

**Report of A Research Review and Planning Meeting on
Cotton Leafroll Dwarf Virus**

Orange Beach, Alabama – October 8, 2019

Steve Brown, Kassie Conner, Austin Hagan, Alana Jacobson,
Jenny Koebernick, & Kathy Lawrence – Auburn University,
Sudeep Bag, Bob Kemeraït & Peng Chee - University of Georgia,
Tom Allen & Sead Sabanadzovic – Mississippi State University,
Bob Nichols - Cotton Incorporated

Executive Summary

Cotton leafroll dwarf virus (CLRDV) is an aphid (*Aphis gossypii*) transmitted Polerovirus. CLRDV was first described in Africa and subsequently infested cotton (*Gossypium hirsutum*) in Asia and South America. Cotton suspected of being virus infected was observed in Alabama (AL) and Florida (FL) in 2017. Genomic sequences of three 2018 isolates from AL were characterized and a whole genome of the AL strain of CLRDV was published in 2019. The Alabama strain differs from those causing cotton blue disease (CBD) and atypical cotton blue disease (ACBD) in Brazil and Argentina. The disease caused by CLRDV in the U.S. should be called Cotton leafroll dwarf disease (CLRDD). Symptoms vary by cultivar: likely by time of infection(s) with respect to cotton's growth, likely by titer of the inoculum, and by our observations, by stage of disease progression and among locations. Moreover, CLRDD symptoms in specific cultivars may inherently differ across locations. In addition, asymptomatic infections occur. The characteristic appearance of symptomatic plants at comparable stages in disease progression differs from Georgia (GA) to Mississippi (MS) to Texas (TX). The first symptoms that may appear are drooping leaves that vary from typically red in AL and GA to dark green MS, respectively. Subsequent growth may show crinkled or rugose leaves – a symptom more commonly observed in AL; such plants may produce green leaves with normal leaf shape in mid-summer, but some plants will remain stunted and suffer fruit deformation and fruit shed and may be altogether barren. Late season symptoms first include stacking of internodes with copious fruiting, but consequent rapid fruit abortion, and then such plants sometimes progress to accentuated elongation (verticality) of the mainstem.

Incidence and Impact: Recorded incidence expanded dramatically in 2019 to many counties in seven states. The virus was frequently detected in plants that appear to be asymptomatic based on the symptoms described above. However, survey of the state Extension plant pathologists indicate overall severity was minor. At this point, severe yield losses in AL, GA, and MS have been potentially recorded only in one, two, and seven fields respectively, although this is by no means an exhaustive count. Common factors among severely damaged fields should be identified. In the Southeast, CLRDV has also been detected in several summer and winter annual weeds and in some perennials and appears broadly distributed in the environment. The publication of these

hosts is pending. Although severity appeared minor in 2019, preparation for this disease is imperative, lest it become a major concern in future years.

Management Options: Cotton aphids have a ubiquitous presence in cotton with a pronounced influx shortly following the summer solstice. Attempts to reduce virus infection by managing the aphid with zero, one (early July), or ten insecticide applications were all equivalently ineffective. Cultivars bred in Brazil to be resistant to CBD, 'BR 293', and both CBD and ACBD, 'BR 286', were uniformly susceptible in sentinel plots from South Carolina (SC) to East Texas. Variations in the response of cotton genotypes to CLRDV-AL have been observed, but evaluation of the lines are at too early a stage to make definite statements regarding relative susceptibility or the availability of resistance genes.

Conclusions: CLRDV is a newly observed virus in North America. Cotton genotypes resistant to the CLRDV strains of South America are susceptible to CLRDV-AL in the U.S. Management by control of the aphid vector does not seem possible. Much about the virus remains unknown. CLRDV appears to be a potential threat to U. S. cotton. The extent of the damage and how such damage may be averted or mitigated should be investigated in a coordinated and well-funded research program.

Pathogen and Disease

Naming the Virus and Disease – R. L. Nichols for the U.S. virologists working on CLRDV Following the publication of the consensus genome of three isolates originally collected from central Alabama (Avelar et al., accepted by Plant Disease 9/17/19) the four virologists actively working on this invasive virus, Drs. Olufemi Alabi, Texas Agri-Life Research, Judy Brown, Univ. of Arizona, Sudeep Bag, Univ. of Georgia, and Sead Sabanadzovic, Mississippi State Univ. concur that the virus now in the Southeast U.S. should be called Cotton leafroll dwarf virus (CLRDV) and the disease it causes therefore is named as Cotton leafroll dwarf disease (CLRDD).

Further Consideration:

1. The rules of nomenclature for viruses are under review.
2. Additional, related isolates may be otherwise named.

Diagnostics - K. Conner, Auburn Univ. and S. Bag, Univ. of Georgia
Diagnostics is currently the bottleneck of Cotton leafroll dwarf virus (CLRDV) research. Determination of the presence and, for certain applications, the titer of the virus is requisite for acquiring information for breeding, and on the distribution, etiology, epidemiology, ecology, vector relationships, and for evaluation of certain management tactics. Symptoms of the virus vary with location, environment, plant nutrition, host genotype, as well as stage of growth, and may be exacerbated by additional underlying stresses. The presence or absence of the virus cannot be reliably confirmed by the presence or absence of symptoms. Many asymptomatic cotton plants and certain asymptomatic weeds have additionally been confirmed to be hosts of CLRDV.

The current qualitative diagnostic method – RT-PCR (of which there are multiple protocols) – is expensive (> \$25/sample) and time consuming (at least 1.5 days, with

processing time depending on the sample size). There is an immediate need for alternative diagnostic protocols to allow for greater throughput and reduced costs. There is also a need for the development of multiple diagnostic techniques to provide flexibility between labs. A qPCR protocol is currently being developed by Dr. Bag, which will allow for quantitative estimation of titer, with slightly higher throughput (1 day) and reduced cost (\$20/sample). Other labs are planning to help validate this protocol. For the longer term, several labs are working towards developing an ELISA protocol using various techniques. After development, the ELISA protocols will be tested for specificity and sensitivity, and then validated. These protocols may not be available until 2021 and ELISA is less sensitive than PCR. Other diagnostic methods currently under development or planning include isothermal amplification. **Large-scale research of CLRDV may not be feasible until diagnostic progress has been made.**

Identification of possible CLRDV variants will be extremely important for breeding. Publication of the whole genome sequence from AL isolates was recently accepted. Now is the time for virologists who have whole genome sequences to compare genomes from different areas. The 2019 regional sentinel plot participants have agreed to compare the P0 protein (coded by ORF0) from their respective states to determine if multiple strains occur across the cotton belt. A comparison of the P0s will be completed by the end of 2019.

Cotton leafroll dwarf disease - Observations in Sentinel Plots Austin Hagan summarizing for the reports presented

Sentinel plots were established in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Texas. Plots were established to determine the presence of CLRDV at the survey locations as well as describe symptom patterns and assess the reaction of four commercial U.S. cotton cultivars along with cotton cultivars resistant to the typical and atypical Brazilian CLRDV strains. The experimental design was a factorial arranged as a split-plot with planting date (one, two or three depending on the location) and cultivar (PhytoGen 480 W3FE, Deltapine 1646 B2FX, Stoneville 5471 GLT, NexGen 5711 B3XF, BRS 286, and BRS 293).

In Alabama, presence of CLRDV was confirmed in all cotton cultivars, including the Brazilian lines resistant to the typical (BRS 293) as well as atypical and typical (BRS 286) strains, in the primary sentinel plots located at the Brewton Agricultural Research Unit (BARU) and Prattville Agricultural Research Unit (PARU). The disease was also confirmed via symptoms and RT-PCR at additional Alabama study locations in one or more cultivars, but disease incidence was very low in these latter studies. At the BARU, symptomatic plants were noted and CLRDV infections confirmed in several plantings of NexGen 5711, BRS 286 and BRS 293 at the 2 July rating date. Disease incidence based on visual symptoms did not exceed 4% (BRS 293) in any of the cultivars screened. At the 29 July rating date, disease incidence was greater at the 3rd compared with the 1st planting date. For the PARU, CLRDD incidence was lower compared with that at the BARU. The presence of CLRDV at PARU was first confirmed via RT-PCR in BRS 293 on 9 July. At any rating date, CLRD incidence did not exceed 1.5% in any cultivar. Observed symptoms included leaf crinkle on seedling and maturing plants,

flagging of the terminal leaves or on an entire plant with some reddening/bronzing of the terminal leaves, red veins on the leaves, red to maroon petioles and stems in the upper canopy, and stacked nodes with shortened internodes. At both locations, a handful of symptomatic plants also failed to set fruit. Based on these results compared to that in a date of planting × cultivar study at BARU, disease incidence was much greater in the date-of-planting experiment compared with that observed in the adjoining sentinel plot experiment at BARU. In addition, CLRDD incidence was only significantly greater in the later than early planting dates in the date of planting experiment.

At the time of the meeting at Orange Beach, AL (10/8/2019) information from sentinel plots in other survey states was anecdotal, with no data being presented concerning CLRDD incidence or cultivars affected. However, each of the sentinel plot locations had been evaluated for the presence of CLRDD, and the symptoms observed were similar to those previously described. Based on PCR analysis of samples submitted to the AU Plant Diagnostic Laboratory, CLRDV was identified in all cultivars at the South Carolina location. In addition, the disease was also diagnosed in seedling cotton in Tennessee. Based on remarks at the meeting, overall, CLRDV incidence may have been greater in Mississippi than at any other sentinel plot location.

Recommendation:

For 2020, the sentinel plot program should continue with some modifications to the trial protocol. Since BRS 286 and BRS 293 proved susceptible to the endemic CLRDV strain present in the southern U.S., and neither expressed *Bt* or were herbicide-resistance traits, both cultivars should be replaced with Dynagro DG 3615 B3XF, which may not only may be highly susceptible to this virus but also contains the needed Bt and herbicide technology traits. Adding a Pima cotton selection with Bt and herbicide technology is also an option. A Pima cultivar, which proved to be highly susceptible to CLRDV in a breeding line study at GCREC, is also an option. One additional proposal may be to monitor the sentinel plots for aphids. Observing the aphids may be possible if the plant pathologist will partner with an entomologist to develop a simplified protocol for scouting for the aphid vector.

Symptoms and Impact of Cotton leafroll dwarf virus in the Southeastern United States

R.C. Kemera Jr., Univ. of Georgia; A. Hagan, Auburn Univ. and

T.W. Allen, Mississippi State Univ.

Impact and Incidence

Cotton leafroll dwarf disease (CLRDD) was observed in Georgia, Alabama, Mississippi, and other states from South Carolina to as far west as Texas in 2019. At the time of this meeting (8 Oct 2019), yield losses as a result of CLRDV are thought to be extremely low for this season. The greatest impact may be concern growers have for future management strategies.

In **Georgia**, fields where significant losses are likely to have occurred have been documented at only two locations. The fields are in central Georgia and in close

proximity to one another. Observations from Georgia, indicate that at least a few plants symptomatic for CLRDV can be detected in every field planted to cotton in the state. The incidence of symptomatic plants is quite low in the vast majority of fields in Georgia. However, it is also believed that as many as 60 to 70% of plants in a field may be infected with CLRDV, yet not exhibit any symptoms.

In southwest **Alabama**, CLRDV incidence in a late-maturing cotton cultivar at the Brewton Agriculture Research Unit exceeded 95% of the 55 plants sampled at cut-out, with many plants not exhibiting noticeable stunting or reduced boll set. However, preliminary results indicated a 41% reduction in the seed cotton yield of individual infected, symptomatic plants compared with non-CLRDV infected cotton plants. In addition, reductions in open, unopen, and rotted bolls were recorded for the symptomatic plants. In comparison, disease incidence in a mid-maturity cotton cultivar in a trial at the Sand Mountain Research and Extension Center in north central Alabama reached 54%, where a significant reduction in plant height, fruiting node numbers and total boll count were recorded for symptomatic plants than for non-CLRDV infected cotton plants.

In **Mississippi**, fields where significant yield losses were believed to have occurred are in seven locations. One, four, one and one of these fields are in Calhoun, Holmes, Leflore and Tishomingo counties, respectively. The incidence of symptomatic plants in five of these fields appeared to be 100%. Based on a fairly comprehensive survey of cotton throughout the state (n≈50 counties), symptomatic plants were observed in every field scouted. Incidence of the virus in those fields varied from low (visually < 10%) to high (approximately 100% of the plants presenting one or more of the symptoms associated with the disease).

Symptoms of CLRDV

Cotton plants testing positive exhibit one or more of the symptoms described below. Symptom expression appears to differ by cultivar and by time of infection and subsequent disease progression.

1. In the early season, seedlings and young plants often show significant reddening, stunting, and downward cupping of the leaves. Additional symptoms include yellowing around the leaf margins and rugosity along the veins of the youngest leaves. In severe cases, death of the seedling/young plant may occur. With good growing conditions, some of these plants determined to contain the virus show signs of recovery following the initial symptoms; however, they will likely remain stunted and set few fruit.
2. Symptoms observed in mid-season progress to more reddening and cupping of the foliage, beginning first in the uppermost parts of the canopy. Stems and petioles may be red around their complete circumference and red veins may be observed in the leaves. New leaves are often crinkled or rugose in appearance. In extreme cases, large portions of the canopy are affected, and the foliage develops yellow, bronze, or red. Whereas extreme reddening has been observed in Georgia, such coloration has not been observed in Mississippi.

Additional symptom expression is likely to include significant compression of internodes near the terminal and little-to-no fruit production on certain symptomatic plants. Flowers on symptomatic plants may be devoid of pollen and have misshapen or elongated flower parts (e.g., elongated stigma with stamens wrapped around the stigma in a whorl pattern). With good growing conditions, symptomatic plants may recover their green color; however, they may remain stunted and suffer fruit loss.

3. Symptoms associated with CLRDV late in the season include **a)** compression of internodes, **b)** excessive elongation of vegetative growth in the upper mainstem that produces a “whip” appearance, **c)** excessive, compact flowering, and in most cases flower/square abortion in the upper canopy, **d)** excessive vegetative branching from the bottom of the plant, **e)** stems that appear thicker than normal, **f)** misshaped (parrot-beaked) and abnormal boll size and shape, **g)** dark green coloration of the entire plant, sometimes with a bluish to purplish cast **h)** leaf tissue that feels thicker than normal (in some cases rubbery), **i)** reduced fruit set on severely infected plants, and **j)** excessive square production followed by rapid square abortion.
4. Symptoms associated with CLRDV on regrowth following defoliation include visible crinkling of the leaves, single-lobed or three-lobed leaves and, at times, reddening of petioles.

Diagnosing the presence of CLRDV-infected plants in the field can be difficult.

There are a number of reasons for this including:

1. Some symptoms associated with CLRDD can be the result of other causal factors. For example, several forms of stress and certain nutrient deficiencies can cause reddening of the foliage. Seedling diseases and Fusarium wilt can also produce symptoms similar to those of CLRDD early in the season.
2. Symptoms associated with CLRDD can be variable and may be associated or exacerbated with the stress.
3. Symptoms of CLRDD can diminish over time. Plants appear to recover.
4. Symptoms associated with CLRDD appear to differ by cultivar. Some varieties present mild symptoms (e.g., leaf rolling, limited node stacking, no elongated terminal growth); Others present severe symptoms (e.g., leaf rolling, node stacking, enhanced terminal elongation, extreme vegetative branching from the bottom of the plant, a high rate of flower/square production followed by abortion).

Possible Management Options

The Aphid Vector- Alana Jacobson, Auburn Univ. and Phil Roberts, Univ. of Georgia

Knowledge regarding aphid population dynamics in relation to virus spread is critical to understanding epidemiology and devising effective management strategies because

CLRDV may be transmitted from weed hosts to cotton exclusively by aphids. Ongoing research trials in Alabama and Georgia are currently investigating aphid species present in cotton agroecosystems, flight patterns and colonization of cotton by *A. gossypii*, and the impact of aphid management practices on the final incidence of CLRDV and yield. Studies conducted in 2019 have confirmed that the cotton aphid, *A. gossypii*, is a vector of CLRDV in the US. Preliminary data from field trials have also shown that colonization of cotton is greatest during peak flights of cotton aphids, and that during this time 100% of cotton plants examined were infested with aphids. Weekly insecticide sprays targeting aphid populations reduced populations but did not eliminate aphid colonization or reduce final incidence of CLRDV in experimental plots. Future research studies are needed to better understand aphid population dynamics in relation to timing of virus spread into cotton. Although chemical control measures are not effective at reducing final incidence of CLRDV, more information is needed to understand the impact of cultural control practices and other crop management practices on disease severity and yield loss.

Breeding for Resistance to the North American CLRDV

Peng Chee, Univ. of Georgia and Jenny Koebernick, Auburn Univ.

History in South America

In Brazil and Argentina, deployment of resistant cultivars is the most efficacious method for managing Cotton Blue Disease (CBD). Resistance to the South American strain of CLRDV that causes CBD has been identified from the variety 'Delta OPAL', which confers complete resistance to the virus. Inheritance studies have shown that resistance to CBD is conditioned by a single dominant gene (designated *Cbd*) located in the telomeric region of chromosome 10, and DNA markers tightly linked to the resistance gene have been developed allowing the use of marker-assisted selection to quickly develop new cultivars with high levels of resistance. However, disease management was complicated by the emergence of a resistance breaking strain of the virus named atypical CLRDV in 2010. Isolates of CLRDV identified in Alabama and Georgia generally show 98-100% sequence similarity and they also are closely related to the atypical CLRDV isolates reported from South America. While most cultivars in Brazil are susceptible to the atypical form of CLRDV, a small number of elite germplasm lines including Delta OPAL and 'BRS 286' are resistant to this virus.

Current Breeding Efforts in the U.S.

University of Georgia: Current research efforts to develop host-plant resistance have focused on screening a large, diverse set of cotton germplasm lines. In addition, because DNA markers linked to the R-gene for atypical-CLRDV have not been developed, several genetic mapping populations were developed using the line BRS 286 that is resistant to CBD in South America in an effort to identify new DNA markers linked to the resistance gene. However, observations from the sentinel plots now indicate that BRS 286 exhibits symptoms similar to atypical CLRDV infection such as stunting due to internodal shortening, leaf rolling, petiole and vein reddening, distorted new growth, and red and withered leaves. The susceptibility of BRS 286 to probable CLRDV-AL has now been

confirmed in several genetic populations developed using this line. Segregation data suggests that while BRS 286 may be carrying a gene for susceptibility, a number of germplasm lines utilized in the population development may be carrying genes for resistance, and the inheritance for susceptibility appears to be recessive.

The implication of an additional atypical-type strain of CLRDV in Alabama and Georgia capable of infecting the resistant cultivars from Brazil is that breeding for host-plant resistance will not be a straight forward process, but will necessitate additional research to identify new sources of resistance and DNA markers linked to the resistance genes. Moving forward, a significant effort should focus on testing additional germplasm as well as verifying those putative resistance lines identified in the 2019 disease nursery. In addition, the available data from 2019 suggests that resistances to the three South and North American strains of CLRDV are conditioned by different R genes, and BRS 286 may be carrying a susceptible gene for Alabama and Georgia strain of CLRDV. We will test this hypothesis by performing marker trait association and transcriptome profiling analysis on the segregating populations previously developed using BRS 286 as the susceptible parent.

Auburn University: In 2019, a collection of breeding lines, elite material, germplasm accessions and commercial breeding material was planted in two locations with two replications. In addition, a genome-wide association study (GWAS) diversity panel was planted in the Tallassee location, while F₂ populations were planted in the Fairhope location, each with two replications but only one location. In total, ~1242 unique lines were screened at this location an additional 100 plots were planted to okra to encourage aphids and over 300 plots served as check controls (BRS lines and Delta Opal). Preliminary results from the 1st replication show that only a fraction of the lines did not test positive for the virus. The second replication of these select lines will be tested to confirm the negative results. Additional testing, in greenhouse transmission studies, will be performed on lines that test negative in both replications.

Acknowledgments:

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Literature Cited:

Avelar, S., R. Ramos-Sobrinho, K. Conner, R. L. Nichols, K. Lawrence, and J. K. Brown. Characterization of the complete genome and ORF0 protein for a previously unreported strain of Cotton leafroll dwarf virus, an introduced polerovirus in the USA. (accepted by Plant Disease 9/17/19)