

INVESTIGATING THE INTERACTION BETWEEN CROP AGE AND TIMING OF *COTTON LEAFROLL DWARF VIRUS* INOCULATION ON DISEASE SEVERITY AND YIELD LOSS

Autumn McLaughlin

Department of Entomology and Plant Pathology, Auburn University
Auburn, AL

Kassie Conner

Alabama Cooperative Extension System Plant Diagnostic Lab, Auburn University
Auburn, AL

Kira L. Bowen

Department of Entomology and Plant Pathology, Auburn University
Auburn, AL

Austin K. Hagan

Alabama Cooperative Extension System
Auburn, AL

Will Groover

Kathy Lawrence

Alana Lynn Jacobson

Department of Entomology and Plant Pathology, Auburn University
Auburn, AL

Introduction

Cotton leafroll dwarf virus (CLRVDV) (genus *Polemovirus*, family *Luteoviridae*) is an emerging plant virus in the U.S. cotton belt that was first identified in samples collected from Alabama in 2017 (Alevar et al., 2018). CLRVDV was first identified in Africa in 1949 (Cauquil, 1977.) and has since been found in Argentina, Brazil, India, Thailand, and Timor-Leste (Corrêa et al. 2005, Mukherjee et al. 2012., Sharman et al. 2015., Ray et. Al 2016). In the U.S., CLRVDV has been reported in NC, SC, GA, AL, MS, and TX (Aboughanem-Sabanadzovic et al. 2019, Alabi et al. 2020, Ayelar et al.2018, Huseeth et al. 2019, Tabassum et al. 2019, Wang et al. 2020). Further testing has confirmed this to be genetically distinct from CLRVDV isolates in South America (Avelar et al. 2020). CLRVDV is a positive single stranded RNA virus. The known vector of this virus is the cotton aphid (*Aphis gossypii*). The cotton aphid transmits the virus in a persistent, circulative manner. Alate aphids are reported to transmit in 40 seconds and can retain the virus for up to 12 days (Michelotto and Busoli, 2003, 2009). The geographic range of the cotton aphid in the U.S. extends across the cotton belt, and infestations occur annually (Abney et al. 2008, Gore et al. 2013, Kerns et al. 2015). The symptoms attributed to the disease caused by CLRVDV in the U.S. are highly variable and include: leaf distortion, red tinting of the leaves, greenish-blue leaf color, red veins, red petioles, red stem, upward cupping, downward cupping, drooping, vein clearing, shortening of internodes, abnormal top growth, square abortion, and reduced size of bolls (Brown et al. 2020, Hagan et al. 2019). There is some data showing variation of CLRVDV symptoms among cultivars (Brown et al. 2019), but based on our understanding of plant viruses we know that there are a variety of factors that can influence symptom development including plant age at the time of infection, interactions with other abiotic and biotic plant stress factors, and environmental conditions. The objective of this study was to compare disease severity and yield loss among cotton plots that were infected with CLRVDV at three different growth stages.

Methods

In 2019, a field study was conducted at the E.V. Smith Research Center in Shorter, Alabama. Cages made of thrips-proof netting were used to cover cotton plots to confine insects released into the plots, and exclude natural infestations of early-season insects. Two-row plots that were 20' long were planted with DP1646 on May 30. The day after plant, plots were covered with insect cages, and all plots were caged for the same amount of time to reduce cage-related effects on symptom expression and yield across treatments. A randomized complete block design with 4 replicates was used to investigate the following treatments: 1) Control plots that were not infested with aphids; 2) Control plots in which non-viruliferous aphids were released (aphids only, no CLRVDV); 3) Plots infected with CLRVDV by releasing viruliferous aphids 1 week after emergence at the 3-4 true-leaf stage; 4) Plots infected with CLRVDV by releasing viruliferous aphids 2 weeks after emergence at the 5-7 true-leaf stage; 5) Plots infected with CLRVDV by releasing viruliferous aphids 3 weeks after emergence at the first-pinhead growth stage. Viruliferous aphids that were released into plots were generated by infesting CLRVDV-infected cotton plants with *Aphis gossypii* 2-3 weeks prior to release. Plants infested with aphids were grown in a greenhouse and in cages made from thrips-proof screen until they were

placed in field cages. In field cages alates were allowed to disperse for one week, and infested leaves were distributed across the plot to promote rapid colonization and virus transmission. Non-viruliferous aphids reared on healthy cotton in a separate greenhouse were reared and released the same. Four border rows that were not covered in cages were planted to monitor natural infection of CLRDV at this location. Aphids were eliminated from infested plots by spraying insecticide 2 weeks after infestation, and once the week before cage removal to prevent unintended virus spread among plots. Cages were then removed 8 weeks after plant. Ten plants were marked in each plot and monitored weekly for aphid numbers, plant mapping, and symptom presence. Plant mapping consisted of plant heights, presence/absence of 1st and 2nd position bolls, first fruiting node, and number of total nodes. Weekly spectral imaging was performed to determine early detection of disease. Virus was confirmed by Auburn's plant disease diagnostic lab in August, and yield was evaluated for each plot.

Results

PCR testing confirmed virus transmission in these plots, but not all plants tested positive for the virus. Analyses are underway to examine multispectral imaging data, plant mapping data, CLRDV symptoms, and aphid numbers. Preliminary analyses conducted on plot yield showed a trend in which the earlier a plot was infested with viruliferous aphids, the lower the yield. Non-infested plots and plots infested with non-viruliferous aphids (control plots) were numerically higher than plots infested with viruliferous aphids. Yield was significantly lower in plots infested with viruliferous aphids on week one, than in control plots ($P=0.05$) (approx. 400 lb. difference). Preliminary analyses of plant mapping data show that there are no significant differences in the number of first and second position bolls between plants testing positive for CLRDV and those that tested negative for CLRDV ($P=0.05$). The preliminary data from this one-year study show that plant age at time of infection impacts yield, and suggests that early CLRDV infection impacts lint production. This study will be repeated in 2020.

Acknowledgements

Funding of this project was provided by the Alabama Agricultural Experiment Station, Cotton Incorporated and the Foundation of Food and Agriculture Research. Data collection conducted by the members of the Jacobson lab is greatly appreciated.

References

- Abney, M.R., Ruberson, J.R., Herzog, G.A., Kring, T.J., Steinkraus, D.C., Roberts, P.M., 2008. Rise and Fall of Cotton Aphid (Hemiptera: Aphididae) Populations in Southeastern Cotton Production Systems. *Journal of Economic Entomology* 101, 23–35.
- Aboughanem-Sabanadzovic, N., Allen, T.W., Wilkerson, T.H., Conner, K.N., Sikora, E.J., Nichols, R.L., Sabanadzovic, S., 2019. First Report of Cotton Leafroll Dwarf Virus in Upland Cotton (*Gossypium hirsutum*) in Mississippi. *Plant Disease* 103, 1798.
- Alabi, O.J., Isakeit, T., Vaughn, R., Stelly, D., Conner, K.N., Gaytán, B.C., Villegas, C., Hitzelberger, C., De Santiago, L., Monclava-Santana, C., and Brown, J.K. 2020. First Report of *Cotton leafroll dwarf virus* Infecting Upland Cotton (*Gossypium hirsutum*) in Texas. *Plant Dis.* doi.org/10.1094/PDIS-09-19-2008-PDN
- Avelar, S., Ramos-Sobrinho, R., Conner, K., Nichols, R.L., Lawrence, K., Brown, J.K., 2020. Characterization of the Complete Genome and P0 Protein for a Previously Unreported Genotype of Cotton Leafroll Dwarf Virus, an Introduced Polerovirus in the United States. *Plant Disease*.
- Avelar, A.S., Sobrinho, R.R., Conner, K., Nichols, R.L., Lawrence, K.S., Brown, J.K., 2019. Characterization of the complete genome and P0 protein for a previously unreported genotype of cotton leafroll dwarf virus, an introduced polerovirus in the USA. *Plant Disease*.
- Brown, S., Conner, K., Hagan, A., Jacobson, A., Koeppernick, J., Lawrence, K., Bag, S., Kemerait, B., Chee, P., Allen, T., Sabanadzovic, S., Nichols, B. 2019. Report of A Research Review and Planning Meeting on Cotton Leafroll Dwarf Virus. <https://www.cottoninc.com/wp-content/uploads/2019/11/10-19-CLRDV-Research-Review-Meeting-Report-Nichols.pdf>, last accessed 1/22/2020

Cauquil J. 1977. Etudes sur une maladie d'origine virale du cotonnier: la maladie bleue. *Cot Fib Trop* 32: 259–278.

- Corrêa, R.L., Silva, T.F., Simões-Araújo, J.L., Barroso, P.A.V., Vidal, M.S., Vaslin, M.F.S., 2005. Molecular characterization of a virus from the family Luteoviridae associated with cotton blue disease. *Archives of Virology* 150, 1357–1367.
- Gore, J., Cook, D., Catchot, A., Leonard, B.R., Stewart, S.D., Lorenz, G., Kerns, D., 2013. Cotton Aphid (Heteroptera: Aphididae) Susceptibility to Commercial and Experimental Insecticides in the Southern United States. *Journal of Economic Entomology* 106, 1430–1439.
- Hagan, A., Conner, K.N., Lawrence, K., Koebernick, J., Jacobson, A., Bowen, K.L., Sikora, E.J. 2019. A New Virus Disease in Alabama Cotton. ANR-2539. https://www.aces.edu/wp-content/uploads/2019/04/ANR-2539-NewVirusDiseaseInAlabamaCotton_041119Lg-copy.pdf, accessed 1/22/2020
- Huseth, A., Reisig, D., Collins, G., and Thiessen, L. 2019. Detection of Cotton Leafroll Dwarf Virus (CLRDV) in North Carolina. go.ncsu.edu/readext?639597, last accessed 1/22/2020.
- Kerns, D.L., Yates, J.A., Baugh, B.A., 2015. Economic Threshold for Cotton Aphid (Hemiptera: Aphididae) on Cotton in the Southwestern United States. *Journal of Economic Entomology* 108, 1795–1803. doi:10.1093/jee/tov161
- Michelotto, M.D., Busoli, A.C., 2009. Biologia de aphid gossypii em plantas infectadas pelo vírus do mosaico das nervuras do algodoeiro. *Bragantia* 68, 1018–1024.
- Michelotto, M.D., Busoli, A.C., 2003. Eficiência de ninfas e adultos de Aphis gossypii Glov. na transmissão do vírus do mosaico das nervuras do algodoeiro. *Bragantia* 62, 255–259.
- Mukherjee, A., Chahande, P., Meshram, M., Kranthi, K., 2012. First report of Polerovirus of the family Luteoviridae infecting cotton in India. *New Disease Reports* 25, 22.
- Ray, J.D., Sharman, M., Quintao, V., Rossel, B., Westaway, J., Gambley, C., 2016. Cotton leafroll dwarf virus detected in Timor-Leste. *Australasian Plant Disease Notes* 11.
- Sharman, M., Lapbanjob, S., Sebunruang, P., Belot, J.-L., Galbieri, R., Giband, M., Suassuna, N., 2015. First report of Cotton leafroll dwarf virus in Thailand using a species-specific PCR validated with isolates from Brazil. *Australasian Plant Disease Notes* 10.
- Tabassum, A., Bag, S., Roberts, P., Suassuna, N., Chee, P., Whitaker, J.R., Conner, K.N., Brown, J., Nichols, R.L., Kemerait, R.C., 2019. First Report of Cotton Leafroll Dwarf Virus Infecting Cotton in Georgia, U.S.A. *Plant Disease* 103, 1803.
- Wang, H., Greene, J., Mueller, J., Conner, K., and Jacobson, A. 2020. First Report of Cotton Leafroll Dwarf Virus in Cotton Fields of South Carolina. *Plant Disease*, *in press*.