Analyzing the Infection Efficiency of CLRDV in *G. hirsutum* and *N. benthamiana* by Agroinoculation

Rachel Livingston[1,] and Kathleen Martin[2]*

[1] Undergraduate Student, Department of Entomology and Plant Pathology, Auburn University
[2] Assistant Professor, Department of Entomology and Plant Pathology, Auburn University

Cotton Leafroll Dwarf Virus (CLRDV) is a newly emerging plant pathogen in the United States and is the causative agent of Cotton Blue Disease (CBD). This virus has been previously described in Africa and South America (Cauquil et al., 1971). CLRDV was first detected in the United States in Alabama in 2017 and has been detected in 11 states across the Cotton Belt. CLRDV mainly infects cotton but has also been detected in 23 different weed species that are commonly found near commercial cotton fields (Sedhain et al., 2021). *Aphis gossypii* (cotton-melon aphid) is a vector for CLRDV and can acquire and transmit this virus within 75 minutes (Heilsnis et al., 2023). Cotton was worth over $346 million dollars in Alabama in 2022 and is the most common fiber utilized in textile manufacturing (USDA 2022). This crop has worldwide importance; therefore, studying CLRDV is a necessary step in preventing its spread and the destruction it may cause.

Viruses are unable to survive without their host and because of this studying them in a lab can be difficult. A copy must be made of the virus so that it can be stored in the lab and easily used in experiments. A CLRDV clone was previously made in Dr. Kathleen Martin's lab at Auburn University. The efficiency of this copy and its rate of infection were characterized in this study.

The CLRDV clone was grown in *Agrobacterium tumefaciens* for one to two days at 28°C. A 10mM MgCl2, 10mM MES buffer was made and the bacteria was mixed into it until an OD of 0.6-1.0 was reached. The solution then sat for one to two hours before the infiltration was completed with a needless syringe. Cotton (*Gossypium hirsutum*) and benth (*Nicotiana benthamiana*) were used in this study. Three replicates of ten cotton plants and two replicates of ten benth plants were infiltrated. For each replicate, for both cotton and benth, two plants were infiltrated with an empty plasmid backbone with no CLRDV insert as a negative control.

After infiltration, the plants were moved to a greenhouse and each replicate was stored in its own mesh cage to prevent infestation from aphids and other insects. After one month, root and leaf samples were collected from cotton and benth, respectively. The samples were stored in 2mL screw-cap tubes and immediately placed in liquid nitrogen. The samples were then stored at -80°C until RNA extraction was completed with a Total RNA Mini Kit (plant) from IBI Scientific. After extraction, RNA was stored at -20°C. Complementary DNA (cDNA) was synthesized using a Verso cDNA Synthesis Kit (ThermoScientific) from the RNA to run detection assays on the samples.

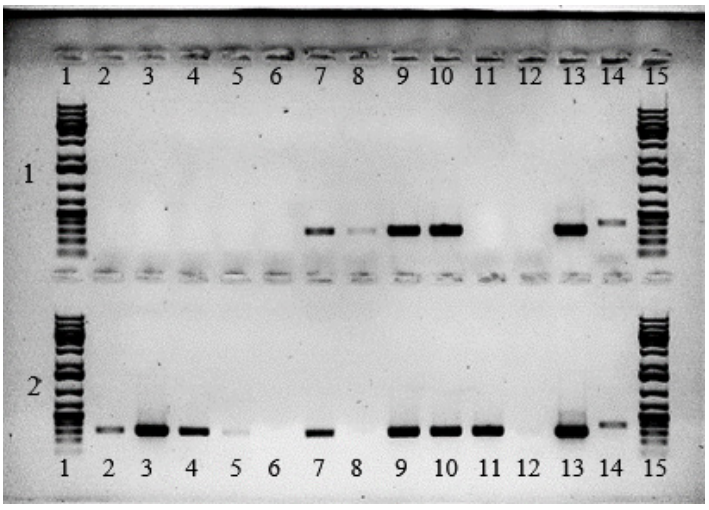

Fig. 1 This image shows a 1% agarose gel after PCR products have been run through it. In columns 1 and 15 on each row is a GeneRuler 1 kb Plus ladder, which helps denote the base pair size of bands in the gel. Columns 2-14 of both the top and bottom of the gel represent test and control samples.

* Corresponding author: rml0048@auburn.edu

Polymerase chain reaction (PCR) was used to assess the infection status of each plant. A nested detection method, using two rounds of PCR, was utilized for maximum amplification of the products (Mahas et al., 2022). After the second round of PCR, the products were analyzed with a 1% agarose gel. A band with a size of 300 base pairs confirmed CLRDV infection in samples. One negative control and two positive controls were run through the nested detection assays to confirm the PCR. In **Fig. 1**, the first row of samples is acceptable because the negative control, located in column two, does not have a band. The second row of samples is not acceptable because there is a band in the negative control, meaning that the PCR products are likely contaminated. The bands in columns 13 and 14 on both rows are the positive controls that confirm both rounds of PCR were successful.

Table 1 This table represents the results of the nested detection PCRs of the CLRDV clone in both *G. hirsutum* and *N. benthamiana.*

Host	Experiment	Systemically infected plants/Infected plants	Percent of infection
G. hirsutum	Empties	1/6	16.6%
	Set A	4/10	40%
	Set B	8/10	80%
	Set C	9/10	90%
N. benthamiana	Empties	1/4	25%
	Set A	6/10	60%
	Set B	8/10	80%

The accumulated results of the nested detection PCRs are represented in **Table 1**. The rate of infection in cotton ranges from 40-90% but the rate of infection in benth ranges from 60-80%. In the empty samples, there was one cotton plant and one benth plant that each tested positive for CLRDV. All empty samples were expected to be uninfected since they were not infiltrated with CLRDV. Due to these results, work is still being done to confirm the rate of infection of this clone.

Statement of Research Advisor

Rachel has performed key studies to test the infection rate of a cloned version of Cotton leafroll dwarf virus (CLRDV) in *Nicotiana benthamiana* and *Gossypium hirsutum*. Before her contributions, it was difficult to determine the rate in which this viral copy could infect these two plant species. However, Rachel was able to optimize the RNA extractions, clean up both the cDNA synthesis and the PCRs to determine positives and negatives with more accuracy. Rachel's experiments have been critical in determining the differences and similarities of the United States CLRDV compared to the South American strains.

- Kathleen Martin, Department of Entomology and Plant Pathology, College of Agriculture

References

[1] Cauquil, J., Vaissayre, M. (1971). La "maladie bleue" du cotonnier en Afrique: transmission de cotonnier à cotonnier par *Aphis gossypii* glover (The "blue disease" of cotton in Africa: transmission cotton to cotton by *Aphis gossypii glover*). *Coton et Fibres Tropicales,* 26.

[2] Corrêa, R.L., Silva, T.F., Simões-Araújo, J.L., Barroso, P.A., Vidal, M.S., & Vaslin, M.F. (2005). Molecular characterization of a virus from the family *Luteoviridae* associated with cotton blue disease. *Archives of Virology,* 150.

[3] Heilsnis, B., Mahas, J.B., Conner, K., Pandey, S., Clark, W. (2023). Characterizing the vector competence of *Aphis gossypii, Myzus persicae and Aphis craccivora* (Hemiptera: Aphididae) to transmit cotton leafroll dwarf virus to cotton in the United States. *Journal of Economic Entomology*, Vol. 116, Issue 3.

[4] Mahas, J.W., Hamilton, F.B., Roberts, P.M. & Ray, C.H. (2022). Investigating the effects of planting date and *Aphis gossypii* management on reducing the final incidence of cotton leafroll dwarf virus. *Crop Protection*, vol. 158.

[5] Sedhain, N., Bag, S., Morgan, K., Carter, R., Triana, P., Whitaker, J., Kemerait, R.C., & Roberts, P.M. (2021). Natural host range, incidence on overwintering cotton and diversity of cotton leafroll dwarf virus in Georgia USA. *Crop Protection*, vol. 144.

[6] United States Department of Agriculture. 2022 State Agriculture Overview (Alabama). Retrieved July 31, 2023, from https://www.nass.usda.gov/Quick_Stats/Ag_Overview/

stateOverview.php?state=ALABAMA, (July 31, 2022).

Authors Biography

Rachel Livingston is a Senior pursuing a B.S. in Applied Biotechnology. She has been working in the Martin lab since Fall '22 and will continue to work until graduation. Since she began working in the Martin lab, Rachel has discovered an interest in plant pathology and virology and intends on pursuing an M.S. in Plant Pathology as her next challenge.

Dr. Kathleen Martin is an Assistant Professor in the Department of Entomology and Plant Pathology. She started her lab in vector entomology at Auburn in 2019. She works on the molecular aspects of insect transmission of plant viruses in the field. Her work focuses on Cotton leafroll dwarf virus, Soybean vein necrosis virus and Tomato spotted wilt virus. She started working on plant viruses during her Master's program at the University of Arizona and continued to work on viruses that also infect their insect hosts/vectors at the University of Kentucky where she completed her PhD in 2011.