

National Watermelon Association Annual Report 2021

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

Introduction

Within the past 15 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; Gadhavre 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 soilborne diseases. Nevertheless, movement of nursery material or even infected alternate host plants among regions can lead to the introduction of pathogens, including plant viruses, and many 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, more recently, Georgia. However, it is critical to determine the prevalence of whitefly-transmitted viruses throughout the southeastern U.S. where watermelon is grown, including the Central Gulf Coast region. Limited information is available throughout this region situated between areas where yellowing viruses have become established in Florida, Georgia, South Carolina, and 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, it was important to determine if these rapidly spreading viruses are present in the Central Gulf Coast region as well. 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 the COVID-19 pandemic, but we were able to obtain a large number of samples from Alabama and some from Mississippi and followed that with a much more extensive sampling throughout the region

during 2021. Although we have not requested additional funding, we will continue with an additional year of sampling during the summer and fall of 2022.

Grant 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

Progress Summary

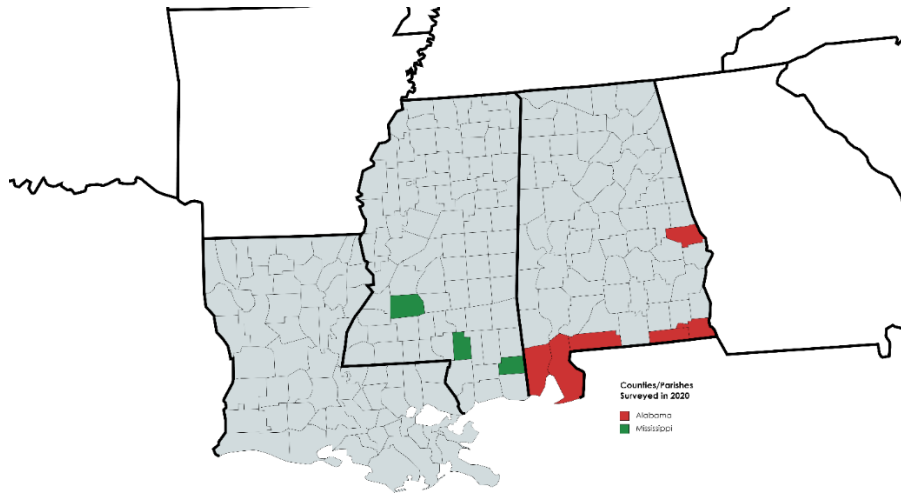
Objective 1: Survey watermelon fields as well as fields of other cucurbit crops in Alabama, Louisiana, and Mississippi for symptoms of whitefly-transmitted viruses.

Surveys were conducted for virus incidence in Alabama, Louisiana, and Mississippi. Travel limitations due to the COVID-19 pandemic 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. All three states were sampled thoroughly in 2021. Most sampling in Alabama was conducted across five counties in the southern part of the state during both years, with some sampling conducted in one county in central Alabama in 2020 (**Fig. 1AB; Table 1**). Samples from Mississippi in 2020 were limited, as previously indicated, to three fields/plantings in three counties (**Fig. 1A; Table 1**), whereas in 2021 samples were collected from growing areas throughout Mississippi, covering 13 counties (**Fig. 1B; Table 1**). Samples collected from Louisiana during the 2021 growing season were from four parishes in eastern Louisiana, south of the Mississippi border (**Fig. 1B; Table 1**). In total, samples were collected from 52 fields/plantings across six counties in Alabama, 23 fields/plantings across four parishes in Louisiana, and 43 fields/plantings across thirteen counties in Mississippi from a broad range of cucurbit crops including watermelon, cantaloupe/melon, pumpkin, squash, and cucumber (**Tables 1, 2, and 3**).

Plants were sampled predominantly 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 (**Fig. 2**), because such symptoms are typical in plants infected by whitefly-transmitted yellowing viruses. Leaves of several plants were collected per field to provide a representation of what was present in each field. Sampled leaves were collected, placed in bags 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. Testing for CYSDV, CCYV, SqVYV, and CABYV, which produces symptoms nearly identical to those of CYSDV and CCYV, was conducted using the multiplex RT-PCR system. Plants

were tested separately for beet pseudoyellows virus (BPYV), which also produces yellowing symptoms but is transmitted by a different whitefly (greenhouse whitefly). The lone DNA virus evaluated, CuLCrV, involved a separate testing method (PCR), as sensitivity of detection is best for RNA and DNA viruses when extracted separately.

A. Counties and Parishes surveyed in 2020.



B. Counties and Parishes surveyed in 2021.

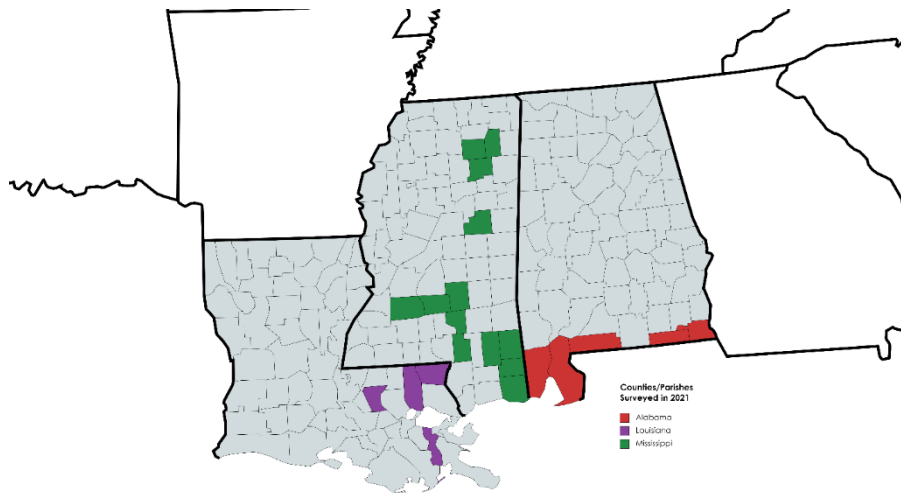


Figure 1. Counties surveyed for yellowing viruses of cucurbit crops in Alabama, Louisiana, and Mississippi in 2020 and 2021. Maps created with mapchart.net ©.

Table 1. Counties/Parishes Surveyed/Sampled for Virus Testing in Alabama, Louisiana, and Mississippi in 2020 and 2021	
State	Counties/Parishes
Alabama	Baldwin, Escambia, Geneva, Houston, Lee, Mobile
Louisiana	East Baton Rouge, Jefferson, Tangipahoa, Washington
Mississippi	Chickasaw, Copiah, Covington, George, Greene, Jackson, Lamar, Lee, Perry, Pontotoc, Simpson, Smith, Winston

Table 2. Number of Commercial Cucurbit Fields, Gardens, and Research/Demonstration Plantings Surveyed/Sampled in Alabama, Louisiana, and Mississippi in 2020 and 2021			
State	2020	2021	TOTAL
Alabama	25	27	52
Louisiana	0	23	23
Mississippi	3	40	43
TOTAL	27	90	118

Table 3. Cucurbit Crops Surveyed/Sampled and Tested for Viruses in Alabama, Louisiana, and Mississippi in 2020 and 2021								
State	Host							TOTAL
	Cantaloupe/ melon	Cucumber	Pumpkin	Squash	Watermelon	Unknown Cucurbit	Weeds	
Alabama	114	25	34	35	206	0	0	414
Louisiana	8	4	0	14	104	0	2	132
Mississippi	35	10	47	26	120	4	13	255
TOTAL	157 (19.6%)	39 (4.9%)	81 (10.1%)	75 (9.4%)	430 (53.7%)	4 (0.5%)	15 (1.9%)	801

The majority of plants sampled were watermelon (53.7%, followed by cantaloupe (19.6%) and pumpkin, (10.1%) (Table 3). Watermelon and cantaloupe/melon were sampled primarily in early summer, whereas squash and pumpkin were sampled primarily in the fall. In addition to

cucurbit crops, some weeds were collected from in and around fields, primarily based on whether or not virus-like symptoms were observed on leaves of the weed plants.



Figure 2. 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 characteristic of infection by the target viruses.

Alabama. Results of testing in 2020 identified the presence of CYSDV and CCYV in Alabama for the first time. This was published in the journal, *Plant Disease* (Mondal et al., 2022; <https://doi.org/10.1094/PDIS-05-21-0922-PDN>). As in 2020, most positives in 2021 were from Alabama. Over the two-year period, 72 plants were determined to be infected with CYSDV and 31 plants were determined to be infected with CCYV, but many yellowed plants were identified to be infected by CABYV. One plant was also determined to be infected with SqVYV (**Table 4**).

Mississippi. Results in Mississippi from 2020 identified a single plant infected by the aphid-transmitted virus, CABYV, but no whitefly-transmitted viruses (**Table 4**). CYSDV was identified for the first time in Mississippi in 2021 in a much lower number of plants compared to what was found in Alabama (**Table 4**). Two plants were suspected to be infected with CCYV, but infection was not able to be confirmed with available secondary testing methods, possibly due to low virus titer in the samples. Although CABYV was identified in one plant in Mississippi in 2020, that virus was not detected in 2021. **Louisiana.** No whitefly transmitted viruses were identified

in Louisiana; however, similar to the two samples in Mississippi, one plant was suspected to be infected with CCYV but was unable to be confirmed (**Table 4**).

Table 4. Viruses identified (confirmed) in cucurbit crops from in Alabama, Louisiana, and Mississippi in 2020 and 2021							
State	BPV	CABV	CCV	CYSDV	CuLCrV	SqV	Potyvirus
Alabama	0	31	30	72	0	1	6 WMV, 1 CMV
Louisiana	0	0	0	0	0	0	--
Mississippi	0	1	0	4	0	0	14 WMV 6 PRSV (w/WMV)
TOTAL	0	32	30	76	0	1	27

In addition, plants with symptoms typical of mosaic viruses, namely a mosaic pattern of light and dark green on leaves and leaf distortion, were present in some fields. These samples were tested for a number of mosaic viruses (cucumber mosaic virus (CMV), papaya ringspot virus (PRSV), watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV)) in addition to the target whitefly-transmitted and yellowing viruses. The aphid-transmitted PRSV and WMV were identified in plants in Mississippi and CMV and WMV were identified in Alabama (**Table 4**).

Furthermore, plants present at two different locations in 2021 in Mississippi exhibited classic symptoms of known yellowing viruses but did not test positive using standard or alternate methods of detection for any of these viruses. Further studies are being conducted on representative samples to determine potential virus-related causes of these symptoms. State project leaders are in the process of notifying producer collaborators whose fields were surveyed of the results from 2020 and 2021. Surveys will continue in 2022 to continue to monitor emergence of target whitefly-transmitted and yellowing viruses across the region.

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. Although sampling was not restricted during 2021, we saw very few whiteflies during the watermelon season in Mississippi and Louisiana, and limited numbers in Alabama. Based on these observations, we focused our efforts on virus detection in plants during 2021. Plans for 2022 include monitoring for the presence of whiteflies in selected fields to determine when populations begin to build.

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

The planned in-person educational programs for Louisiana, Mississippi, and Alabama watermelon producers in April 2020 were cancelled due to the COVID-19 pandemic. As a result, a webinar was held in May 2020 to educate stakeholders on whitefly-transmitted and yellowing viruses in cucurbits and this NWA-funded initiative to survey for these viruses. A recording of the webinar, as well as the developed educational resources, were posted online on the Mississippi State University Extension website (project page: <http://msuext.ms/ipl6c>). The project and whitefly-transmitted and yellowing viruses were also discussed during other county agent or producer trainings held during 2020 and 2021. The developed educational resources, including the fact sheet and photo guide, were distributed to stakeholders through a variety of means, including mail, e-mail, and in person. A grower-collaborator interest form (<http://msuext.ms/h27md>) was also set up to try to identify potential grower-collaborators in the three states.

Extension Publications/Deliverables:

- **Poster** - Survey of whitefly-transmitted viruses in watermelon and other cucurbit crops in Alabama, Louisiana, and Mississippi
Displayed at producer advisory council meetings held in Mississippi in 2020.
- **Publication (factsheet)** - [Whitefly-transmitted and yellowing viruses in watermelon and other cucurbits](#)
Over 200 copies printed for distribution.
Award Recognition: State (Mississippi) and Regional (Southern Region) winner in the 2021 National Association of County Agricultural Agents Communications Awards – Publication category
- **Publication (photo guide)** - [A photo guide to whitefly-transmitted and yellowing viruses in watermelon and other cucurbits](#)
Three hundred copies printed for distribution.
- **Project webpage** – [Survey of Whitefly-transmitted Viruses in Watermelon and Other Cucurbit Crops in Alabama, Louisiana, and Mississippi](#) (URL: <http://msuext.ms/ipl6c>)
Reported 330 unique page views.
- **Voice-over presentation** - available on Mississippi State University Extension YouTube Channel at https://www.youtube.com/watch?v=yn_OepNuDB0 (posted June 18, 2020) and on project webpage (link above)
Reported 382 views as of 01/28/2022.
- **Webcast** - available on Grow: Plant Health Exchange’s Focus on Cucurbits at <https://doi.org/10.1094/GROW-CUC-08-20-006> (posted August 2020)

Peer-reviewed Publication:

Mondal, S., Hladky, L.J., Melanson, R. A., Singh, R., Sikora, E., and Wintermantel, W. M. 2021. First report of cucurbit yellow stunting disorder virus and cucurbit chlorotic yellows virus in cucurbit crops in Alabama. *Plant Disease*. <https://doi.org/10.1094/PDIS-05-21-0922-PDN>.

Presentations for Scientific Audiences

Wintermantel, W. M., Melanson, R. A., Sikora, E., and Singh, R. 2022. Update on a survey of whitefly-transmitted and yellowing viruses in the Central Gulf Coast states. 2022 Watermelon Research & Development Group Annual Meeting (hybrid; virtual presentation), New Orleans, LA, February 11-12, 2022.

Melanson, R. A., Wintermantel, W. M., Sikora, E., and Singh, R. 2020. Survey of whitefly-transmitted viruses in watermelon and other cucurbit crops in Alabama, Louisiana, and Mississippi. (poster) Coastal Mississippi Producer Advisory Council Meeting, Biloxi, MS, February 27, 2020.

Melanson, R. A., Wintermantel, W. M., Sikora, E., and Singh, R. 2020. Survey of whitefly-transmitted viruses in watermelon and other cucurbit crops in Alabama, Louisiana, and Mississippi. (poster) Central Mississippi Producer Advisory Council Meeting, Raymond, MS, February 19, 2020.

Webinar

Melanson, R. A. (organizer and host), Wintermantel, W. M. (presenter), Sikora, E. (panelist), and Singh, R. (panelist). 2020. Identifying threats from virus diseases in watermelon and other cucurbits. Mississippi State University Extension Service. May 13, 2020.

Video

Wintermantel, W. M., Melanson, R. A., Sikora, E. J., and Singh, R. 2020. Identifying threats to virus diseases in watermelon and other cucurbit crops. Mississippi State University Extension Service. Online: https://www.youtube.com/watch?v=yn_OepNu_DB0&t=3s. Available: June 18, 2020.

Awards

State winner (Mississippi) in the 2021 National Association of County Agricultural Agents Communications Awards – Publication category (publication entry: Whitefly-transmitted and Yellowing Viruses in Watermelon and Other Cucurbit Crops by Melanson, R. A., Wintermantel, W. M., Sikora, E. J., and Singh, R.)

Regional winner (Southern Region) in the 2021 National Association of County Agricultural Agents Communications Awards – Publication category (publication entry: Whitefly-transmitted and Yellowing Viruses in Watermelon and Other Cucurbit Crops by Melanson, R. A., Wintermantel, W. M., Sikora, E. J., and Singh, R.)

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