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Cotton leafroll dwarf disease: An emerging virus disease on cotton in the U.S.

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Symptoms associated with cotton leafroll dwarf virus: blistering in new leaf growth (left) and downward cupping (right). Photo courtesy of Dr. Judith K. Brown, University of Arizona.

In 2017, symptoms of leaf crinkling, downward curling, and deformation resembling virus-like diseases were observed in Alabama. Partial gene

sequences of the virus identified as cotton leafroll dwarf virus. By 2019, the virus has been detected from all the cotton-growing states east of Texas, suggesting its widespread presence in the Cotton Belt of the continental U.S. This article discuses the virus's history, symptoms, diagnosis, host range, properties, and genome.

In 2017, symptoms of leaf crinkling, downward curling, and deformation resembling virus-like diseases were observed in Alabama. Since there was a high incidence of whitefly in the fields where the symptoms were observed, they were tested for the presence of whitefly-transmitted viruses; however, none were detected. In 2017, cultivated cotton in Alabama was affected with an estimated economic loss of $19 million. Further investigations revealed the association of an aphid-transmitted RNA virus with symptomatic plants. Partial gene sequences of the virus identified as cotton leafroll dwarf virus, the same virus associated with a disease of cotton commonly known as "cotton blue disease" prevalent in South American and Asian countries (Avelar et al., 2019).

In late 2018, extensive surveys were carried out, and the presence of the virus was confirmed from 23 counties in AL and 14 counties in GA (Figure 1). The virus's presence was also confirmed from other cotton-growing regions in the Southeast, including Mississippi, South Carolina, and Florida. By 2019, the virus has been detected from all the cotton-growing states east of Texas, suggesting its widespread presence in the Cotton Belt of the continental U.S.

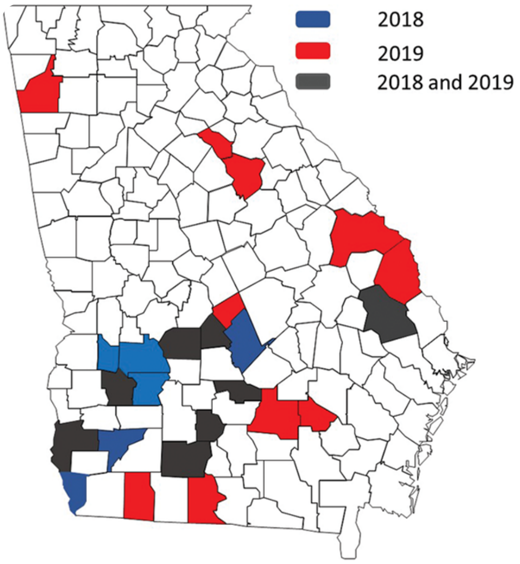

Figure 1, *Counties in Georgia where the presence of cotton leafroll dwarf virus (CLRDV) has been confirmed from commercial fields and University of Georgia research farms during 2018–2019.*

Cotton Leafroll Dwarf Virus History

Cotton plants showing reddening of leaves and petioles, shortening of internodes, and stunting were first described in 1949 from central Africa, and the condition was commonly called cotton blue disease (CBD; Cauquil & Vaissayre, 1971). In 1938 and 1962, similar symptoms were also described from cotton in Brazil known as vein mosaic "var. Ribeirao Bonito." Transmissibility of the disease by cotton aphids (*Aphis gossypii*) under controlled studies suggested the association of the virus with the disease (Cauquil & Vaissayre, 1971). Based on the partial genome sequence, it was identified as a new virus and named cotton leafroll dwarf virus (CLRDV) (Correa et al., 2005). Later, the full-length viral genome was sequenced and characterized (Distefano et al., 2010). Since then, the virus has been reported in cultivated cotton (Mukherjee et al., 2012) and chickpea (Reddy & Kumar, 2004) from India, Thailand (Sharman et al., 2015), East Timor (Ray et al., 2016), and Uzbekistan (NCBI GenBank MK461137). Although not from plant hosts, the virus was also detected from soybean aphids (*Aphis glycines*) collected from soybean in China (Feng et al., 2017). Cotton blue disease has been endemic in Argentina and Brazil with up to 80% yield loss (Silva et al., 2008; Distefano et al., 2010). Host plant resistance against the virus was the most effective method for managing CBD in South America. However, in 2006, “resistance-breaking” atypical strains of CLRDV capable of infecting CBD-resistant varieties have been reported from Argentina and Brazil.

Symptoms

In Georgia and other cotton-growing regions in the U.S., various types of plant symptoms were observed. The symptoms’ diversity depends on the biotic and abiotic factors, including variety, location, planting date, aphid population density, and other environmental factors.

In Georgia, early in the planting season, symptoms as reddening of leaves and petiole and downward drooping of leaves as an inverted “V” were observed (Figure 2a–d). The plants shade the leaves (Figure 2e), and in many cases, the symptoms diminish, and the plants revive later in the season (Figure 2f).

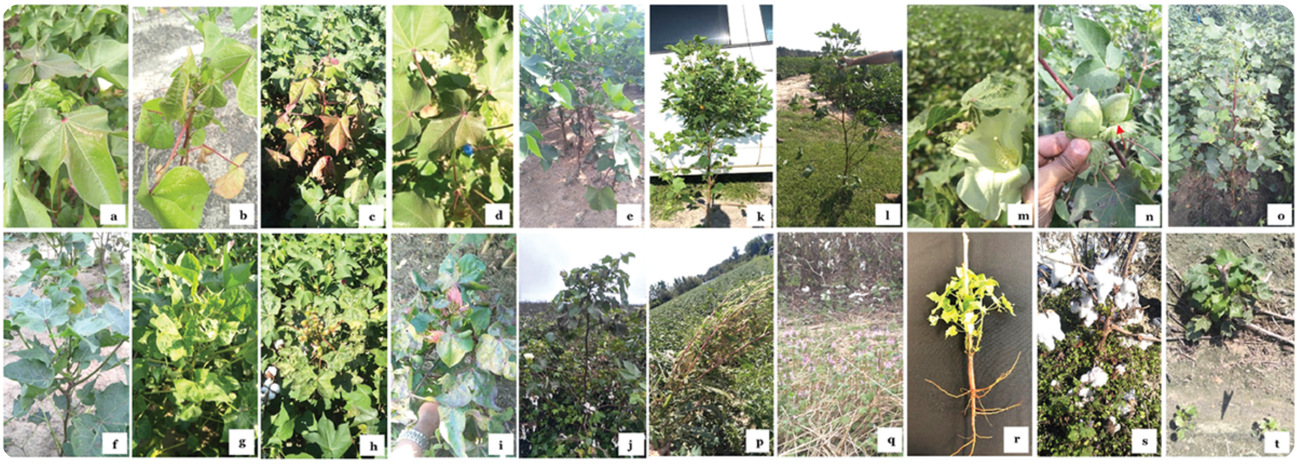

Figure 2, Different symptoms observed in cotton plants in the early and late stages of plant growth. Cotton leafroll dwarf virus (CLRDV) has been detected from all these symptomatic plants, volunteer cotton, and weeds.

In late infections, the plants show leaf deformation (Figure **2g**), crinkling (Figure **2h**), leaf rugosity (Figure **2i**), shorter internodes (Figure **2f,j**), bushy foliage (Figure **2k**), fewer boll retentions (Figure **2l**), deformed flowers (Figure **2m**), and “parrot-shaped” bolls (Figure **2n**). In some cases, plants with extended nodes of 32–36 internodes were observed (Figure **2o**) with no harvestable bolls (Figure **2l,o**). Similar symptoms were also observed from other cotton-growing regions in the U.S. On volunteer cotton and basal regrowth, symptoms like leaf deformation, shorter leaves, dwarf, and shorter internodes were observed (Figure **2p,q**).

Cotton leafroll dwarf virus has been detected from these symptomatic plants using a molecular assay, but a high percentage (>90%) of asymptomatic cotton plants also tested positive for the virus, suggesting that it remains latent in the plant and potentially expresses under certain favorable conditions. The symptoms observed due to CLRDV are highly variable and need to be further investigated. The presence of the virus from symptomatic plants does not suggest that it is the causal agent for the particular symptoms unless replicated under controlled conditions. Research efforts are ongoing to understand the disease under controlled conditions.

Diagnosis and Host Range

Since a large number of asymptomatic cotton plants test positive for CLRDV, symptoms alone are not reliable for accurate detection of the virus. Nucleic acid–based RT-PCR (reverse transcription–polymerase chain reaction) detection assays targeting multiple genes were developed and standardized to detect the virus in plant tissues (Tabassum et al., **2020**). The qRT-PCR assay has been designed to quantify the virus in the plants that will be applied to estimate the virus titer in symptomatic and asymptomatic plants.

Since the report of CLRDV in the U.S., the virus has been detected from common weeds such as Palmer amaranth (*Amaranthus palmeri*; Figure **2p**), carpetweed (Mollugo verticillata), evening primrose (Oenothera biennis), henbit deadnettle (Lamium amplexicaule; Figure **2q**), perennial peanut (Arachis glabrata), and white clover (Trifolium repens). Another potential source of the virus inoculum is the overwintering cotton that survives mild winters in no- or reduced-tilled cropping systems. A very high percentage of over-wintering cotton was found positive for CLRDV in 2019–2020 in Georgia (Figure **2r–t**).

Minimal information is available on the host range of CLRDV. The virus is reported from several *Malvaceae* species as *Gossypium arboretum*, *G. barbardense*, *G. hirsutum*, *Hibiscus sabdariffa* var. *altissima*, *H. rosa sinensis*, and *Sida acuta*. Similarly, the CLRDV genome was also sequenced from soybean aphids (Feng et al., **2017**), suggesting that the virus could be present in other hosts, including leguminous and oilseed crops. Chickpea stunt disease-associated virus is a Luteovirus with a very high level of sequence similarity that has been reported from chickpea in India (Reddy & Kumar, **2004**). The extensive host range of the CLRDV isolates of the U.S. is currently not known.

Virus Properties and Genome

Cotton leafroll dwarf virus is a phloem-limited virus. The virion particles are non-enveloped, spherical, and approximately 23 nm in diameter. The virus is efficiently transmitted by cotton aphids (*Aphis gissipii*) in a circulative, non-propagative manner**3**. The aphids acquire the virus during feeding, and it remains within the aphids for a few weeks to their entire life period. Although cotton aphids more efficiently transmit CLRDV on cotton, other aphids species as cowpea aphids (*A. carcciora*) and green peach aphid (*Myzus persicae*) are also reported to transmit the virus (Reddy & Kumar, 2004).

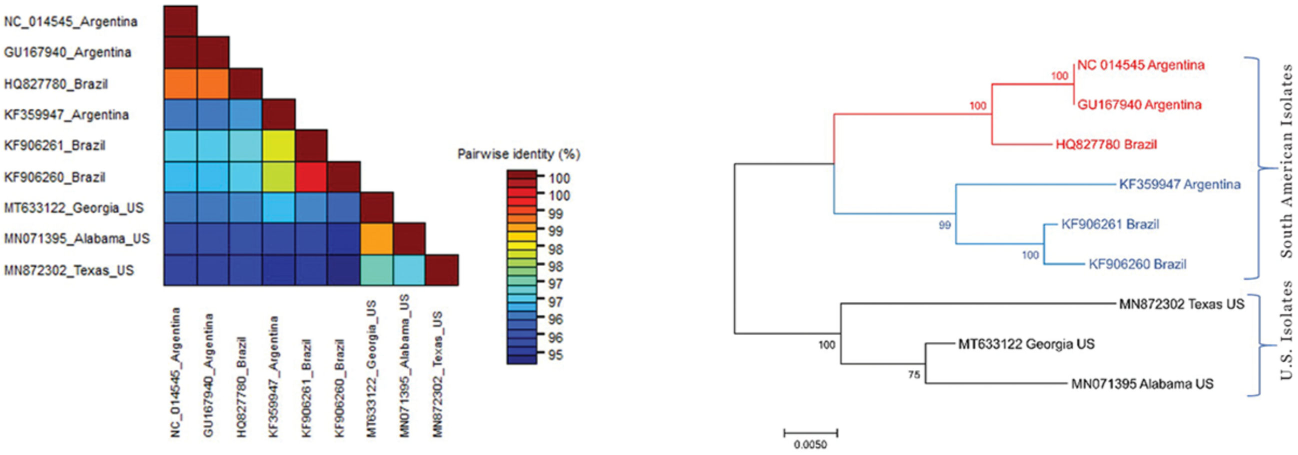

Figure 3, *Pairwise sequence identity matrix (left) and maximum likelihood phylogenetic tree of complete nucleotide sequences of cotton leafroll dwarf virus (CLRDV) from Georgia and other sequences reported in GenBank (right). The U.S. isolates form a separate clade from other South American isolates.*

The genome of CLRDV is linear, monopartite, ssRNA (+) of 5,866 bp in length. In the U.S., the genome sequence of CLRDV from Alabama, Georgia, and Texas has been elucidated and is publicly available in the GenBank (**www.ncbi.nlm.nih.gov/genbank**). The genome sequence from Georgia is 95 to 98% identical to the genome of other CLRDV isolates from the United States (Alabama and Texas) and South America (Figure **3**). In the phylogenetic analysis based on full-length nucleotide sequences, the U.S. isolates formed a clade separate from that of the South American isolates. Further, the isolates from Alabama and Georgia are very similar to each other. In contrast, the Texas isolate is similar to the South American isolate, suggesting two different strains of the virus present in the U.S. (Figure **3**) (Tabassum et al., **2020**).

Conclusion

Cotton leafroll dwarf virus is widely prevalent in cotton-growing regions in Georgia and other cotton-growing states in the U.S. A wide range of symptoms are associated with CLRDV on cotton; however, a very high percentage of asymptomatic cotton has also been found positive for the virus. The virus is transmitted efficiently by cotton aphids. Overwintered cotton and common weeds could host viruses and the vector and potentially act as an inoculum source for the consecutive planting season. Cotton leafroll dwarf virus has the potential to cause a significant threat to the cotton industry. Currently, there is no host resistance available against the virus. There is a need to apply conventional and advanced tools to understand the disease and develop integrated disease management strategies. Further surveillance and investigation are needed to increase our understanding of the virus biology and prevalence in these regions as well as potential yield loss.

Acknowledgments

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