COTTON LEAFROLL DWARF VIRUS – AL POLEROVIRUS IDENTIFICATION, SYMPTOMATOLOGY, AND OCCURRENCE IN ALABAMA

Kathy S. Lawrence
Alana L. Jacobson
Ed Sikora
Austin Hagan
Kassie Conner
Auburn University
Auburn, AL
Drew Schrimsher
Agri-Afc, LLC
Slocomb, AL
Judith K. Brown
University of Arizona
Tucson, AZ

Abstract

Cotton plants displaying symptoms of a possible virus disease were observed in producer fields across the gulf coast area of Alabama (Schrimsher et al, 2018). On August 29, 2017, Drew Schrimsher, Agronomist with AGRI-AFC and alumni of Auburn University conveyed his concern about the cotton symptoms he was observing across the acreage of the southern region of his territory to Kathy Lawrence, Professor in the Department of Entomology and Plant Pathology. August 31, 2017 we met in Barbour County, AL to look at some symptomatic cotton fields. Symptoms consisted of foliar distortion, leaf crinkling at the center of the leaf with 'rolling' in the direction of the leaf edge (Figure 1). The veins were sometimes reddish toward the petiole but chlorotic, extending toward the edges of leaves. Whiteflies (*Bemisia tabaci* B type) were abundant in all the fields. As we walked through the fields, whiteflies were swarming. Initial thoughts were herbicide damage, but with further investigation, this possibility was ruled out. Symptomatic cotton leaves with the petiole attached and/or stems with 6-8 leaves attached were collected from plants in several locations.

Figure 1. Cotton virus symptom on August 31, 2017.

Cotton viruses have not been reported to occur in the southeastern region of the cotton belt. John Murphy, Professor and virologist, Department of Entomology and Plant Pathology looked at the symptomatic samples, and suggested contacting Judith Brown, Professor in the School of Plant Sciences, The University of Arizona, a well-known expert in cotton viruses. In the southwest cotton region, the whitefly-transmitted *Cotton leaf crumple virus* (CLCrV), a begomovirus (*Geminiviridae*) infects the cotton crop in most years, causing damage primarily when plants become infected at young growth stages. Ultimately, three labs, Dr. Brown's, Auburn University Diagnostic, and AGDIA Inc., were contacted for assistance. Dr. Brown's lab tested multiple symptomatic cotton foliar samples and whiteflies but were unable to detect the leaf crumple virus or the best-known species associated with the cotton leaf curl disease

complex, also whitefly-transmitted geminiviruses, that infect cotton in China, India, and Pakistan. The results from AGDIA testing for CLCrV were negative.

Dr. Brown's lab continued to work with us to attempt to identify the virus-like causal agent suspected to be associated with the disease. We returned to the symptomatic cotton fields bi-weekly during September through October of 2107, collecting symptomatic cotton samples using varying approaches to preserve the samples to protect putative viral RNA or DNA. During this time period, approximately 30 to 60 days after the initial indications of a disease, the foliar symptoms advanced to include distorted juvenile leaves with severe foliar crinkling and distortion, shortening of the internodes, vein-reddening, a dark-bluish coloration of some leaves, with 1 to 2 mm leaf necrotic lesions observed on some of the plants (Figure 2).

Figure 2. Cotton symptoms from September through October 2017. Entire plant on left, upper foliage in the center and a single leaf on the right.

Cotton bolls were not present on the symptomatic portion of the plant; however, bolls were maturing normally at the base of the plant below the symptomatic leaves. At the end of the 2017 season, the symptomatic plants covered an estimated 50,000 acres across Alabama, with similar symptoms observed in fields in Georgia and the panhandle of Florida.

March 16, 2018, Dr. Brown's lab identified the suspect virus using Illumina RNAseq sequencing and *de novo* sequence assembly of an ~1140 nt fragment when compared with sequences available in the GenBank database, was found to be most closely related to isolates of *Cotton leafroll dwarf virus* (CLRDV) (genus, *Polerovirus*) known to occur in Argentina and Brazil (Avelar et al., 2018). The fragment represented only about one-fifth of the genome, permitting a tentative diagnosis. In Brazil, the common name of CLRDV is cotton blue disease, and the virus is known to be aphid-transmitted. Dr. Brown contacted the United States Department of Agriculture-APHIS to report the identification of this presumed exotic (introduced) virus in the U.S. Dr. Lawrence contacted Drs. Bob Nichols, Cotton Inc. and Don Parker, Cotton Foundation to share the findings of a new cotton disease.

Monitoring for this new virus began before cotton planting in 2018. In April, we tested for the virus in possible over-wintering hosts using reverse transcriptase-polymerase chain reaction (RT-PCR) amplification with primers designed based on the partial virus sequence, including weeds from the fields were symptoms were present the previous year. Over 30 weed genera were collected and sent to Dr. Brown's lab, however, no virus was detectable. This does not mean that they were definitely not hosts of the virus, and so they were not eliminated as suspects. Timing and virus concentration affect the ability to detect plant viruses. June 7, 2018 the first cotton aphid outbreak occurred in Barbour Co, and symptomatic cotton leaf tissue was collected (Figure 3 left), and shipped to Dr. Brown's lab; however, no virus was detected.

Figure 3. Cotton on June 7, (left) and June 25 (right), 2018 with aphid damage and virus like symptoms.

On June 25, 2018, samples were collected (Figure 3 right), and tested with similarly negative results. On August 16, 2018, Dr. Brown visited Auburn University to observe the symptoms first-hand, and to share her experience with cotton viruses with Auburn cotton geneticists/breeder, pathologists, and entomologists. She shared the CLRDV-AL primers with Dr. Kassie Conner, Auburn University Diagnostic lab to enable virus identification in cotton samples collected throughout the region. In August 28, 2018 cotton virus symptoms were observed at the Plant Breeding Unit of the E. V. Smith Research and Experiment Station of Auburn University on cotton varieties Phytogen 800 and 72 (Figure 4), and samples were sent to Drs. Brown and Conner. Both labs confirmed the presence of our CLRDV-AL virus.

Figure 4. Phytogen 800 and 72 cotton cultivars collected August 28, 2018 which tested positive for the CLRDV-AL virus.

August 29, 2018, Drs. Bob Nichols, Cotton Inc. and Don Parker, Cotton Foundation organized a meeting at the Auburn University Wiregrass Research and Extension Center with Austin Hagan, Cotton Extension Plant Pathologist hosting. The objective of the meeting was to share all the information we know about the virus on cotton with extension and research scientists, private industry, and growers.

September 19, 2018, Dr. Conner and I met in the Plant Breeding Unit field were the cotton varieties Phytogen 800 and 72 had previously tested positive for our CLRDV-AL virus. Dr. Conner collected samples, confirmed the virus and reported the finding to APHIS. In September through November 2018, university and extension scientists and Drew Schrimsher from AGRI AFC collected symptomatic samples and send them in to both labs to confirm and map the current location of the virus by counties. There were 20 counties in Alabama are the virus had been confirmed by December 6, 2018 (Figure 5.)

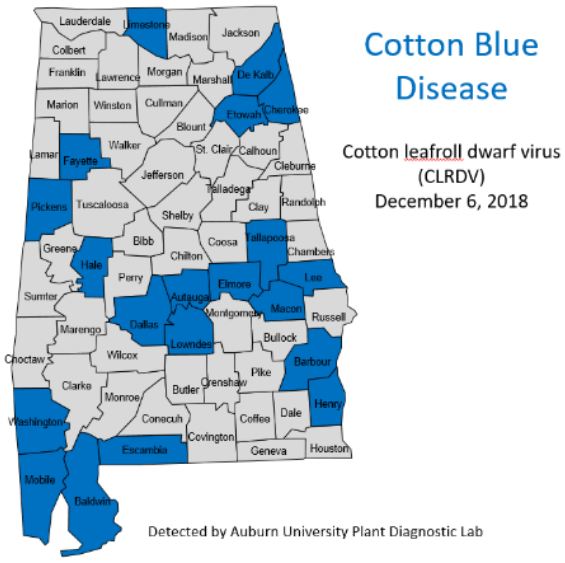

Figure 5. Counties in Alabama, which tested positive for CLRDV-AL as of December 6, 2018.

References

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