

Annual Report 2020

Survey of Whitefly-transmitted Viruses in Watermelon and Other Cucurbit Crops in Alabama, Louisiana, and Mississippi

Within the past 14 years, cucurbit growers in many states of the southeastern and southwestern U.S. have experienced losses as a result of introduced whitefly-transmitted viruses. Specifically, the emergence of cucurbit leaf crumple virus (CuLCrV; Guzman et al., 2000; Hagen et al., 2008) and cucurbit yellow stunting disorder virus (CYSDV) in California (Kuo et al., 2007; Brown et al., 2007; Wintermantel et al., 2009; 2016; 2017) was followed by their identification in Florida, Georgia, and South Carolina within a decade (Polston et al., 2008; Akad et al., 2008; Gadhav et al., 2018). Similarly, the identification and characterization in Florida of squash vein yellowing virus (SqVYV; Adkins et al. 2007; 2013) was followed by its introduction to California in 2014 (Batuman et al., 2015). In 2019, cucurbit chlorotic yellows virus (CCYV) was identified in California, and further testing of frozen archived melon nucleic acid extracts demonstrated that CCYV had been present in California since 2014 (Wintermantel et al., 2019). Importantly, the increase in distribution of cucurbit viruses has been correlated with the transition to using cucurbit transplants, an important production resource that has improved crop performance against soil-borne diseases. Nevertheless, movement of nursery material among regions can lead to the introduction of pathogens, including plant viruses. Many of these viruses have latent periods making it difficult to detect early virus infections prior to shipment or planting in fields or greenhouses. Most information to date has been generated in California and Florida, with some additional information from South Carolina and Georgia. However, it is critical to determine the prevalence of whitefly-transmitted viruses throughout the southeastern U.S. where watermelon is grown, as limited information is available throughout the region situated between areas where yellowing viruses have become established in Florida, South Carolina, Georgia, and southern Texas. A previous survey of viruses present in watermelon was conducted nearly a decade ago (Ali et al., 2012), shortly after the identification of CYSDV in California and Florida and the identification of CuLCrV in Florida. At that time, cucurbit aphid borne yellows virus (CABYV) was found to a limited degree in Alabama, but CYSDV, SqVYV, and CuLCrV were not found in the southeastern U.S. aside from areas where they had previously been reported, and CCYV had not yet been found in the Western Hemisphere. However, considerable time has passed since that survey, the use of transplants has expanded, and additional viruses have been identified in U.S. cucurbit production, including CCYV and SqVYV in California. With the presence of CYSDV and CuLCrV in Georgia and Florida, we believe it is important to determine if these rapidly spreading viruses are present in the Gulf Coast region. Therefore, we began surveys of watermelon and other cucurbit crops in the region during the summer and fall of 2020. This work was complicated by restrictions on travel due to COVID-19, but we were able to obtain a large number of samples from Alabama and some from Mississippi. Due to travel restrictions, we were not able to obtain samples from Louisiana but hope to sample all three states in 2021.

Objectives/Goals

1. Survey watermelon fields as well as fields of other cucurbit crops in Alabama, Louisiana, and Mississippi for symptoms of whitefly-transmitted viruses

2. Collect samples of whiteflies associated with watermelon and other cucurbit fields to determine primary whitefly species or biotypes associated with cucurbit production in the region
3. Educate growers and stakeholders, through Extension programming, on identification of whitefly-transmitted virus symptoms on cucurbit crops and, after completion of surveys, their management options

Objective 1: Survey watermelon fields as well as fields of other cucurbit crops in Alabama, Louisiana, and Mississippi for symptoms of whitefly-transmitted viruses.

Surveys were conducted for virus and whitefly incidence in Alabama and Mississippi. Travel restrictions prevented collection from Louisiana during 2020, and delayed sampling in Mississippi until very close to the end of the watermelon season. Therefore, most samples collected were from Alabama during 2020. Most sampling in Alabama was conducted in the southern part of the state, as well as some sampling in central Alabama. Samples from Mississippi were limited due to COVID-related travel limitations with 14 plants sampled from three watermelon fields/plantings that remained available when travel was allowed. Plants were identified based on the presence of mottling and yellowing, particularly leaf yellowing with veins remaining green and symptoms spreading from the crown area outward along vines (**Figure 1**). Leaves with this type of symptom were collected and maintained on ice, then shipped overnight to the USDA-ARS in Salinas, CA for testing. Testing included analysis for four whitefly-transmitted viruses with RNA genomes using a test that evaluates for all four viruses simultaneously, which we refer to as a multiplex RT-PCR test. Testing was conducted for CYSDV, CCYV, SqVYV, and CABYV, which produces symptoms nearly identical to those of CYSDV and CCYV using the multiplex RT-PCR system. Due to COVID limitations this year, plants have not yet been tested for beet pseudoyellows virus (BPYV), which also produces yellowing symptoms but is transmitted by a different whitefly (greenhouse whitefly); however, this must be done separately from the multiplex as the BPYV detection primers are not compatible with the multiplex system. The lone DNA virus evaluated, CuLCrV, involved a separate testing method (PCR).

Results in Mississippi identified a single plant infected by the aphid-transmitted virus, CABYV, but no whitefly-transmitted viruses. A much more extensive sampling was conducted in Alabama, with 230 cucurbit plants sampled, including 70 watermelon plants, 51 melon, 25 cucumber, and 84 squash/pumpkin plants. Of these, 62 plants tested positive for at least one whitefly-transmitted virus (**Figure 2**).



Figure 1. Melon plant (A) and watermelon plant (B) showing interveinal yellowing, symptoms of CYSDV. Melon plant showing typical pattern of yellowing progressing from crown toward vine ends due to CYSDV infection (C). These symptoms are all characteristic of infection by criniviruses on cucurbits.

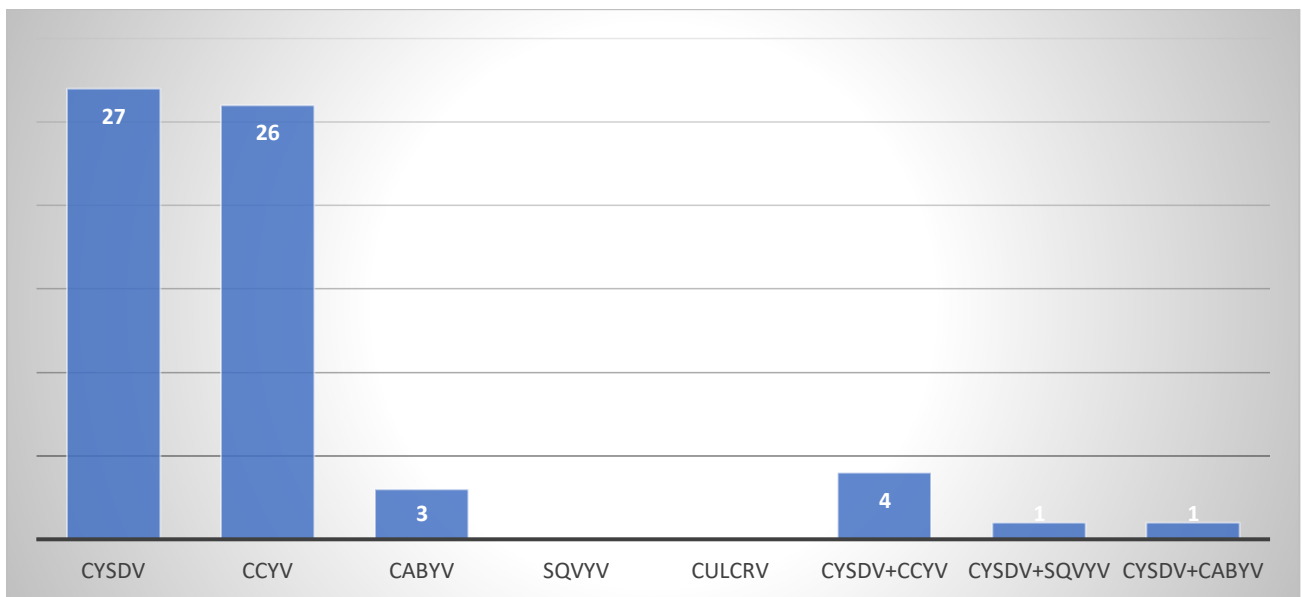


Figure 2. Numbers of plants infected with each of five whitefly-transmitted viruses in Alabama. CYSDV = cucurbit yellow stunting disorder virus; CCYV = cucurbit chlorotic yellows virus;

CABYV = cucurbit aphid-borne yellows virus; SqVYV = squash vein yellowing virus; CuLCrV = cucurbit leaf crumple virus

Objective 2: Collect samples of whiteflies associated with watermelon and other cucurbit fields to determine primary whitefly species or biotypes associated with cucurbit production in the region

Due to COVID-19 travel restrictions and limitation on our ability to sample, we were not able to collect whiteflies during 2020. If we are able to conduct on-site visits ourselves during 2021, whitefly sampling can occur to determine species and biotype of sampled whiteflies from watermelon and other cucurbit crop fields.

Objective 3: Educate growers and stakeholders, through Extension programming, on identification of whitefly-transmitted virus symptoms on cucurbit crops and, after completion of surveys, their management options

Because the presence and prevalence of whitefly-transmitted viruses is not currently known in many of the southeastern states, many growers and Extension personnel were likely not familiar with the symptoms and potential impact of these viruses. Although our original plan was to conduct Extension meetings in Louisiana and Mississippi (in a region that would allow attendance from growers in both Alabama and Mississippi), this was not possible due to COVID-19 restrictions on public gatherings and travel. Since we were not able to have in-person meetings, our team arranged for an online presentation by Dr. Wintermantel providing information on identification of virus-infected plants, differences in symptoms among whitefly-transmitted and other viruses, as well as production risks posed by these viruses. The presentation was attended live by approximately 45 individuals. In addition, the team developed a recorded voice-over PowerPoint presentation (video) by Dr. Wintermantel, hosted by Mississippi State University Extension and made available online (https://www.youtube.com/watch?v=yn_OepNuDB0&feature=youtu.be), that can be viewed by growers and stakeholders everywhere (total 239 views to date).

In order to assist in the identification of symptoms in the field, a fact sheet (<http://extension.msstate.edu/publications/whitefly-transmitted-and-yellowing-viruses-watermelon-and-other-cucurbit-crops>) and photo guide (http://extension.msstate.edu/sites/default/files/publications/supportfiles/p3440_final_72120.pdf) were developed through Mississippi State University Extension that complement the online video and webinar. The fact sheet describes symptoms and management recommendations relevant to whitefly-transmitted viruses, and the photo guide can be taken to the field for direct comparison with symptoms that may be present on plants to assist with diagnosis. The original plan was to distribute these resources, including a laminated copy of the photo guide, to meeting attendees and to others in 2020. Due to the necessary changes in program delivery, distribution of these resources was largely through electronic means. Efforts to distribute hard copies of these resources, including the laminated photo guide, will be a focus in 2021. In addition, a scientific-style poster that describes the project and presents background information on the whitefly-transmitted viruses and the risks they pose to watermelon and other cucurbit crops in Alabama, Louisiana, and Mississippi was designed and displayed at two regional producer meetings in Mississippi.

Although there were limitations in sampling due to COVID-19 restrictions, and our team was not able to hold in-person meetings, most outreach goals, including deliverables, were met. The development of the fact sheet and photo guide will be useful during the upcoming 2021 season and in future years toward monitoring spread of these target viruses, and will also be useful for identification of whitefly-transmitted viruses in other watermelon growing regions. Similarly, the availability of the voiceover presentation will provide easily accessible answers to assist in virus identification and sampling.

In addition, a project website (<http://msuext.ms/ipl6c>), hosted on the Mississippi State University Extension webpage, where all of the electronic resources are posted and available to website visitors was developed.

Continuing Research

This project was granted a one-year no cost extension due to COVID-19 limitations. We plan to expand sampling this coming year (2021) even with continuing COVID-19-related limitations. This is necessary following the identification of not only CYSDV in Alabama, but also CCYV, which first emerged in North America in California in 2014 and was identified in Georgia for the first time in 2020 (Kavalappara et al., 2020). As a part of this continuing research we are still seeking growers to join us as collaborators on this project to assist us with identifying the locations of fields with yellowing virus symptoms for sampling. Growers interested in working with us, even simply to learn more about these diseases, can sign up at the following link: <http://msuext.ms/h27md>.

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