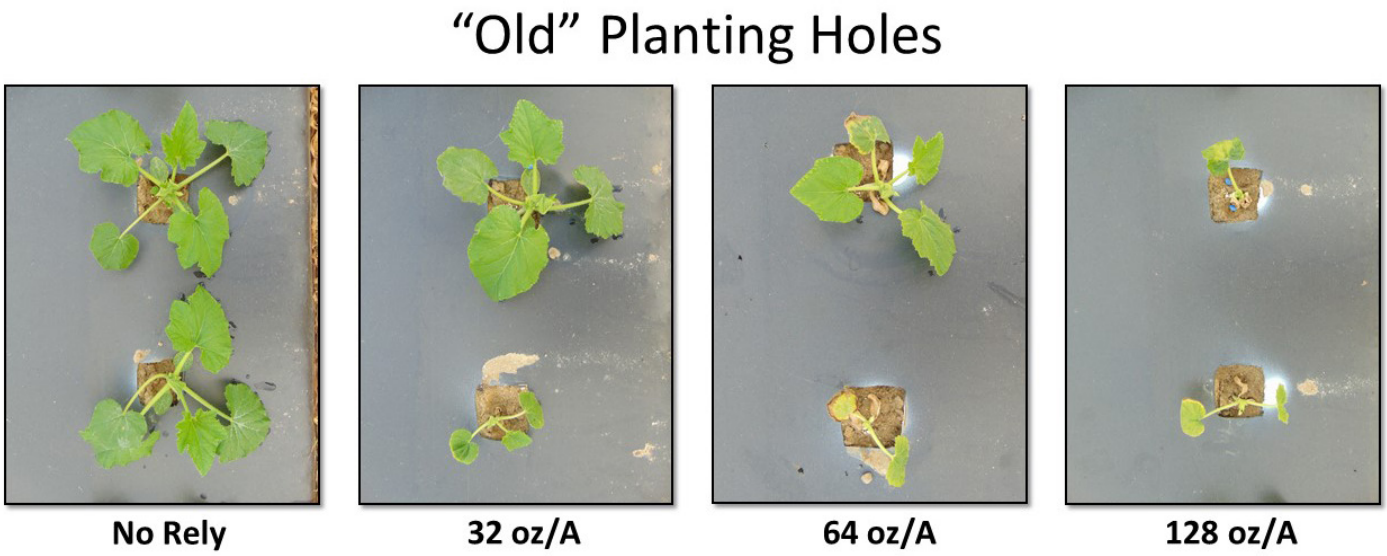

Figure 2. Influence of Rely rate on squash injury at 16 days after transplanting.

Vegetable response to Rely: Crop injury was less than 8% from all treatments when plants were placed in new holes or adjacent to old holes; no impact on crop growth, biomass, fruit number, or fruit weights was recorded (*as noted in the methods, the herbicide was washed off the mulch prior to punching holes or transplanting and plants did not contact treated soil*).

Tomato plants placed in old holes were not injured regardless of rate. In contrast, cucumber and squash injury ranged from 35-76%, biomass reductions ranged from 44-88%, and fruit yield losses ranged from 25-70% with Rely at 64 oz/acre or 128 oz/acre. Although cucumber and squash injury from 32 oz/acre was less than at higher rates, injury was significant and must be avoided.

Conclusion

Results of this experiment suggest that tomato, cucumber, and squash can be safely planted into raised beds covered with plastic mulch treated with Roundup or Rely, **but only if**:

1. The mulch is washed with an 0.5 inch of rainfall or irrigation after the herbicide application but before hole punching.
2. Transplants are placed 6 inches from old holes or tears in the mulch at the time of the herbicide application. Transplants placed in areas where the herbicide has contacted the soil may be damaged, depending on herbicide rate and the crop planted.

Support of the UGA Georgia Weather Network

P. Knox

Introduction

The UGA Weather Network provides 15-minute weather data and monitors soil conditions at 86 locations around the state, mainly in agricultural areas. The support from the Vegetable Commission helps us maintain the stations, store the data, calibrate instruments, and work with Extension agents to monitor crop conditions, pest and disease pressure on vegetables, and allow us to explore expansion of the network.

Material and methods

Our network of 87 Campbell Scientific automated stations is maintained by one full-time and one part-time technician, an electronics engineer who provides IT support and manages the network, and a quality control specialist who monitors the data for errors and makes appropriate corrections. The technicians visit the stations every four to six weeks to clean and repair equipment and ensure that quality siting conditions are maintained. Instruments are rotated out and calibrated on a regular schedule. The IT specialist maintains the network and is working on moving the historical data files from a Griffin-based service to online cloud storage for improved access.

In 2020, we added a station at Glennville and temporarily removed the station at Alpharetta due to siting issues. In 2021, we installed a station at Sparks and restored the Alpharetta station at a new location.

Results

In 2020, our network maintained nearly continuous availability of current weather data other than some temporary delays due to cell network outages. This continued into 2021 in spite of personnel reductions and travel time due to the pandemic from mid-March until August. When cell service was disrupted, all stations stored the weather data, which was restored as soon as the cell service was reactivated.

In 2020, the work of moving the data storage of the network from server-based text files to a cloud-based database was continued. The migration has been temporarily delayed due to the hiring freeze at UGA. We hope that the migration will be complete by the end of 2021.

Cell modems were scheduled to be replaced due to the imminent demise of 3G cellular service, and that process was completed in early 2020.

Conclusion

Thanks to the support from the Vegetable Commission as well as other commodity commissions in Georgia, the network performed well and consistently provided continuous, current high-quality data to Extension agents and producers around the state on demand.

We provided additional archived data to scientists and students for specialized studies of disease and pest management on request. We hope to continue this service to vegetable producers and expand our range of tools in the coming years.

MiniRAE 3000 prevents fumigant damage to vegetable crops on a large scale

J. Shealey

Experiment overview

In 2020, a MiniRAE 3000 (Honeywell, Charlotte, NC) was purchased by UGA Extension in an effort to help vegetable growers better understand the importance of fumigant placement and lifespan when shank injected into the soil within a plasticulture raised bed system. Specifically, the meter is used to determine when the soil fumigant has dissipated to a level that is safe to plant so long as the labeling restrictions have been met. Another goal of using the fumigant meter was to assist with worker safety.

With the loss of methyl bromide, alternative fumigant systems have been developed and adopted. These alternative fumigant systems are effective but are more challenging than methyl bromide primarily because the time interval between fumigation and planting is extended. Plant back intervals for methyl bromide never exceeded 10 days while current fumigants have been documented to stay in the soil in some cases for over 45 days. It is not possible to accurately predict how long current fumigants will stay in the soil because soil moisture and environmental conditions, which change daily, greatly influence the fumigant stability.

Because of this challenge, UGA bought a fumigant meter in 2014 for the Echols County area. This fumigant meter allows Extension personnel to check grower fields prior to planting to help determine whether it is safe to plant. This program addressing grower requests to check fields has grown significantly, with over 50 fields checked for soil fumigants in 2014 in the Echols area alone. In 2015, the number increased to over 120 fields checked. Additionally, the meter is in such demand it is being used across Echols, Lowndes, Clinch, Berrien, Cook, Brooks, Colquitt, Tift, Grady, Decatur, and Turner counties. Simply put, Extension was not able to meet the requests of our growers with a single fumigant meter. Since 2015, the Georgia Commodity Commission for Vegetables has awarded two grants to UGA Extension, placing new meters in Lowndes, Berrien, Grady and Tattnall counties. Since February 2017, the fumigant meters are being used in the previously discussed counties as well as Mitchell, Seminole, and Thomas counties.

Growers can see the immediate impact of these meters. One producer laid plastic on 40 acres over a two-day period with a small rain in between. When the field was checked with the fumigant meter, it was found that half of the field was ready to plant and the other half still had fumigant levels high enough to kill the transplants. Had the producer proceeded on a calendar schedule alone, or had the field not been properly checked, the producer would have lost roughly 20 acres of bell pepper transplants. The loss would have been around $38,500 for the plants alone, not including labor, fertilizer, and other costs associated with transplanting. The grower said, "The fumigant meter is one of the best tools county agents can have to help vegetable growers. There is no way at my size I could have overcome a $38,500 loss at the beginning of a crop." Producers also shared that they were able to get into the fields a week and a half earlier, on average, which gave them a longer growing season and better return on investment.

There is a science behind using the fumigant meters correctly and understanding all the factors that influence fumigant stability in the soil. Agents have been trained on how to properly use the meters to ensure success as well as how environmental factors influence what is happening in a producer's field. The education of growers and farm workers is extremely important because it allows them to better understand the products they are using and find the fumigant system for each farm to ensure product stability and maximized yields.

Criticial factors to understand when determining plant back intervals:

Fumigant – The fumigant product(s) used influences plant back intervals.

Rate – The amount of fumigant injected in the bed directly impacts the lifespan of fumigant in the bed.

S**oil type and texture** – Heaver (higher clay content) soils tend to hold fumigants longer than sandier soils.

Plastic – LDPE, VIF, TIF mulches influence fumigant life; the greatest gas escapes occur through LDPE mulch with the least occurring through TIF.

Moisture – Soil moisture, time of application, and low lying field areas that hold water can either increase or decrease plant back depending on product.

Weather – Air temperature, soil temperature, and humidity can influence degradation and volatilization of fumigants applied.

Educational objectives:

- Work with vegetable growers in Georgia to gain a better understanding of the new fumigant systems.
- Use the meter as a tool to show growers how fumigant rate, plastic selection, weather, and soil type determine the amount of time needed between application and planting.
- Help determine area in a field where fumigants tend to last longer or escape faster.
- Help ensure worker safety.

Survey of food safety pathogens found on produce imported to Georgia

R. Raad, M. Aaron, Y. Ortega, G. Hirsch, C. Rodrigues, A. da Silva, L. Dunn

Introduction

Increased costs associated with domestic vegetable production, including labor and required implantation of food safety best practices, have impacted the profitability of growing specialty crops in Georgia. Meanwhile, farms in parts of Mexico and Central America have increased exportation to the U.S., which may have resulted in diminished returns on Georgia-grown vegetables. Farms importing produce into the U.S. are subject to the same food safety regulations as domestic entities but concerns regarding enforcement of these policies have been raised, making consumers question the safety of produce grown outside the U.S. To address these concerns, a microbial survey of vegetables imported from these regions was conducted in Georgia grocery stores and farmers markets.

Material and methods

Fruits of different vegetable crops (Table 1) were sampled from three farmers markets in Atlanta, Georgia, and three grocery stores in Athens, Georgia. Because *Cyclospora cayetanensis* infections most frequently occur in the U.S. during the warmer months, sampling occurred during July, August, and October of 2020 (Table 2). Most vegetables were imported from Mexico or Central America (Table 3).

Table 1. Imported vegetables sampled for detection of *Cyclospora cayetanensis* or *Salmonella enterica* in summer 2020.

Vegetable	Number of Samples	
	Cyclospora	*Salmonella*
Cucumber	0	33
Tomato	96	33
Bell Pepper	21	33
Chile	93	0
Other*	15	0
Total	**225**	**99**

*Cilantro, sweet pea, sugar snow pea, radish

Table 2. Number of vegetable samples collected each month.

Sampling month	Number of Samples	
	Cyclospora	Salmonella
July	78	72
August	99	27
October	48	0

Table 3. Country of origin and number of samples collected for detection of *Cyclospora cayetanensis* or *Salmonella enterica*.

Country of Origin	Cyclospora	Salmonella
	Number	Number
Mexico	210	93
Guatemala	9	0
Costa Rica	3	0
Dominican Republic	3	0
Mexico/Canada*	0	6
TOTAL	**225**	**99**

*Country of origin not specified, either Mexico or Canada

To detect *Cyclospora cayetanensis*, 265 vegetable samples were washed with elution buffer in filter bags. The DNA was extracted with the FastDNA Spin for Soil Kit (MP Biomedicals, Irvine, CA) and tested using a nested PCR described by Li et al. (2019; 18S rDNA of 500bp). For detection of *Salmonella enterica*, vegetable samples (n=99) were incubated in Universal Pre-Enrichment Broth overnight before a 1 mL aliquot was added to tubes containing Tetrathionate broth or Rappaport-Vassiliadis broth and again, incubated overnight. Ten µL from each broth tube was streaked onto Xylose Lysine Tergitol-4 agar; presumptive positive colonies were confirmed using polymerase chain reaction (Rahn et al., 1992). Samples for both organisms were examined in triplicate.

Results

Two hundred twenty-five vegetables were screened for the presence of *C. cayetanensis* during July, August, and October 2020 from three Atlanta farmers markets. None of the samples examined tested positive for *Cyclospora*.

Ninety-nine vegetable samples were screened for *S. enterica* in July and August of 2020 from three Athens-area grocery stores. *S. enterica* was not detected on any of the collected samples.

Conclusion

No *Salmonella* or *Cyclospora* were found on imported vegetables collected from stores or markets in Georgia in 2020. However, the small-scale nature of the study provides only one snapshot of the overall sanitary condition of imported produce. Also, the absence of these two microorganisms does not necessarily mean that other pathogenic microorganisms are not present, including pathogenic *Escherichia coli*.

Additionally, this work did not examine contamination in other commodities frequently implicated in foodborne outbreaks, such as leafy greens. Future work including a larger sample size, more frequent sampling, additional commodities, and less localized sampling (i.e., imported product collected outside of Georgia) may give a more representative picture of the sanitary quality of imported produce. For more comprehensive surveillance for *Cyclospora*, future sampling should also include leafy greens and herbs imported from Mexico.

References

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Requesting funds for advanced diagnostic support to benefit Georgia vegetable growers

J. Brock, B. Dutta

Introduction

The Extension programs in the Department of Plant Pathology are designed to reduce plant disease losses by educating and assisting UGA Extension county faculty and producers. The Tifton Plant Disease Clinic supports plant disease management programs through diagnostic services for commercial vegetables for the entire state of Georgia. Pathogen groups to be diagnosed or identified include fungi, bacteria, and viruses. A fast, accurate diagnosis not only helps reduce yield losses, but also allows for elective treatments that can reduce or eliminate unwarranted extra chemical applications.

Material and methods

Funds from this request were used to maintain diagnostic capabilities, especially for hard-to-identify pathogens such as bacteria and viruses. Detecting and identifying a pathogen might depend on isolation of fungi or bacteria on growth media, the use of immunological test kits (strip tests), sequencing of DNA, or the use of polymerase chain reaction (PCR).

Results

During 2020, the Tifton Plant Disease Clinic received approximately 475 vegetable samples from nearly 45 counties and represented over 40 vegetable crops. The top 10 most common crop samples are in Figure 1. Approximately 200 samples required the isolation of either a fungal or bacterial pathogen, 55 required the use of an immunological test kit, and 20 were submitted for DNA sequencing and testing.

Conclusion

The funds provided by the Georgia Commodity Commission for Vegetables (2020) were instrumental in providing advanced, accurate, and timely diagnoses of vegetable samples. Advanced level testing allowed for confirmation of bacterial diseases such as bacterial fruit blotch of cucurbits or black rot of crucifers. Viruses are also difficult to diagnose without these advanced diagnostic tools. White-fly transmitted viruses of crucifers and beans are an emerging problem that also require molecular testing to confirm. These are just a few of many pathogens that have diagnoses dependent on advanced testing. Without the funding, the confidence level and turnaround time for vegetable sample diagnoses would both suffer. In large part due to the funding, Georgia vegetable growers and the vegetable industries receive plant health information that helps shape management strategies.

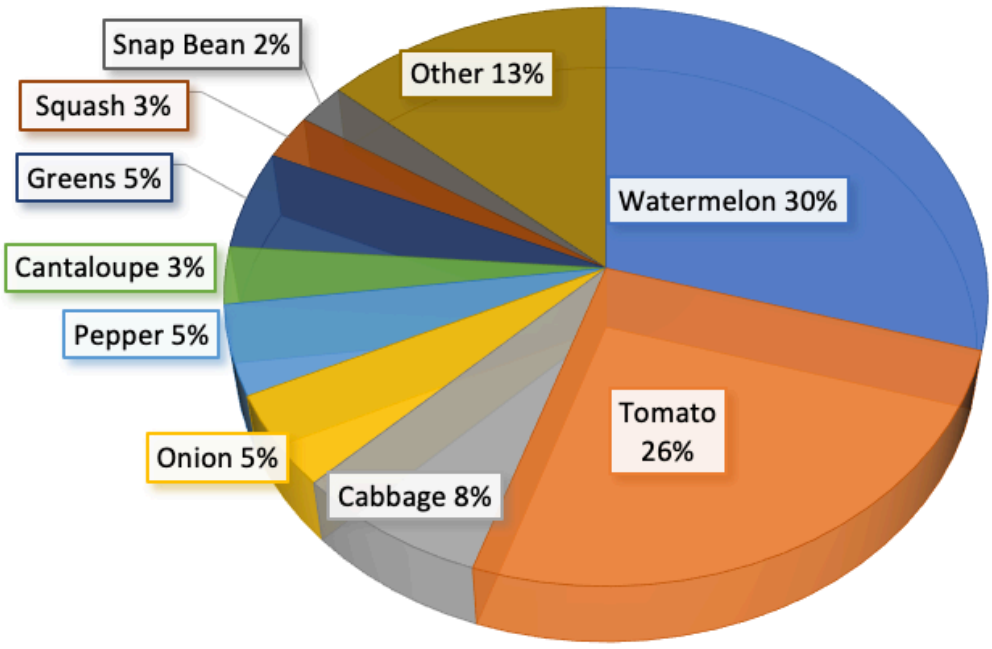

Figure 1. Percentage of samples by crop.

Development of early and rapid LAMP detection of downy mildew caused by *Pseudoperonospora cubensis* on vegetables

E. Ali, S. Waliullah, P. Ji

Introduction

Downy mildew caused by *Pseudoperonospora cubensis* is one of the most destructive foliar diseases on vegetables. Downy mildew can affect vegetable plants of all ages. Initial symptoms are small chlorotic spots on the upper leaf surface and gray- to purple-colored downy mold growth that develops on the undersides of leaves. In favorable weather conditions (warm and wet), the lesions expand, and the infected tissue eventually becomes necrotic (dead) leading to leaf death and defoliation.

At present, chemical control is the most effective remedy for controlling downy mildew at the early stage of infection. However, sometimes downy mildew can be hard to diagnose accurately during early infection, when symptoms can easily be confused with other diseases or nutritional problems. Rapid and sensitive detection techniques for monitoring the presence and severity of infection would help growers to apply chemicals with more accuracy and at a higher efficiency without the risk of crop loss. Therefore, a rapid and low-cost on-site molecular detection method would be of great significance for the proper management of vegetable downy mildew. Molecular diagnosis based on polymerase chain reaction (PCR) methods exists for this pathogen. However, these methods are time-consuming and require access to sophisticated and bulky laboratory equipment. In particular, the complexity of the thermal cycling equipment required for PCR restricts the use of these methods mainly to well-equipped laboratories. The objective of this study was to develop a loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Pseudoperonospora cubensis* at the early stage of infections.

Material and methods

P. cubensis-specific LAMP primer set was designed using Primer Explorer version 5 based on the published genomic region (c2555.3e7) unique to and conserved in *P. cubensis* isolates (Withers et al., 2016). The assay condition was optimized, and amplified products were detected using relative florescence values provided by the Genie® III instrument.

Results

The resulting assay successfully identified *P. cubensis* samples only — CDM1 and CDM2 (Figure 1). The samples were tested with extremely low concentrations of DNA for sensitivity. The assay specificity was carried out using various closely related oomycetes for specificity including *Pseudoperonospora humuli*. It was shown to specifically amplify DNA of *P. cubensis* and the detection limit was 100 picograms (Figure 2).

Conclusion

Pseudoperonospora cubensis is the causal agent of downy mildew and the most economically destructive disease to cultivated cucurbits. This disease causes significant damage to cucurbit production in the United States as well as worldwide. An advanced early detection method of this oomycete pathogen is essential for effective chemical controls with more accuracy and at a higher efficiency.

In this study, we have developed a LAMP technique that is suitable for early detection and has the potential to overcome many of the limitations of traditional diagnosis. The sensitivity of LAMP is 100 times higher than a regular PCR assay. The LAMP assay can be carried out rapidly (often 30 minutes) with minimal equipment (a water bath or heated block). Compared to all other existing methods, this assay is highly effective for on-site detection because of its low cost, high specificity, efficiency, simplicity of operation, rapidness, and ability to be used in broad applications.

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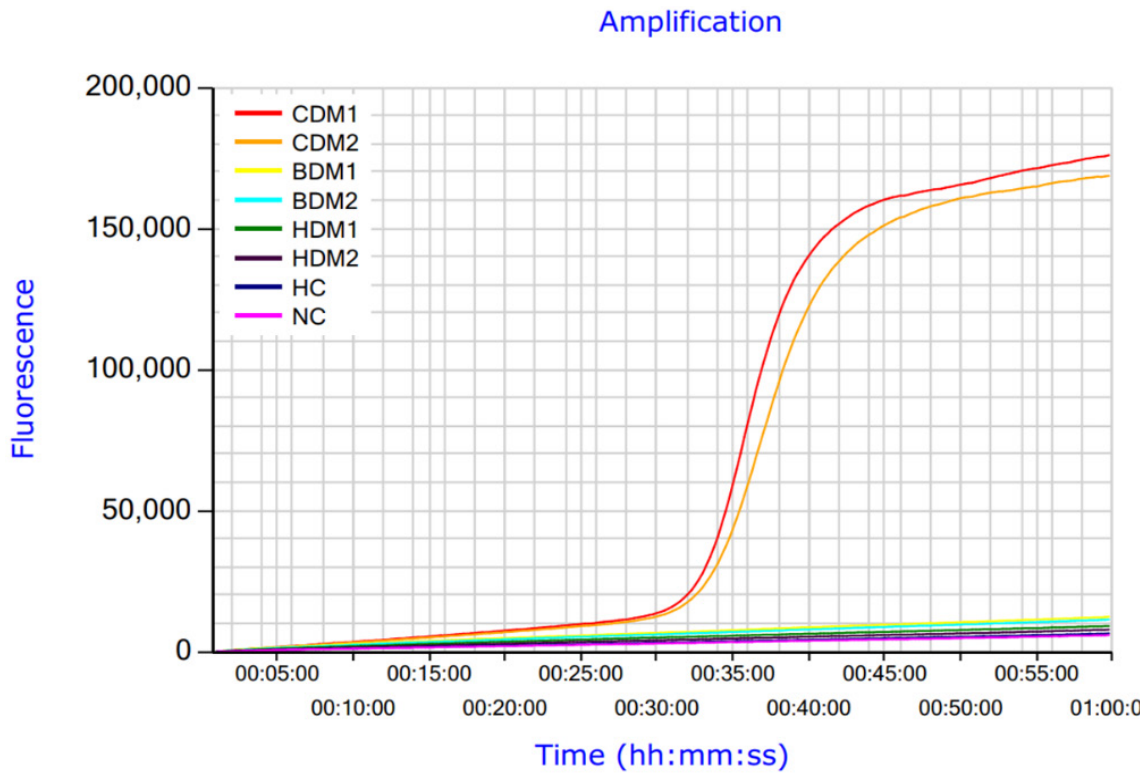

Figure 1. LAMP assay results. Here CDM1 and CDM2 are *Pseudoperonospora cubensis* samples from cucurbits; BDM1 and BDM2 are *Peronospora belbahrii* from basil; HDM1 and HDM2 are *Pseudoperonospora humuli* from hop downy mildew; HC for a health control, and NC for a negative control (dH_2O).

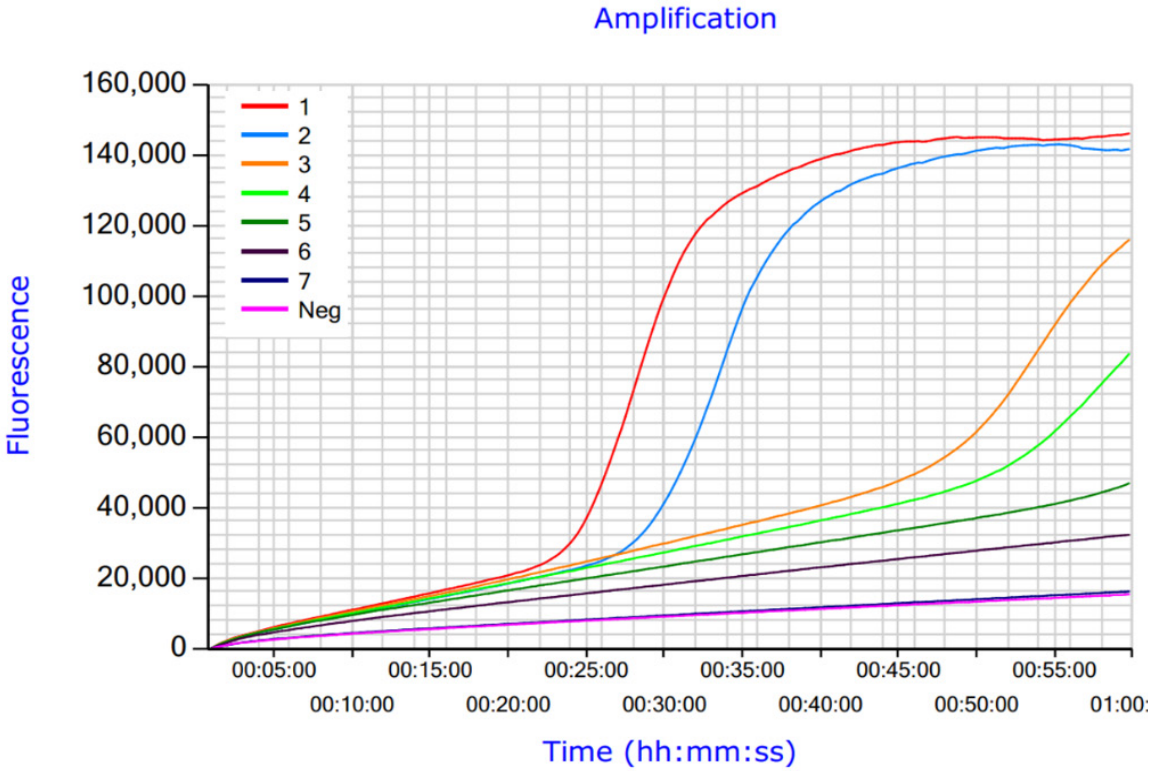

Figure 2. LAMP amplification of serially diluted DNA extracted from *P. cubensis* spores. The results were viewed using relative florescence values provided by the Genie® III instrument. Concentrations are as follows: 1 (10 ng), 2 (1.0 ng), 3 (0.1 ng), 4 (0.01 ng), 5 (1.0 pg), 6 (0.1 pg), 7 (0.01 pg), and Neg (Negative Control-Water).

Statewide monitoring of *Phytophthora capsici* in irrigation water sources for vegetable production using newly developed loop-mediated isothermal amplification assay

E. Ali, S. Waliullah, P. Ji

Introduction

Phytophthora blight, caused by the plant pathogen *Phytophthora capsici*, is one of the most devastating diseases of solanaceous and cucurbit crops in Georgia. It is a water mold that can attack the roots and foliage of crops causing root rot, crown rot, leaf lesions, and finally, wilting. Heavy rainfall in Georgia makes the disease a widespread problem for vegetable production. This pathogen produces spores (e.g., sporangia and zoospores) on the surface of diseased plant tissues, and the spores can be easily washed out by splashing rain, contaminating nearby irrigation sources like ponds or lakes. For proper identification of *P. capsici* in irrigation water sources, our lab recently developed a filter paper-based loop-mediated isothermal amplification assay (LAMP) which could detect low concentrations of the pathogen in water (unpublished data). Our recent survey of farms in Tift County, Georgia, using this new technique showed that 3 out of 10 irrigation ponds were positive to this pathogen. These findings confirmed that this pathogen can survive in irrigation water that may serve as an inoculum source. To date, there was no extensive statewide survey conducted for monitoring of *P. capsici* in irrigation water sources in Georgia. The objective of this study was to conduct a statewide survey to detect irrigation water contamination by *P. capsici* using newly developed loop-mediated isothermal amplification assay. We also compared the sensitivity of PCR and LAMP assays.

Material and methods

Irrigation ponds were chosen first at the county level (Figure 1). A researcher and Extension agent then visited each site and took 1 liter of water from each site by throwing a 5-liter plastic pail attached to a rope into the pond. Debris, plant material, and heavy sediment were avoided. One liter of water was then added to a pre-sterilized plastic bottle for transport to the laboratory. A novel loop-mediated isothermal amplification (LAMP) primer set was used to amplify *P. capsici*, based on a 1121-base pair (bp) fragment of *P. capsici* using PrimerExplorer V5 software (Hudson et al., 2020). The assay condition was optimized and amplified products were detected with three different methods: relative florescence values provided by the Genie® III instrument, naked eye color change visualization (pink to yellow), and 1% agarose gel visualization on a UV gel doc.

Results

Of the 42 ponds tested, 10 were positive for the presence of *P. capsici* (19%) (Table 1). Of the nine counties surveyed, five had ponds that tested positive (55.5%). An example of LAMP assay results of six samples are shown in Figure 2. Of the samples tested, two were positive for the LAMP assay but negative for the PCR assay, suggesting that assays with increased sensitivity could be put to good use in similar surveys of irrigation water where the ratio of pathogen propagule to water volume is low.

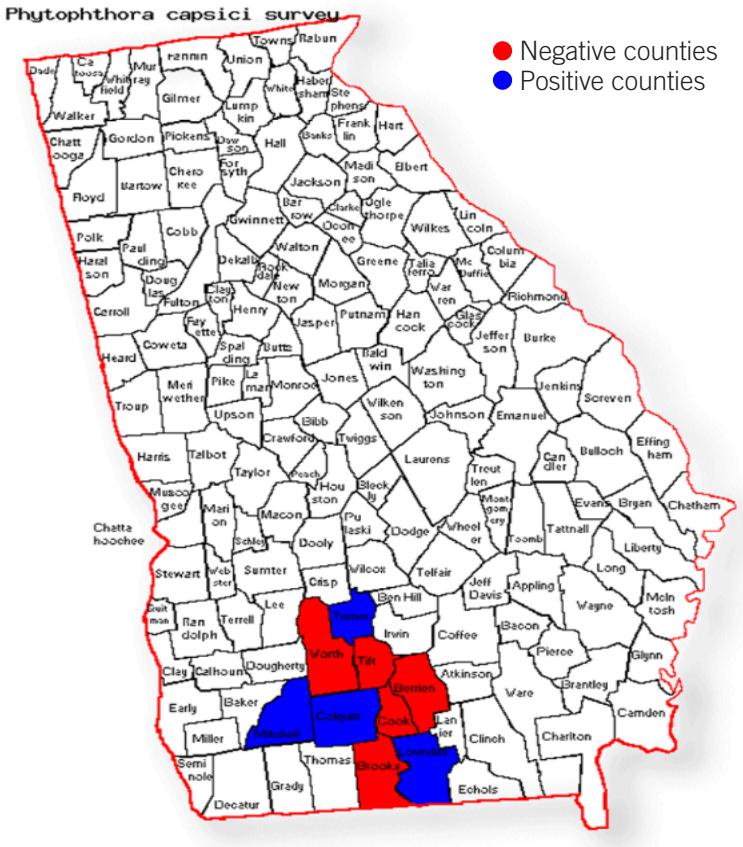

Figure 1. Map of Georgia counties sampled. Blue counties were sampled but negative for *Phytophthora capsici*, and red counties had ponds that tested positive for *P. capsici*.

Table 1. Imported vegetables sampled for detection of *Cyclospora cayetanensis* or *Salmonella enterica* in summer 2020.

County	Sample collected	Positive PCR for *P. capsici*/total	Positive LAMP for *P. capsici*/total
Berrien	5	1/5 (20%)	2/5 (40%)
Brooks	3	1/3 (33%)	1/3 (33%)
Colquitt	4	0/4 (0%)	0/4 (0%)
Cook	6	2/6 (33%)	2/6 (33%)
Lowndes	4	0/4 (0%)	0/4 (0%)
Mitchell	4	0/4 (0%)	0/4 (0%)
Tift	7	3/7 (43%)	3/7 (43%)
Turner	5	0/5 (0%)	0/5 (0%)
Worth	4	1/4 (25%)	2/4 (50%)

*PCR = polymerase chain reaction; and LAMP = loop-mediated isothermal amplification.

Conclusion

In this study, 42 irrigation ponds in nine counties in south Georgia were surveyed for the presence of *P. capsici* using a novel filtration method in conjunction with a LAMP assay specific for *P. capsici*. Ten ponds in five counties were found to have *P. capsici* as detected from the assay. The results of this study suggest that a significant proportion of irrigation ponds that growers actively use in south Georgia are contaminated with potentially damaging plant pathogens such as *P. capsici*.

Growers or nurseries that use irrigation water, particularly in south Georgia or other agriculture-heavy locations, should test their water source or employ the services of a molecular detection laboratory to detect pathogens that could be harmful to their crop. Using the filtration method introduced in this study, other diagnostic laboratories can develop and combine other specific assays for pathogens that commonly occur in irrigation ponds with new molecular assays to assist their local growers or nurseries.

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Alternaria leaf spot in brassica: Determining Fungicide Resistance and Diversity of *Alternaria brassicicola* in Georgia

B. Dutta

Introduction

Cabbage is an important vegetable commodity in United States for both processing and fresh consumption. Cabbage production in the U.S. in 2017 was approximately 1,845 million pounds. Georgia is a leading cabbage producer in the U.S. and the farm gate value of cabbage in Georgia was $53.5 million in 2017. Cabbage production has been negatively impacted by a number of pests and diseases. One such disease that affects cabbage production is Alternaria leaf spot caused by *Alternaria brassicicola*. Alternaria leaf spot has caused millions of dollars in losses annually and is a serious disease affecting cabbage in Georgia. *Alternaria brassicicola* can be seed, soil, or airborne. The pathogen can survive in the form of spores on infected crop debris from year to year. Warm temperatures (60-78 °F) coupled with at least 12 hours of relative humidity of 90% favors the disease. Under these conditions, the pathogen can sporulate profusely and spread throughout the field by wind, rain, splashing irrigation water, farm equipment, and workers. It has also been observed that the pathogen can be transmitted to healthy plants through the feeding of flea beetles. However, the main reason of disease introduction to newer areas is still unknown. Disease management is extremely difficult and often results in severe losses when the conditions are favorable. To control this disease, growers apply azoxystrobin and chlorothalonil at weekly intervals. However, recently, we reported insensitivity of Alternaria isolates to azoxystrobin, which further complicates disease management. In this proposal, we utilized species-specific PCR assay to decipher Alternaria species complex and major species that contribute to disease outbreaks. We also investigated the host range of Alternaria in crops belonging to the brassica family (broccoli, kale, cabbage, and collard) and are currently in the process of measuring the fungicide sensitivity profile.

Material and methods

Eight commercial brassica fields were surveyed. Overall, 108 *Alternaria* spp. were isolated, purified, and single-spore cultures were stored at -80 °C under further use. Each purified isolate was subjected to PCR assay with *A. brassicicola*- and *A. japonica*- specific primers. Further host range tests were conducted on four brassica hosts (cabbage, collard, kale and broccoli) using a modified detached leaf assay. Because the fungicide sensitivity assay needed significant optimization, the assay is currently underway and will be completed by December 2021.

Results

Utilizing *A. brassicicola*-specific primers, 108 Alternaria isolates from Georgia collected during survey were assayed and confirmed as *A. brassicicola*. We developed a detached leaf assay to assess aggressiveness of *Alternaria* spp. in brassicas. One hundred percent of the isolates collected were pathogenic on brassica hosts but aggressiveness varied considerably (Figure 1). Based on this assay, 83.7% of the Alternaria isolates were highly aggressive on broccoli whereas only 10.9% and 4.3% of the isolates were moderately and less-aggressive, respectively (Figure 1). Only 1.1% of the isolates were nonpathogenic on broccoli. The fungicide sensitivity assay required significant optimization and to this end, we have screened 35 *A. brassicicola* isolates for sensitivity to azoxystrobin. We found that the majority of the isolates were either intermediately sensitive or resistant to azoxystrobin.

Conclusion

A. brassicicola is the major driver of the Alternaria infection in brassica crops in Georgia. Based on the host range assay, these isolates are pathogenic on cabbage, broccoli, kale, and collard; however, the level of aggressiveness varied considerably. Based on the limited fungicide sensitivity assay, the majority of the isolates were either intermediately sensitive or resistant to azoxystrobin.

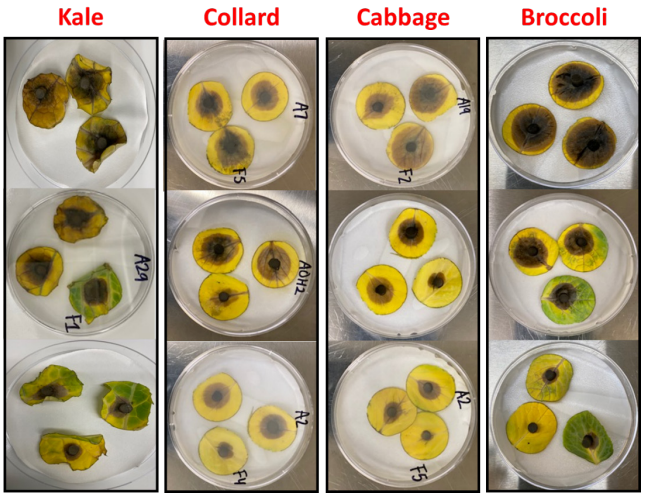

Figure 1. Detached leaf assay to determine pathogenicity and aggressiveness of *Alternaria brassicicola* isolates.

Evaluation of fungicides against Alternaria leaf blight of carrot

B. Dutta, M. Foster, M. Donahoo

Introduction

Alternaria leaf blight, cause by *Alternaria dauci*, is an endemic disease of carrot in Georgia. Yield losses due to this disease can be considerable, especially if it occurs early in the season. The symptoms of this disease include small lesions commonly found on the margins and tips of carrot leaflets. Under severe conditions, the lesions coalesce and give a blighted appearance. In some cases, larger lesions can develop in the petiole, which may result in the girdling and killing of leaves. Severe infection can also result in the death of the entire foliage. Severe defoliation and weakened foliage result in reduced harvest efficiency. Infection is favored by warm to moderate temperature and prolonged leaf wetness. Among management practices, chemical management through fungicides is important. However, due to a limited number of labeled fungicides and monoculture of carrot in some areas, disease management has been difficult. Also, limited information is available on carrot cultivars that are resistant or tolerant to this pathogen in carrot-growing regions of Georgia. In addition, the interaction of different labeled/experimental fungicides and cultivars has never been investigated. Hence, the overall goal of this proposed research is to evaluate different fungicides against Alternaria leaf blight of carrot.

Material and methods

The experiment was conducted at the UGA Blackshank Farm in Tifton, Georgia. Carrot (cv. Bolero) was direct seeded into six-row beds on 10 Dec 2019. Beds were on 6-ft centers with 1-in. plant spacing within rows (522,720 population per acre). Plots were 15 ft long with 10-ft unplanted breaks between plots within the row. The treatments were arranged in a randomized complete block design with four replications. Plots were overhead irrigated weekly as necessary using a pivot-irrigation system. Fertility and insecticide treatments were applied according to UGA Extension recommendations. The field has a history of Alternaria leaf blight infection since 2015, so natural infection was relied upon for this trial. Fungicide treatments were applied with a backpack sprayer calibrated to deliver 40 GPA at 80 psi through TX-18 hollow cone nozzles. Fungicide applications were made on 14-day intervals: 13 Jan., 27 Jan., 10 Feb., 24 Feb., 9 Mar., and 23 Mar. Plots not treated with fungicides served as non-treated check. Disease severity was assessed on 20 Feb., 5 Mar., 19 Mar., and 2 Apr. as percent leaf area with necrosis per plot and area under disease progress curve was calculated for each treatment. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (ANOVA) and the Fisher's LSD test to separate means at P=0.05. The mean rainfall received during Dec. 2019 and Apr. 2020 was 4.2 in. and 8.4 in., respectively. The average high and low temperatures for the month of Dec. 2019 were 58 °F and 44 °F, respectively, and for the month of Apr. 2020 were 82 °F and 63 °F, respectively.

Results

Alternaria leaf blight was first observed on 20 Feb. with 28.8% disease severity in the non-treated check. During the same disease assessment period, disease severity was significantly higher in the non-treated check compared to other treatments. Among the treatments, fungicides alternated with Penncozeb had significantly lower disease severity compared with the same fungicides sprayed without Penncozeb. Disease progressed gradually over the next seven weeks, and the final disease severity ratings were recorded on 2 Apr.

Based on disease ratings on 2 Apr., treatments comprised of Merivon and Penncozeb (35.5%), Luna Sensation and Penncozeb (39.2%), Pristine and Penncozeb (41.8%), Switch and Penncozeb (40.4%), or Miravis Primer and Penncozeb (38.5%) had significantly lower disease severity compared with other treatments and the non-treated check (Table 1). Alternaria leaf blight severity was not significantly different for treatments with the solo application of either Merivon, Pristine, Luna Sensation, Switch or Miravis Prime; however, these treatments had significantly lower disease severity compared to the non-treated check. AUDPC values followed the same trend as that of final disease severity ratings on 2 Apr. Merivon, Luna Sensation, Pristine, Switch, or Miravis Prime in a program with Penncozeb had significantly lower AUDPC values compared to other treatments and the non-treated control. Phytotoxicity was not observed.

Conclusion

Our data indicate that a fungicide program with Merivon, Luna sensation, Pristine, Switch, Miravis Prime, and Penncozeb can effectively manage Alternaria leaf blight in carrot.

Table 1. Summary of treatments, fungicide application frequency, disease severity, and area under disease progress curve (AUDPC).

Treatment and rate per acre	Fungicide applications[z]	Disease severity (percent)[y]		AUDPC[x]
		20 Feb.	2 Apr.	
Merivon 5.5 fl oz	1,3,5	17.5 b[w]	47.2 b	782.8 b
Pristine 10.5 fl oz	1,3,5	19.2 b	52.8 b	728.2 b
Luna Sensation 7.6 fl oz	1,3,5	16.5 b	54.5 b	705.5 b
Switch 11 fl oz	1,3,5	15.2 b	51.2 b	650.2 b
Miravis Prime 9.2 fl oz	1,3,5	14.8 b	55.0 b	798.8 b
Merivon 5.5 fl oz Penncozeb 2 lb	1,3,5 2,4,6	9.8 c	35.5 c	348.2 c
Luna Sensation 7.6 fl oz Penncozeb 2 lb	1,3,5 2,4,6	10.2 c	39.2 c	265.8 c
Pristine 10.5 fl oz Penncozeb 2 lb	1,3,5 2,4,6	7.2 c	41.8 c	382.2 c
Switch 11 fl oz Penncozeb 2 lb	1,3,5 2,4,6	4.8 c	40.4 c	278.5 c
Miravis Prime 9.2 fl oz Penncozeb 2 lb	1,3,5 2,4,6	3.2 c	38.5 c	245.5 c
Non-treated	N/A	28.8 a	64.5 a	1245.2 a

[z]Spray dates were: 1 = 13 Jan; 2 = 27 Jan; 3 = 10 Feb; 4 = 24 Feb; 5 = 9 Mar; and 6 = 23 Mar.

[y]Alternaria leaf blight severity was rated on a 0-100 scale where 0=0% leaf area affected and 100=100% leaf area affected on 20 Feb, 5 Mar, 19 Mar, and 2 Apr.

[x]AUDPC was calculated from ratings taken on 20 Feb, 5 Mar, 19 Mar, and 2 Apr.

[w]Means followed by the same letter in each column are not significantly different according to the Fisher's protected LSD test at P≤0.05.

Effect of deep application of non-fumigant nematicides on root-knot nematodes in tomato

C. Nnamdi, A. Hajihassani

Introduction

Root-knot nematodes (RKN; *Meloidogyne* spp.) are the most significant nematode pests of vegetable crops in Georgia. The use of non-fumigant nematicides is a desirable option for the control of *Meloidogyne* spp. in vegetable crops grown on plastic beds (Hajihassani, 2018). However, applying non-fumigant nematicides at the upper soil surface through drip tape might offer little or no control of the nematode populations that are present deeper in the soil profile. After application, these nematodes could move upward in the soil and cause harm to crops grown on the plastic mulch (Noling, 2016). This research was based on the hypothesis that application of non-fumigant nematicides through both surface (placed 2-3 inches below the plastic mulch) and subsurface (placed 12 inches below the plastic mulch) drip tape would offer better nematode control than the use of surface drip tape.

Material and methods

This experiment was carried out at the UGA Horticultural Hill Farm in the spring of 2019 and 2020 in Tifton, GA. A subsoil rig enabled the insertion of the subsurface drip tape at a depth of 12 inches in the soil. The surface drip tape was placed 2 inches below the soil's surface by a drip tape layer, and the plastic mulch was laid immediately afterward. The drip tapes for each plot were cut and the ends were closed using locking fittings to prevent cross-contamination among treatments. The drip tapes had an emitter spacing of 12 inches, and the plastic used was a white-on-black low-density polyethylene (LDPE) mulch. Fluazaindolizine (Reklemel or Salibro) at 30.1 fl oz/acre and fluopyram (Velum Prime) at 6.5 fl oz/acre were applied at transplanting, while fluensulfone (Nimitz) at 5 pt/acre was applied one week before transplanting. A CO_2 pressurized tank was used to inject each nematicide into a drip irrigation manifold. Prior to nematicide application, the beds were irrigated for two hours to allow for adequate bed moisture, increasing mobility of the nematicides in the soil. After the chemigation event, the irrigation was left running for 30 minutes to flush any remaining nematicides from the drip tape. Tomato seedlings were transplanted to the field with an in-row spacing of 24 inches.

Data on tomato fruit yield, root gall index, and soil population density of root-knot nematodes were evaluated. Tomato fruit were harvested by hand at maturity. Tomato roots were assessed for root-knot nematode galling using a 0-5 gall index (0 = root with no galls, and 5 = roots completely covered in galls) (Bridge and Page, 1980). Soil samples for nematode analysis in each plot were collected before nematicide application and at harvest. Data analyses were performed using SAS software, and treatment means were separated using Tukey's test.

Results

Drip tapes and Nematicide Interaction

There was no drip tape and nematicide interaction for fruit weight and end of trials root gall index and soil population density of *M. incognita* (data not shown).

Drip tapes

There was no drip tape effect on fruit weight, root gall index, and RKN soil population density at the end of trials (Table 1), suggesting that there was no difference in nematode control between surface drip tape vs. surface and sub-surface drip tapes.

Table 1. There was no drip tape effect on fruit weight, root gall index, and RKN soil population density at the end of trials, suggesting that there was no difference in nematode control between surface drip tape vs. surface and subsurface drip tapes.

Treatment	Fruit weight (kg/plot)	Root gall index	*M. incognita* number/100 cm^3
Surface drip tapes	11.17 a	0.6 a	21.19 a
Surface and subsurface drip tapes	9.68 a	0.89 a	27.38 a

Data are the means of two years and four replications (n = 8). Means with same letter in each column are not significantly different ($P < 0.05$).

Nematicides

Root gall ratings at the end of the trial were significantly lower in nematicide treated plots than the control plot (Figure 1). Plants in the fluensulfone plot had less ($P < 0.05$) root galling than in the fluopyram

treatment; however, this was not significantly different from root galling of plants in the fluazaindolizine plots.

The average number of *M. incognita* in the soil prior to year 2019 and 2020 field trials was 6.43 and 0.12 nematodes per 100 cm^3 of soil, respectively. RKN numbers at the end of trials were significantly lower in nematicide treated plots than the control plot (Figure 2). Fluensulfone did better ($P < 0.05$) than the fluopyram in reducing the *M. incognita* populations in the soil. Both fluensulfone and fluopyram had the same impact on the nematode population density (Figure 2).

Conclusion

The main objective of this study was to evaluate the impact of nematicide application through the surface and subsurface drip tapes vs. only the surface drip tape on *M. incognita*. Results showed that the type of drip tape utilized plays a minor role in nematode control in a single-crop plasticulture system. This technique, however, might be effective in multi-cropping systems of vegetables and requires further investigation.

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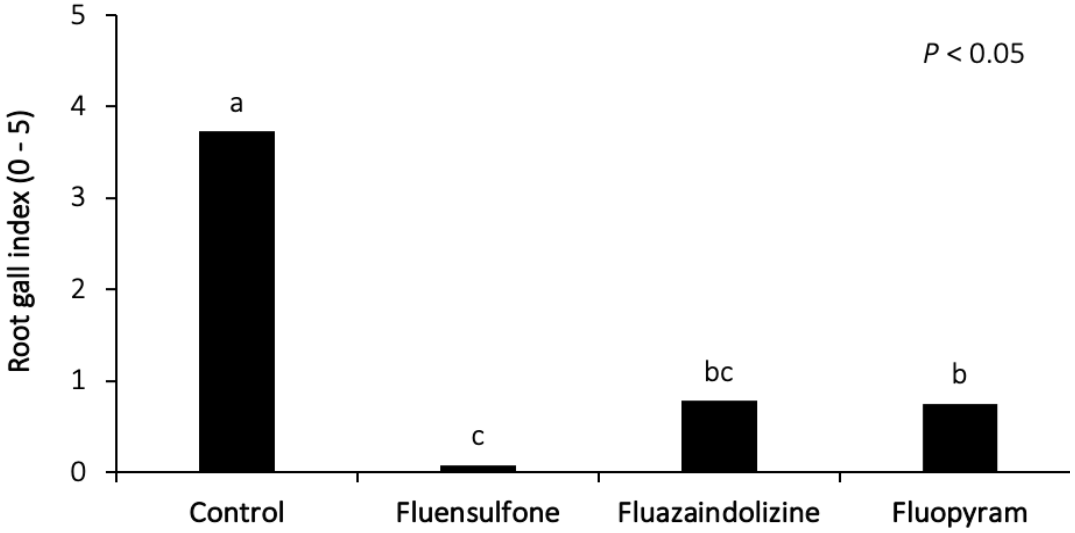


Figure 1. Effect of nematicide on root galling caused by *Meloidogyne incognita* on tomato.

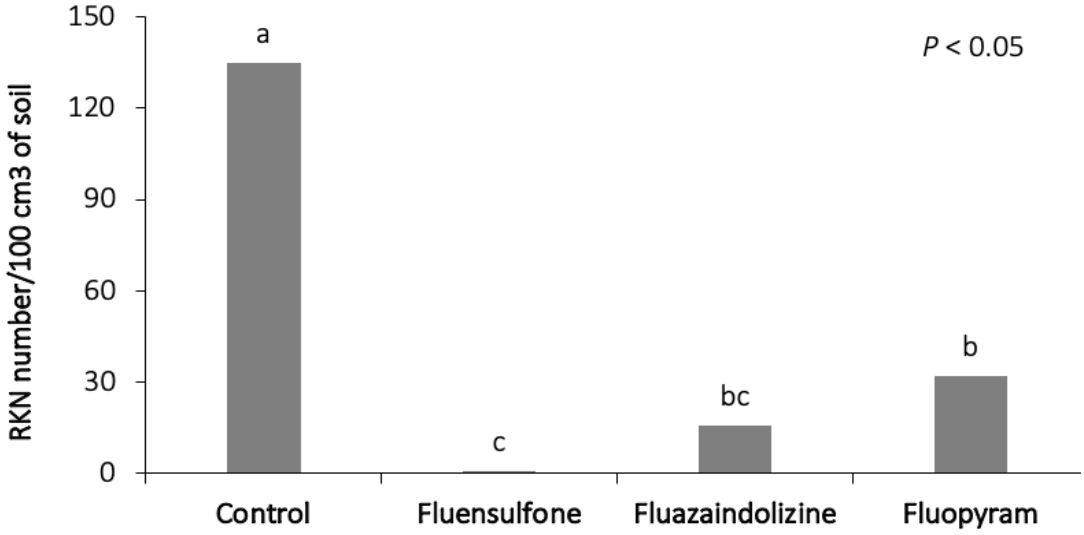


Figure 2. Effect of nematicide on soil population density of *Meloidogyne incognita* on tomato.

Managing whiteflies and whitefly-transmitted viruses in important vegetable crops of Georgia, 2020

R. Srinivasan, B. Dutta, T. Coolong, A. Sparks

Whiteflies and virus incidence in 2020

Whiteflies and a suite of whitefly-transmitted viruses continue to establish in Georgia and are becoming chronic issues. Fall 2020 was a moderate year for populations and viruses with a few sporadic high locations of intense populations. The viruses commonly found in 2020 included the tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) in tomato; cucurbit leaf crumple virus (CuLCrV), cucurbit yellow stunting disorder virus (CYSDV), and cucurbit chlorotic yellows virus (CCYV) in squash; and CuLCrV and sida golden mosaic virus (SiGMV) in snap bean. CCYV in squash was first identified in Georgia in 2020. CuLCrV and CYSDV/CCYV were often found as mixed infection in squash. Similarly, CuLCrV and SiGMV were found as mixed infection in snap bean. The mixed-infected plants are typically more symptomatic than plants infected with one virus and suffer heavy yield losses. Our laboratory is fully committed to studying interactions between viruses, hosts, and whiteflies to comprehensively understand how these viruses are transmitted, how the virus inoculum is maintained year after year, and how best to use this information for short- and long-term management.

Understanding the problem

Our laboratory continues to spend considerable time and resources to understand how these viruses are transmitted by whiteflies, specifically in transmission, whitefly population dynamics, and virus epidemics. Each of these questions requires a multitude of experiments to be precisely addressed. Our goal is to exploit the knowledge gained to better manage whiteflies and viruses in vegetable crops. Management options have centered on host plant resistance (when available) and on cultural and chemical tactics.

Whitefly cryptic species and populations in vegetable crops

Whiteflies form a cryptic species complex. Cryptic species have the same visual appearance but are biologically different. Based on our multiple molecular assays conducted in our laboratory it is sufficient to say that in Georgia, the *Bemisia tabaci* (Gennadius), Middle East Asia Minor 1 (MEAM 1), is the predominant whitefly cryptic species across vegetable and row crops. This scenario has not changed recently. Whitefly populations were moderate in 2020 (when compared with 2016-18). Results from our research on whitefly populations in Georgia were published in 2020.

Results from that study indicated that whitefly populations showed very little variation when collected from multiple hosts throughout Georgia.

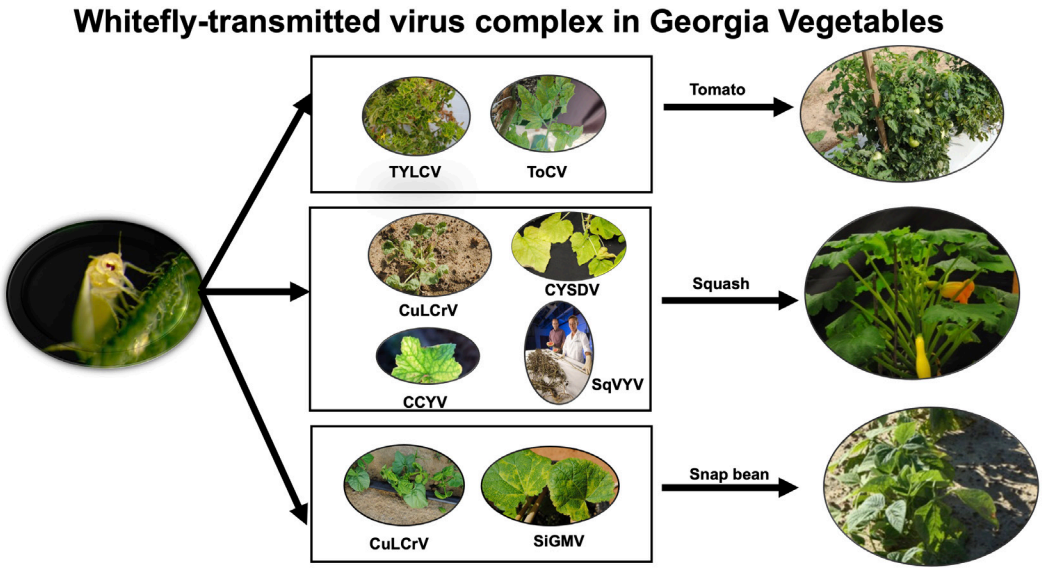


Figure 1. The virus-whitefly web depicts the complexity associated with vegetable hosts and the whitefly-transmitted viruses infecting them. This web structure is getting more elaborate with the addition of new viruses and affected hosts.

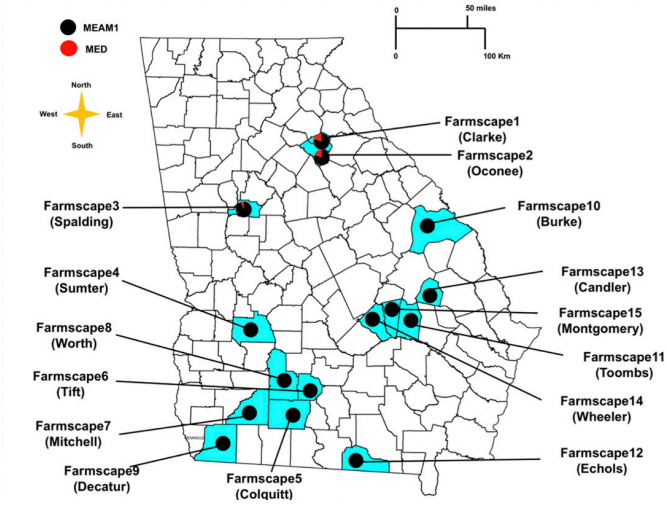


Figure 2. Distribution of *B. tabaci* cryptic species (MEAM1 and MED) in Georgia based on mtCO1 and microsatellite markers' analyses.

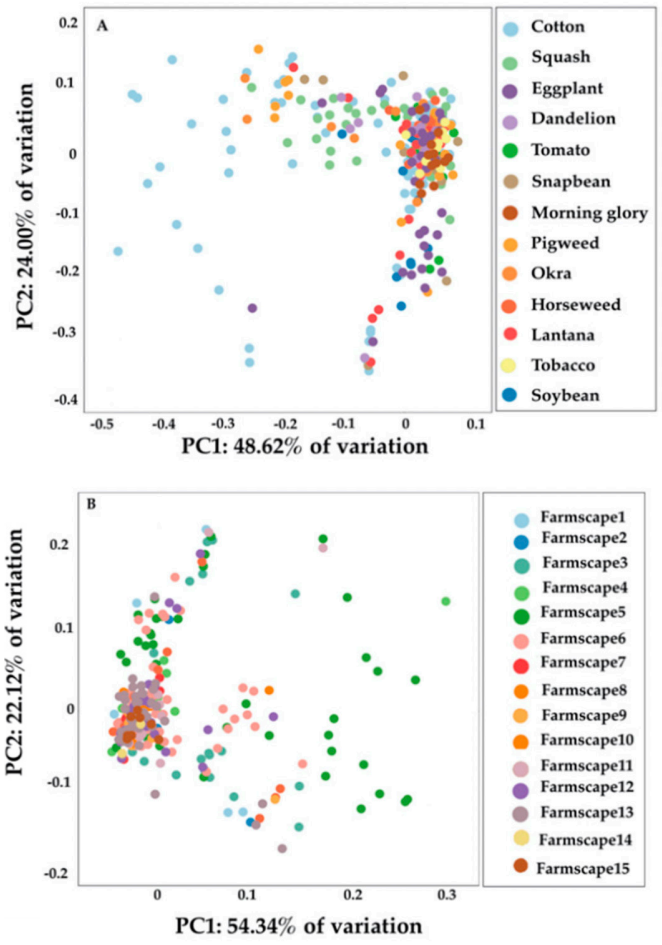


Figure 3. Population structure based on principal component analyses for *B. tabaci* MEAM1 populations collected from (A) different host plants and (B) farmscapes.

Results

Results reiterated the prevalence of a single cryptic species throughout Georgia and high population homogeneity in the landscape. This indicates that whitefly populations across hosts are not different, and that population buildup on one host influences populations on other susceptible hosts nearby, especially when fall vegetables are planted.

Virus transmission by whiteflies

Our lab conducts research on various factors that influence virus transmission, including the effects of whitefly cryptic species and mixed infection of viruses. In 2019 and 2020, research was conducted on mixed infection of CuLCrV and CYSDV in squash.

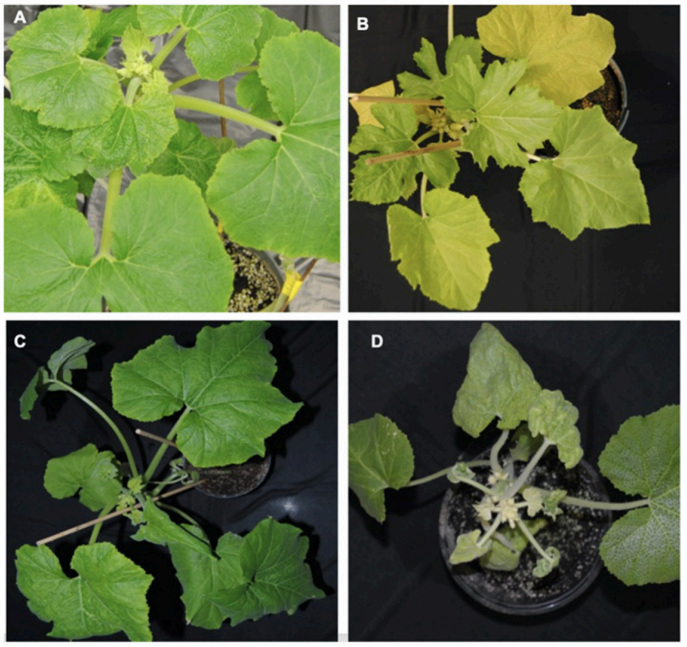


Figure 4. Photographs of (A) non-infected, (B) CYSDV-infected, (C) CuLCrV-infected, and (D) mixed (CuLCrV and CYSDV)-infected squash plants.

Results

The infection of multiple viruses (CuLCrV and CYSDV) in squash makes the crop extremely susceptible and induces heavy yield losses. CuLCrV and CYSDV are both transmitted by whiteflies, albeit in different modes. Virus management should focus on both these viruses, as they are more often present as mixed infection than single infection. The role of the most recent entry to Georgia, CCYV, in mixed infection remains to be evaluated.

Host resistance

Host resistance, when available, is the most effective management option. This is evident in the case of tomato with *Ty* genes conferring resistance to TYLCV. Numerous cultivars are now commercially available with superior horticultural traits. Research over the

years revealed that continuous growing TYLCV-resistant cultivars has not led to development of resistance-breaking strains of whitefly. These cultivars are very effective in mitigating yield losses in the fall season. Our laboratory has also been exploring combining whitefly resistance with TYLCV resistance.

Figure 5. Photographs of a TYLCV-resistant cultivar (left) and a TYLCV-susceptible cultivar (right) planted in Tifton. Both cultivars were planted at the same time

Host resistance against cucurbit viruses, mainly CuLCrV and CYSDV, is currently being conducted in collaboration with Cecilia McGregor in UGA's Department of Horticulture. Research on this aspect is in the initial screening and evaluation phase. Doctoral student Gurjit Singh is leading this effort.

Host resistance against CuLCrV and/or SiGMV is also ongoing in collaboration with Bhabesh Dutta. There has been substantial progress on this front with the identification of commercial cultivars that could be readily used by Georgia growers. In depth evaluations of genotypes with enhanced resistance/tolerance to begomoviruses (like CuLCrV and SiGMV) are currently being conducted in our laboratory. Postdoctoral associate Saioa Legarrea is leading this effort.

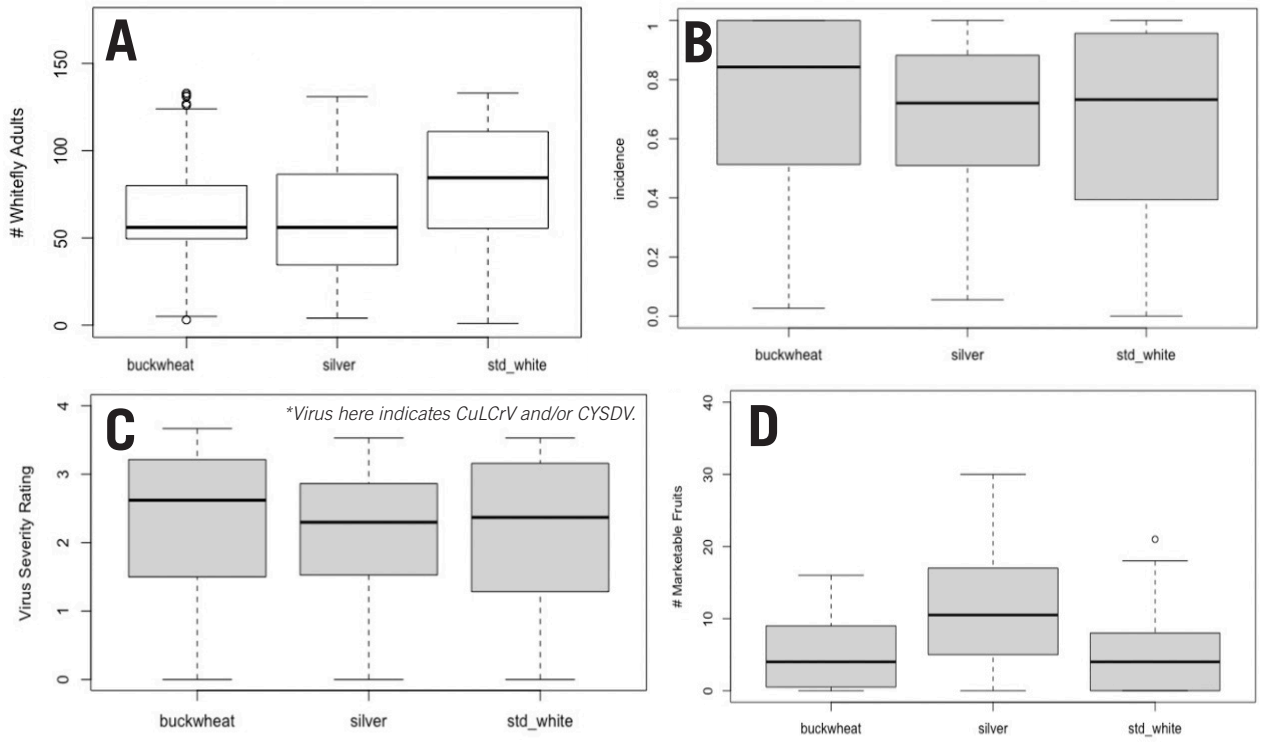
Results

When available, host resistance is the best management tactic. TYLCV resistance in tomato seems to hold up despite continuous usage.

Management of whiteflies and/or viruses

Our research is aimed at understanding basic aspects of virus transmission prevention and using the knowledge gained to develop short- and long-term management strategies. Options such as using insecticides, mulches, and row covers have been evaluated in the last few years. While some insecticides appear effective in reducing whitefly populations, their efficacy in preventing virus transmission has been rather low. This is primarily because of the immense whitefly pressure that we experience in the fall season in south Georgia. Some of our 2020 results are presented below.

Figure 6. Plots of mulch type comparisons on (A) whitefly adults, (B) virus incidence, (C) virus symptom severity, and (D) yields.



Mulch treatments

In 2020, a mulch trial was conducted at the Tifton Vegetable Park at the UGA Tifton campus. Three kinds of mulch were evaluated: white plastic, reflective, and live (buckwheat) mulch. They were evaluated in a randomized complete design with at least four replications for each treatment.

Results

The reflective silver mulch was effective in suppressing whiteflies, and virus incidence and symptom severity varied slightly when compared with live mulch (buckwheat) and standard white mulch. Consequently, there were increased yields per plant when grown on silver reflective mulch compared to the living mulch and standard white mulch. These results have been consistent over the years. The effect, though not very large, could be valuable when combined with other management options. Silver mulch as a management option could become important, especially when there is no host resistance available.

Insecticide treatments

In 2020, seven insecticides with activity against whiteflies were evaluated against whiteflies in squash. A row cover treatment was included along with insecticides. Insecticides were applied at weekly intervals for a period of six weeks consecutively. A row cover was used on squash seedlings to protect them at the most vulnerable stage against whiteflies and viruses. The row cover was removed at first flowering.

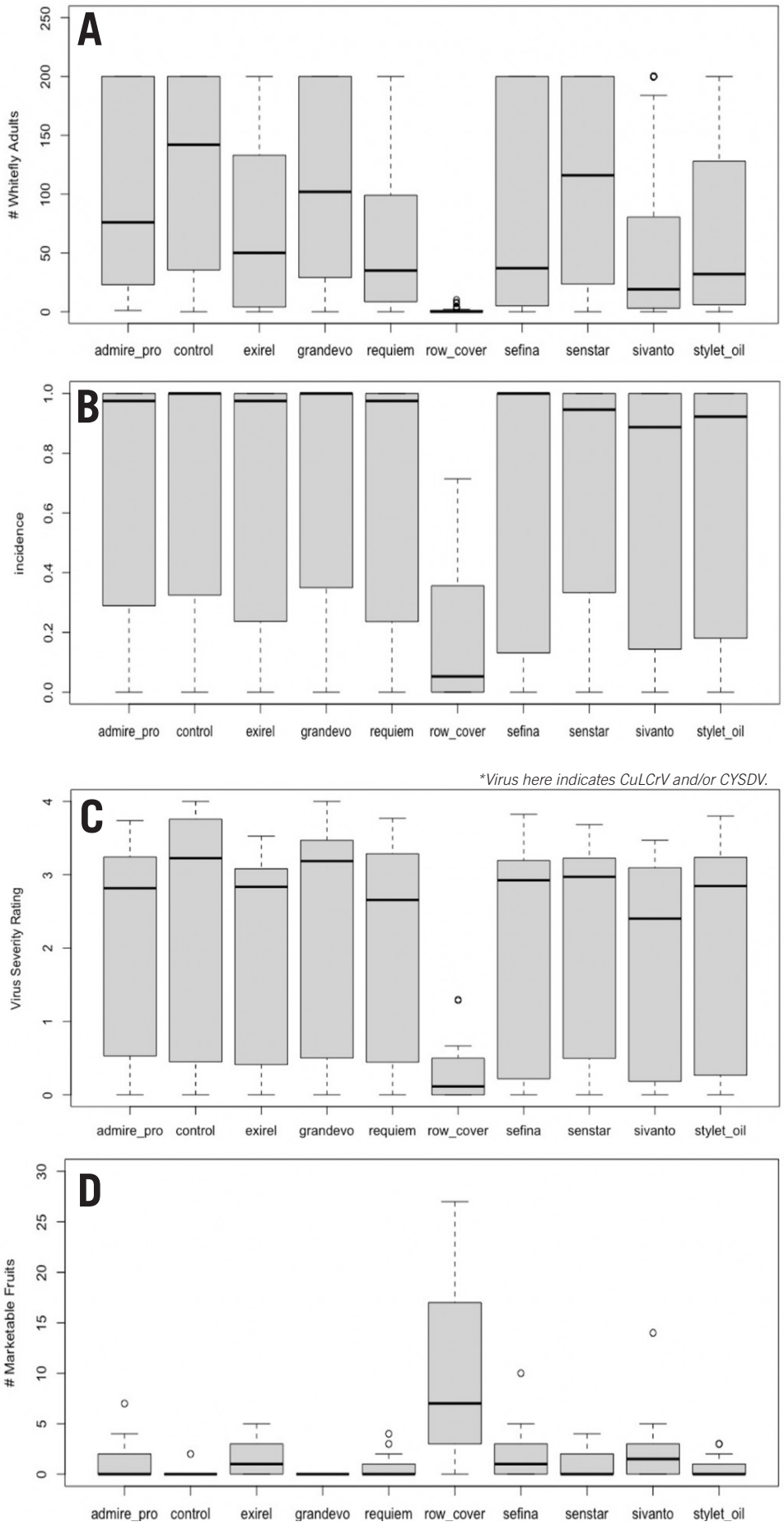


Figure 7. Plots of insecticide and row cover comparisons on (A) whitefly adults, (B) virus incidence, (C) virus symptom severity, and (D) yields.

Results

The application of insecticides suppressed whiteflies, with some insecticides performing better than others. Despite the suppression, insecticides did not prevent virus transmission and/or decrease symptom severity. Some yield benefits were observed in plots treated with insecticides than in non-treated control plots. What stood out in this trial was the row cover. The row cover was very effective in excluding whiteflies in the most vulnerable stages of the crop, reducing overall virus incidence and symptom severity in comparison with insecticide-treated plots and non-treated plots. Consequently, yields from row cover plots were much greater than from insecticide-treated plots and non-treated plots.

Grower demonstration trial, 2020

Grower demonstration trials have been conducted on nearby vegetable farms to determine the impact of different management tactics in a real-world production environment.

What stood out from the trials over the last few years (2017-20) were silver mulch and row covers. They were effective in suppressing whiteflies, reducing virus incidence and symptom severity, and boosting yields. A large demonstration trial was conducted at Lewis Taylor Farms in 2020 to evaluate silver mulch and/or row covers.

Long-term management

The long-term management aspects include using RNAi approaches and host plant resistance. Some research on those fronts have already begun in collaboration with Dr. Bhabesh Dutta, Dr. Cecilia McGregor, Dr. Andre da Silva, and Dr. Timothy Coolong. Also, currently transcriptomics research is ongoing to lay the foundation for RNAi based management.

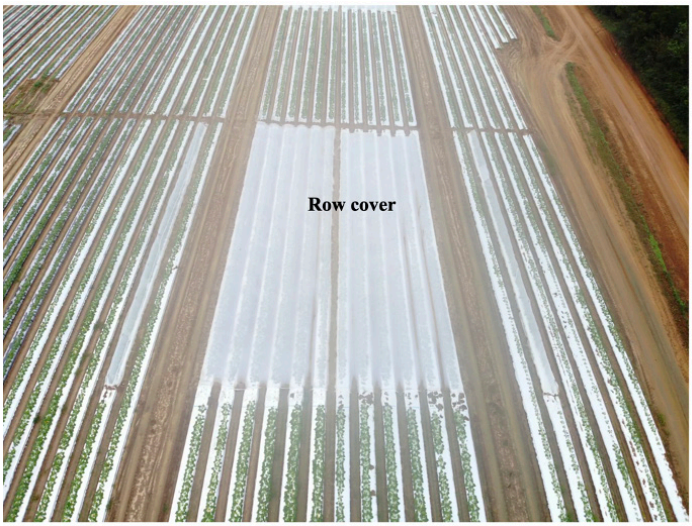
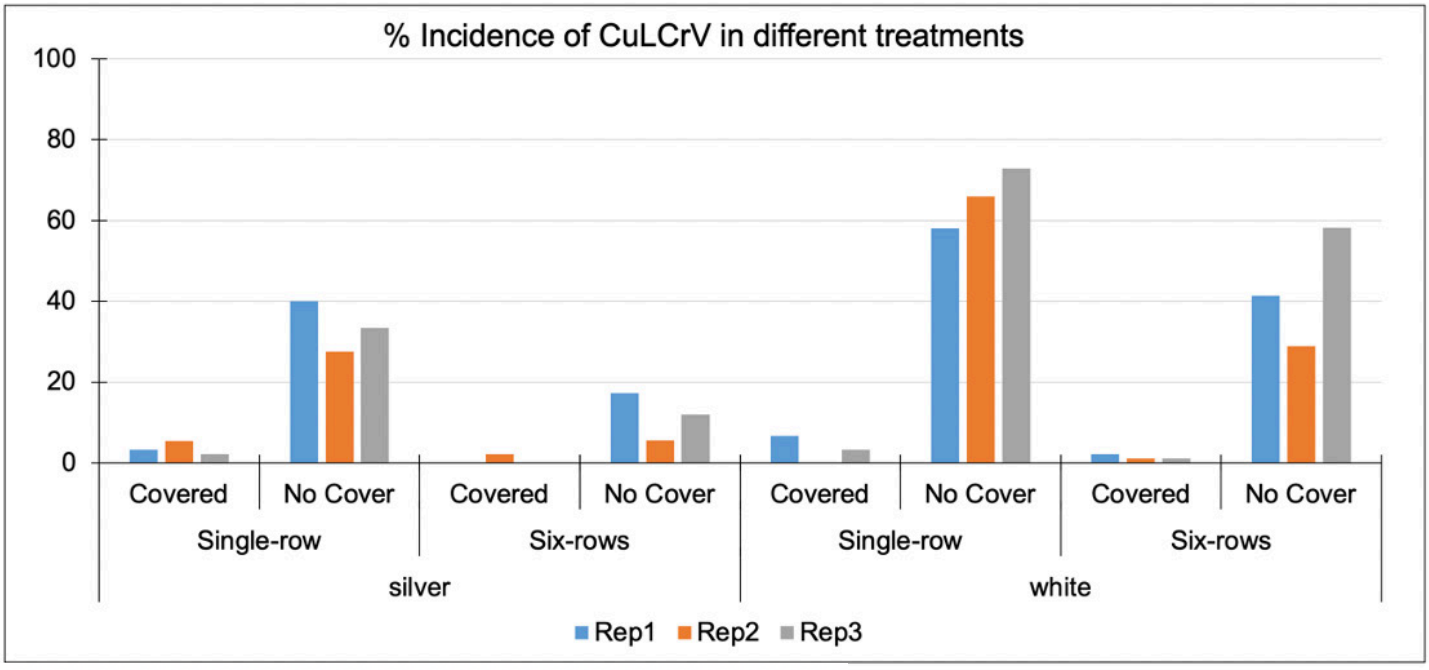


Figure 8. Demonstration trial for whitefly insecticide resistance monitoring in Tifton in 2020 to evaluate row covers and silver mulch against whiteflies and viruses transmitted by them.



*NOTE: % incidence is based upon ratings taken on October 22, 2020

Figure 9. Demonstration trial at Lewis Taylor Farms in 2020. Impact of row cover and silver mulch on virus incidence in squash.

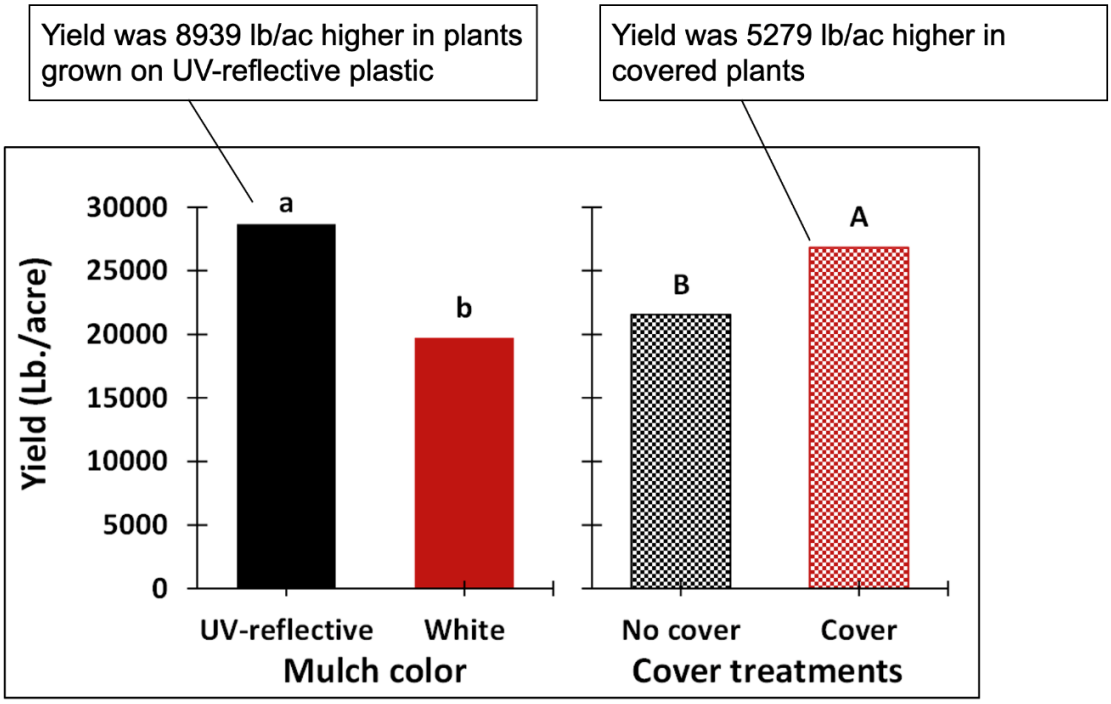


Figure 10. Demonstration trial at Lewis Taylor Farms in 2020. Impact of row cover and silver mulch on yield in squash.

Conclusion

When available, host plant resistance (resistant cultivars) is the best management option for whitefly-transmitted viruses in tomato and squash. In their absence, cultural and chemical options become relevant. With limited success in the case of standalone insecticide applications, other options such as using row covers and reflective mulch seem to be effective in suppressing virus incidence and boosting yields.

Manuscripts generated from this research

- Agarwal, G., Kavalappara, S. R., Gautam, S., da Silva, A., Simmons, A., Srinivasan, R., & Dutta, B. (2021). Field Screen and Genotyping of Phaseolus vulgaris against Two Begomoviruses in Georgia, USA. Insects, 12, 49.
- Gautam, S., Crossley, M. S., Dutta, B., Coolong, T., Simmons, A. M., da Silva, A., Snyder, W. E., & Srinivasan, R. (2020). Low Genetic Variability in Bemisia tabaci MEAM1 Populations within Farmscapes of Georgia, USA. Insects. 11: 834. doi: 10.3390/insects11120834
- Gautam, S., Gadhave, K. R., Buck, J. W., Dutta, B., Coolong, T., Adkins, S., & Srinivasan, R. (2020). Virus-virus interactions in a plant host and in a hemipteran vector: Implications for vector fitness and virus epidemics. Virus Research. 286: 198069. doi: 10.1016/j.virusres.2020.198069
- Marchant, W. G., Gautam, S., Hutton, S. F., & Srinivasan, R. (2020). Tomato yellow leaf curl virus-resistant and -susceptible tomato genotypes similarly impact the virus population genetics. Frontiers in Plant Science. 11:599697. doi: 10.3389/fpls.2020.599697
- Marchant, W. G., Legarrea, S., Smeda, J. R., Mutschler, M. A., & Srinivasan, R. (2020). Evaluating acylsugars-mediated resistance in tomato against Bemisia tabaci and transmission of tomato yellow leaf curl virus. Insects. 11: 842. doi: 10.3390/insects11120842

Monitoring of overwintering pepper weevil in southern Georgia

A. Sparks, T. Weredyk, T. Torrance, J. Shealey, S. Hollifield, W. Gay, J. Kichler, J. Hand

Introduction

The pepper weevil, *Anthonomus eugenii* Cano, is the key insect pest of peppers wherever the crop and pest coexist. In Georgia, the pepper weevil has historically been considered an occasional pest, with infestations generally attributed to man-aided movement. In the fall of 2017, infestations in southern Georgia were widespread, with weevil infestations occurring in the vast majority of pepper fields throughout the region. The wide distribution suggested that random man-aided movement was not the most likely source of these populations. To investigate the likelihood and distribution of pepper weevil overwintering in southern Georgia, pheromone traps were established in commercial pepper fields and monitored from December through March during the winters of 2018-19 and 2019-20.

Material and methods

In 2018-19, 16 fields were monitored, with four fields each in Echols, Grady, Colquitt and Brooks counties. In 2019-20, 15 fields were monitored with four each in Grady and Echols counties, three in Colquitt County, and two each in Brooks and Tift counties. Four pepper weevil traps were established in each field. Traps consisted of a 15.2 cm by 30.5 cm yellow sticky card and were baited with the standard two-part pepper weevil pheromone from Trece Corporation (Pepper Weevil 4-Station Kit, Trece Corp., Adair, OK). Traps were monitored weekly and replaced every two weeks.

Results

Figure 1 presents the number of weevils per trap per week averaged across all locations for each year. While the general trend indicates sustained populations through the winter with decreasing numbers through February and March, of greatest importance is the fact that weevils were continuously present at some level through March. As spring pepper fields are commonly transplanted beginning in March in southern Georgia, this clearly indicates that pepper weevils successfully overwintered and could potentially infest the subsequent spring-planted crop. In fact, at least some of the reduction in catch in March of both years is likely a result of crop competition with the traps. It is likely that overwintering populations were higher than indicated by the reduced numbers on traps after initiation of transplanting.

Figure 2 presents the trap data with counts averaged by county. Colquitt County showed the most consistent catches of weevils in both years, although Tift County had high capture levels late in the single year it was included. Overall, all counties trapped showed similar trends with weevil captures declining in the spring but with some population remaining as the spring pepper production season began.

Conclusion

The pepper weevil trapping data collected during the winters of 2018-20 clearly show that weevil adults overwintered throughout southern Georgia and presented a potential threat to the subsequent year's spring crop. While intensive management practices reduced the impact of pepper weevil in 2019 and 2020, widespread field infestations further suggested that weevils had successfully overwintered throughout the area. These data and field experiences have impacted pepper pest management in this area, with growers generally applying one or more insecticide applications at or near transplanting to prevent weevil establishment followed by additional preventive sprays in combination with intensive targeted scouting. A renewed interest in timely crop destruction after final harvest has been noted with most growers and will hopefully have a significant positive impact on weevil management and reducing overwintering potential.

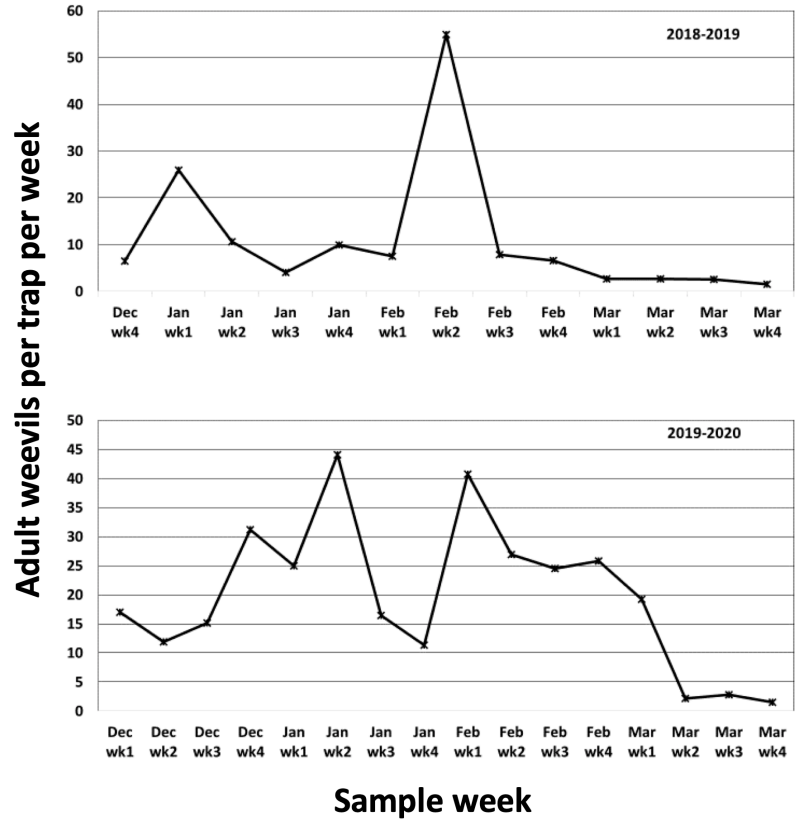


Figure 1. Pepper weevil adult captures on pheromone-baited sticky traps in southern Georgia during the winters of 2018-20. Counts are averaged across all fields (16 in 2018-19; 15 in 2019-20) monitored in each year.

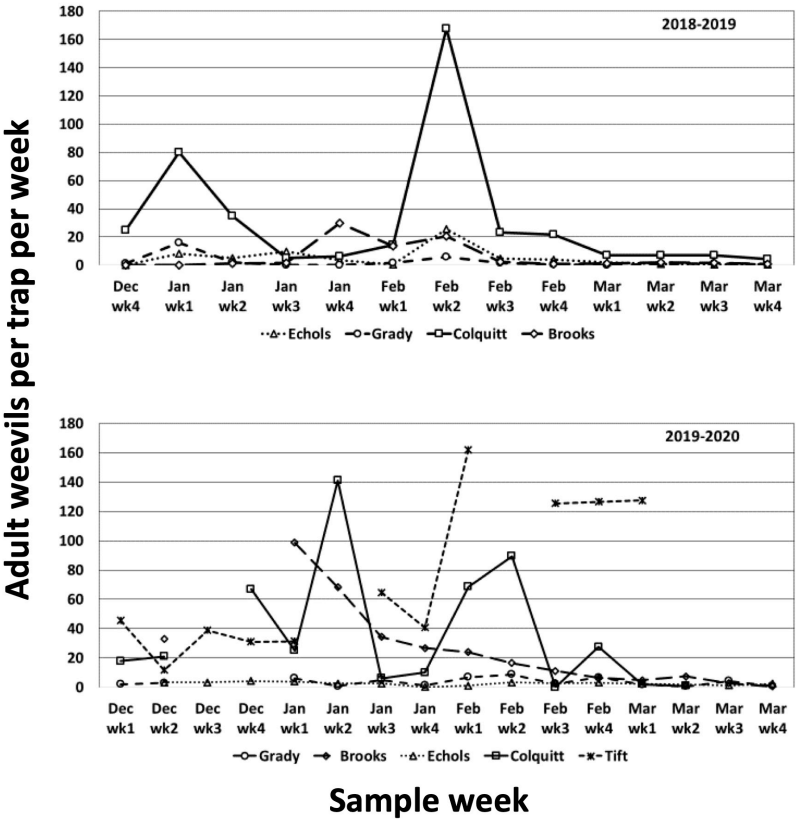


Figure 2. Pepper weevil adult captures on pheromone-baited sticky traps in southern Georgia during the winters of 2018-20. Counts are averaged across all fields monitored in each county in each year.

Whitefly insecticide resistance monitoring, 2020

D. Riley

Introduction

The silverleaf whitefly, Biotype B *Bemisia tabaci* Gennadius (Figure 1), has become one of the most destructive insect pests of vegetable crops in Georgia in the summer and fall seasons due to a buildup of high populations in late summer. This insect is also notorious for vectoring begomoviruses, tomato yellow leaf curl virus in particular. Controls for whitefly are mostly preventative, and the primary means of control is through chemical insecticides. Early intervention with an efficacious insecticide program has been shown to reduce the spread of virus and increase yields in tomato in Georgia. However, resistance to insecticides can reduce this benefit. Resistance in whitefly to neonicotinoids has been observed through work done at the Coastal Plain Experiment Station at Tifton. We developed several methods that can provide a quick bioassay reading (less than 24 hours) for the major insecticides used to control whiteflies in vegetable crops.

Figure 1. Whitefly adults on a squash leaf in Tifton in August 2020. *Photo: D. Riley*

Material and methods

Whitefly field bioassays were developed using clean cotton seedlings in dedicated, insect-free growth chambers as test plant material. We compared cotton leaf bioassay results with standard test results in pumpkin sprayed plots to see if results of whitefly mortality were comparable between the two. The commercial insecticide treatments (per acre rate) evaluated were Assail 30SG (4 oz), Venom 70SG (4 oz), Admire Pro 4.6F (2.2 fl oz), Transform WG (2.25 oz), Sivanto Prime 1.67SL (12 fl oz), Knack 0.86EC (10 fl oz), Beleaf 50SG (4.28 oz), Oberon 2SC (8.5 fl oz), Exirel 0.83SC (13.5 fl oz), and an untreated check. We dipped the cotton seedlings in the same solution as the field test and added Belay and lower concentrations of Admire, Sivanto, and Exirel for comparison. We compared live adults in the 24-hour bioassay to egg and nymph leaf counts on the sprayed pumpkin test one to two weeks after sprays. In a second set of bioassays, we either dipped the leaves into the insecticide test solution or placed the solution into the vial containing the roots. We used a high labeled rate and low 1/10th rate of Admire Pro 4.6F, Sivanto Prime 1.67SL, Knack 0.86EC, Exirel 0.83SC, and a water check. We collected ~30 adult whiteflies in clear plastic testing tube from Tifton and a slightly more susceptible Florida population for comparison. We report the adult whitefly survivorship after 24 hours.

Results

The results of the rapid bioassay method were promising in that most of the treatments from the field spray test that provided improved whitefly control (in terms of reduced egg lay and whitefly nymph development) also had the lowest number of live adults in the cotton leaf bioassay (Table 1). When Exirel, Sivanto, and Venom bio-assayed with the lowest numbers of live adults, we observed a corresponding lower number of eggs and nymphs developing on the field-grown pumpkin crop. The two exceptions were that Beleaf did not provide adult kill in the bioassay but did result in lower eggs and nymphs on the crop. Conversely, the high rate of Admire did control adults in the bioassay but only provided marginal control of eggs and nymphs on the crop. We did see a rate response with the bioassay with Admire, indicating that there was a stronger benefit from increasing the rate with this product. In the second set of bioassays of whitefly adults, we also detected a weaker response to Admire in the more resistant Tifton and more susceptible Florida populations (Figure 2). The significant rate responses for Exirel, Venom, and Sivanto in at least one population means we could use this bioassay to measure rate response.

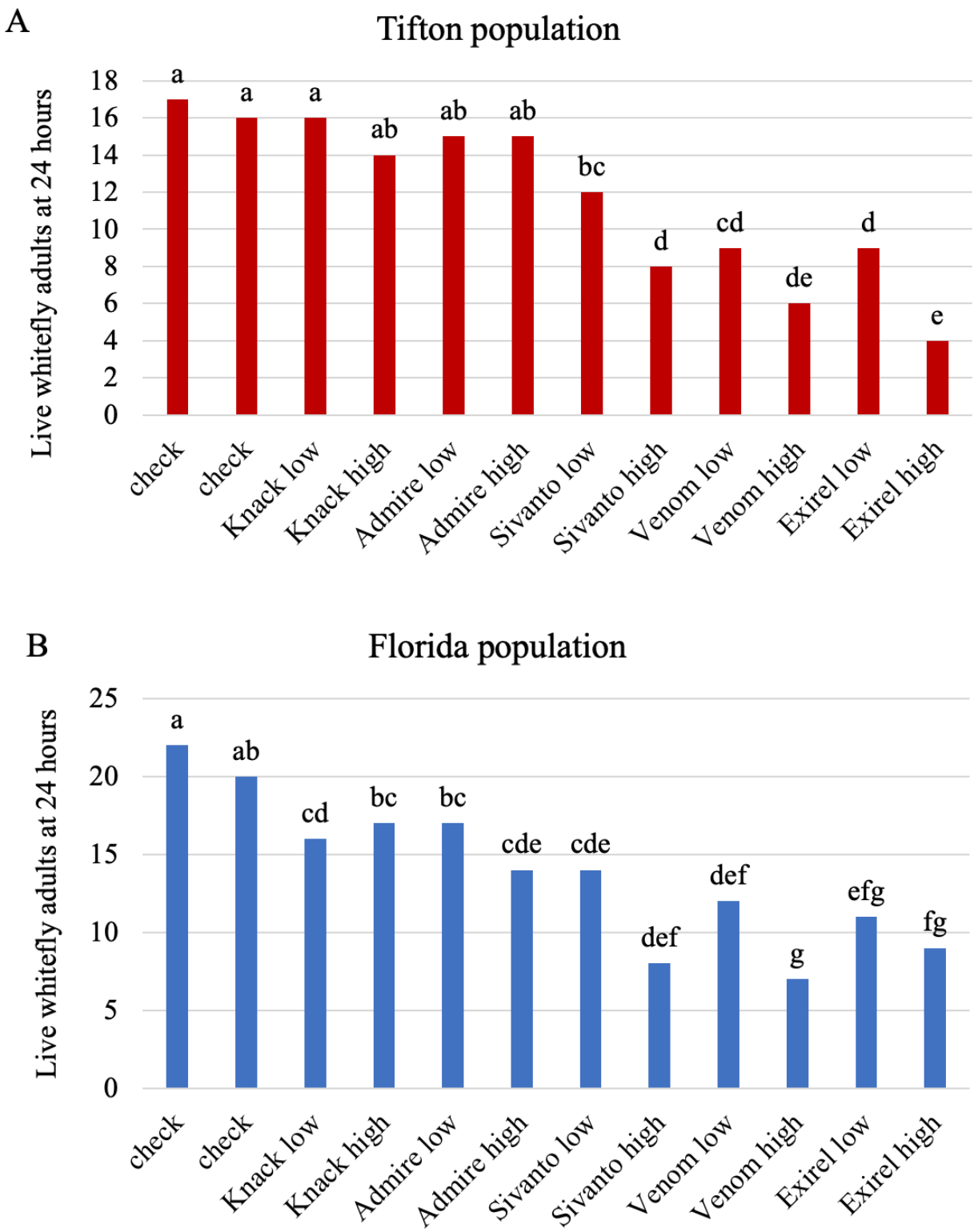


Figure 2. The number of live adults remaining in the bioassay tube after 24 hours of exposure to the indicated insecticide at the highest labeled rate (high) and a one-tenth rate (low) in Tifton (A) and Florida (B) collected populations of whiteflies.

Table 1. Comparison of the rapid cotton bioassay to field efficacy in a pumpkin spray trial at the UGA Coastal Plain Experiment Station in Tifton, Georgia.

IRAC group	Product common name	Commercial product used in test	Cotton bioassay amount/200ml**	Cotton bioassay whiteflies adults live at 24 h	Whiteflies eggs on pumpkin leaves	Whiteflies small nymphs on pumpkin leaves
-	water	Water check	0	54ab*	230a	172a
7C	pyriproxyphen	Knack 0.86EC	0.156 ml	50abc	227a	143a
4A	clothianidin	Belay 50WDG	0.064 ml	59a	-	-
4C	sulfoxaflor	Transform WG	0.034 g	22ef	100bc	89b
4A	acetamiprid	Assail 30SG	0.06 g	27de	111bc	84b
4A	imidacloprid	Admire Pro 4.6F	0.0036 ml	26de	-	-
4A	imidacloprid	Admire Pro 4.6F	0.036 ml	5g	132b	84b
4A	dinotefuran	Venom 70SG	0.06 g	7g	84bc	52bc
23	spiromesifen	Oberon 2SC	0.132 ml	39cd	82bc	51bc
4D	flupydifurone	SivantoPrime 1.67SL	0.0188 ml	7g	-	-
4D	flupydifurone	SivantoPrime 1.67SL	0.188 ml	3g	65c	49bc
9C	flonicamid	Beleaf 50SG	0.064 g	44bc	63c	36c
28	cyantraniliprole	Exirel 0.83SC	0.0212 ml	10fg	-	-
28	cyantraniliprole	Exirel 0.83SC	0.212 ml	8g	54c	28c

*Means within columns followed by the same letter(s) are not significantly different (P<0.05, LSD)

Conclusion

Measuring insecticide efficacy is a critical part of making a management recommendation for the control of whiteflies. However, running field tests limits our ability to test extensively across the state. A rapid field bioassay gets around this restriction and paves the way for widespread efficacy evaluations and the ability to assess insecticide response before sprays are even applied. This could be a tremendous help in managing insecticide resistance in whitefly populations in Georgia. We currently have several products that provide significant control of whiteflies, but heavy whitefly population pressure means heavy selection for resistance. As long as whiteflies continue to build up in the late summer and fall in Georgia, we will need to annually assess mortality response to our best insecticides for control.

Monitoring insecticide resistance in *Plutella xylostella*, the diamondback moth, in cole crops and developing more genetic analysis tools to identify mechanisms

D. Riley, T. Dunn, J. Bennett

Introduction

In 2019, we had some breakthroughs in terms of understanding insecticide resistance in the diamondback moth (DBM), *Plutella xyllostella* (Figure 1), in Georgia. These breakthroughs both came in the insecticide response surveys, as well as the genetic analysis of collected DBM larvae. After genetic sequencing of the ryanodine receptor (RyR) from DBM populations collected in Georgia, a previously discovered mutation of the DBM RyR was identified in all collected samples. This mutation, referred to as the G4946E, is associated with resistance to diamide insecticides, specifically to chlorantraniliprole (Coragen™) (Troczka et al., 2012; Kang et al., 2017). The presence of this mutation in DBM populations from Georgia may explain the outbreaks of Coragen-resistant DBM in recent years. This was the first report of this mutation in Georgia and it appears that this mutation is widespread in southern Georgia DBM populations. We attempted to determine the prevalence of this trait in more populations. We also tested for other mutations reported to be associated with diamide resistance, including E1338D, Q4594L, and I4790M (Guo et al., 2014). We summarized a maximum insecticide dose bioassay survey for both Georgia and Florida that showed interesting insecticide response profiles.

Material and methods

We used a maximum insecticide dose leaf dip assay of DBM larvae to document which of the IRAC groups of insecticides demonstrated efficacy against a sample population of DBM. Along with collaborators, we collected larvae and sent or brought samples to the Vegetable Entomology Research Laboratory at the UGA Tifton campus. Samples were loaded in the bioassay dishes and read at 24 and 72 hours. Mortality results were recorded and placed into a descriptive graph along with a recommended IRAC group rotation sheet. These bioassays were conducted in any

Figure 1. Crop damage caused by resistant diamondback moth larvae in a collard field in southern Georgia with an adult moth (inset A) and late instar larvae (inset B).
Photo: T. Torrance

county where we were contacted by county agents reporting a DBM outbreak. DBM colonies, which were established while collecting for maximum dose insecticide bioassays, were used as a source of DBM larvae for genetic testing. Initially, DBM mRNA was extracted using TRIzolTM (Ambion, Thermo Fisher Scientific, Waltham, MA) following the manufacturer's protocol. Subsequently, the use of a Dynabeads™ mRNA Direct™ Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA) proved to be more successful for mRNA extraction in this study. cDNA was synthesized with SuperScript IV VILO MasterMix™ (Invitrogen) following the manufacturer's protocol and stored in a -20 °C freezer until needed for polymerase chain reactions (PCR). Standard PCR was used to screen for known mutations conferring target-site resistance. Primers were designed to amplify regions of the DBM RyR around the suspected mutation sites, and amplicons were produced using Phusion Taq Polymerase (New England Biolabs). All PCR products were amplified using a BIO-RAD C1000 Touch™ (Life Science, Hercules, CA) thermal cycler.

Results

The insecticide survey was published in the Journal of Economic Entomology (Riley et al., 2020) and the highlights are presented in Figures 2 and 3. The survey showed high levels of resistance to chlorantraniliprole (e.g., Coragen™, an IRAC Group 28 diamide insecticide) and bifenthrin (e.g., Brigade™, IRAC Group 3A pyrethroid insecticide), but, surprisingly, we had significantly better control with cyantraniliprole (e.g., Exirel™, also IRAC Group 28) across the sample areas in Georgia and Florida (Figures 2 and 3). All of the samples tested from Georgia and Florida were positive for the G4946E mutation, but no other known target-site mutations for Group 28 insecticides were detected. In terms of DBM resistance to diamide insecticides, this suggests that the genetics of target-site resistance seems to be limited to a single gene at the present time. However, this particular resistance seems to be unequal for all diamide insecticides based on maximum labeled dose responses. For example, in Georgia and Florida, resistance to chlorantraniliprole was fairly widespread providing less than 40% control, but cyantraniliprole provided significantly better control over most locations (Figure 3). We could potentially rely on PCR tests of a few larvae to tell us whether or not a DBM population would be controlled with a diamide insecticide spray.

If a high frequency of the G4946E mutation is detected in a DBM population, a poor response to chlorantraniliprole would be expected. However, the same could not be said for other insecticides, including cyantraniliprole. In addition, the resistance to the pyrethroid insecticides has typically been associated with metabolic detoxification, which likely also contributes to resistance to other insecticide IRAC groups, including diamide insecticides. Thus, DBM resistance is likely to be more complex than the single G4946E mutation for populations in the Southeastern U.S.

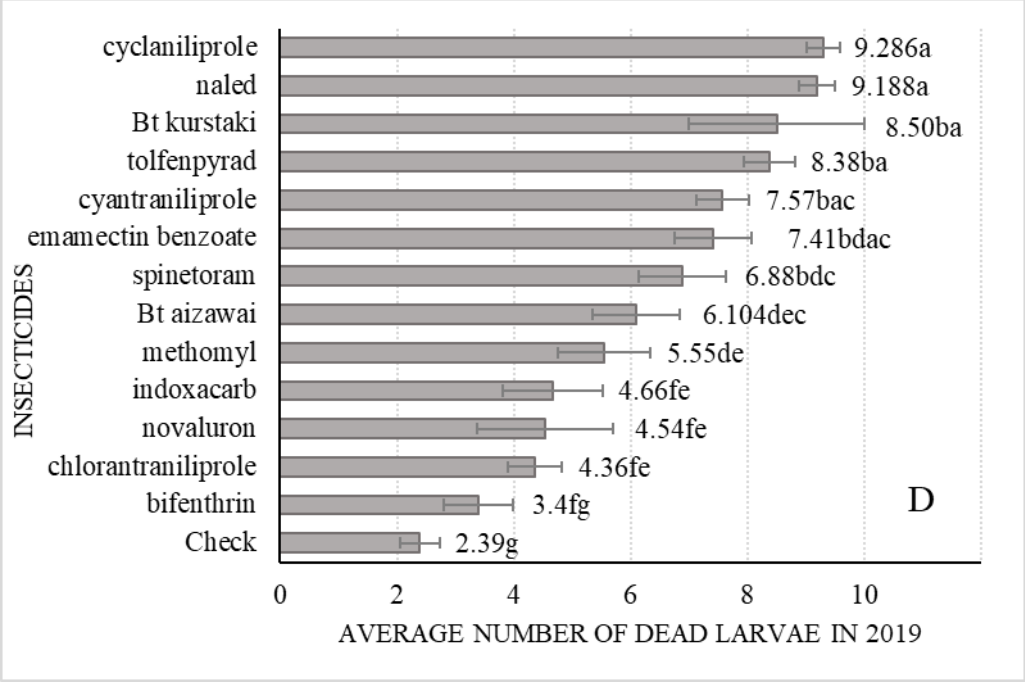


Figure 2. The average 72-hour mortality response of diamondback moth larvae in Georgia and Florida in 2019, assessed in a laboratory bioassay conducted at the University of Georgia Coastal Plain Experiment Station in Tifton, Georgia.
Reproduced with permission from Riley et al., 2020.

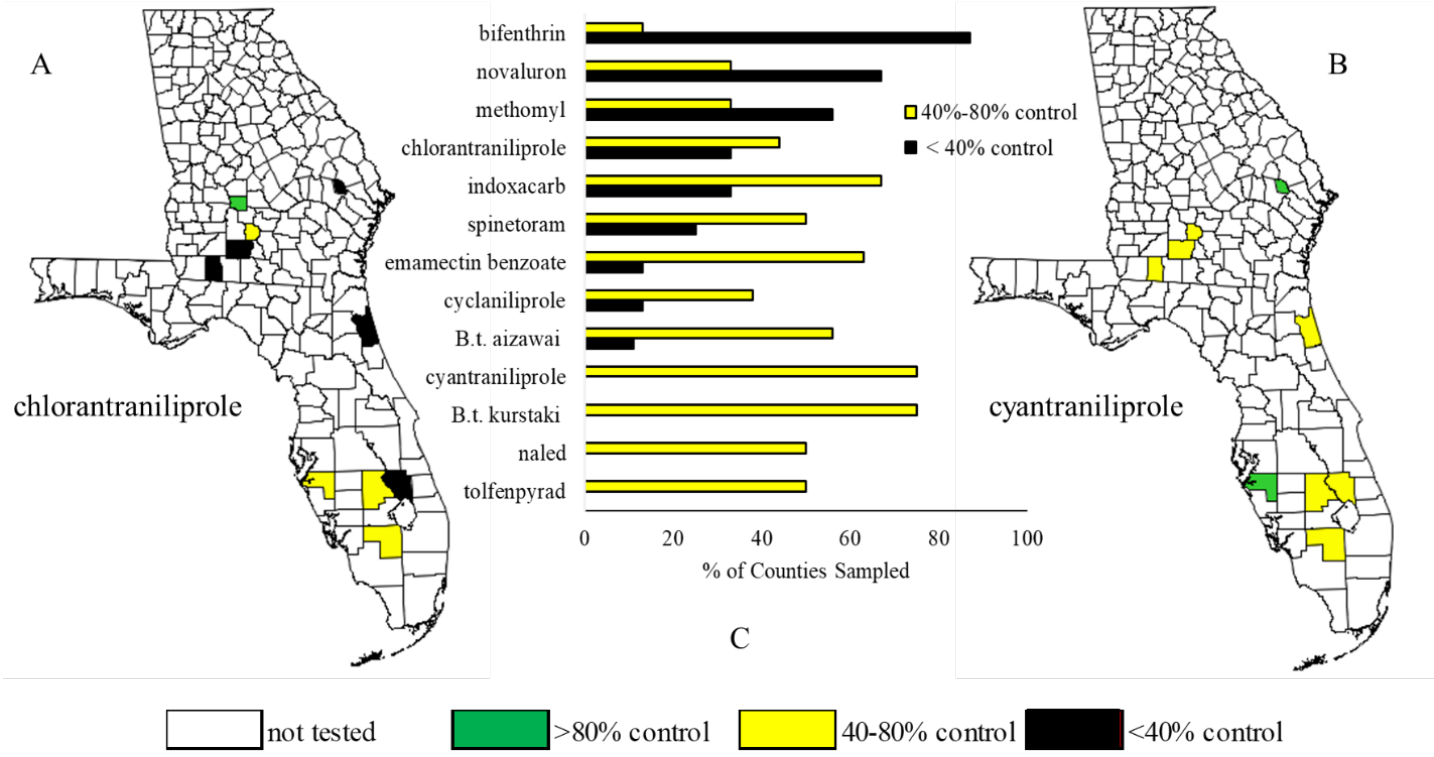


Figure 3. County maps showing the average mortality response of diamondback moth larvae to insecticides chlorantraniliprole and cyantraniliprole. The center bar graph is a summary of the percent of counties sampled with either intermediate or poor DBM control—the most concerning categories to pest managers. The counties were colored based on control category: **good control** = 80-100% = green, **intermediate control** = 40-80% = yellow, no to **very poor control** 0-40% = black, and **no report of DBM control failure** so not sampled = white.
Reproduced with permission from Riley et al., 2020.

Conclusion

Our work to characterize resistance in DBM populations in Georgia is still ongoing because the genetics of resistance appears to be complex. We will continue to provide bioassay services at the UGA-Tifton Entomology Lab for growers to get an insecticide response profile similar to Figure 2 for their DBM population so that effective insecticides can be selected. Future work will attempt to develop PCR tests that can provide a better profile of resistance mechanisms.

As we have recommended in the past, insecticide rotations and knowledge of which insecticides are effective are the best preventative measures for reducing the selection for insecticide resistance in DBM populations.

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