TRANSMISSION OF COTTON LEAFROLL DWARF VIRUS BY APHIS GOSSYPII

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Abstract

Cotton leafroll dwarf virus (CLRDV), the causal agent of Cotton leafroll dwarf disease (CLRDD), is the first cotton virus reported to reduce cotton yields in the Southeastern United States. CLRDV has been identified in seven southern states including: Alabama, Texas, Georgia, Mississippi, South Carolina, Tennessee, Louisiana, North Carolina, and Florida (Aboughanem-Sabanadzovic et al. 2019, Avelar et al. 2019, Alabi et al. 2020, Huseth et al. 2019, Tabassum et al. 2019, Personal Communication, Kassie Conner). CLRDV was first suspected to be a *Bemisia tabaci*-transmitted disease due to the appearance of CLRDV symptoms concurrent with a whitefly outbreak in Southern Alabama in 2017 (Schrimsher et al. 2018). However, de novo assembly of both DNA and RNA sequences found no match for whitefly transmitted DNA viruses, and instead found a single match for the RNA contig in GenBank for *Cotton leafroll dwarf virus* "atypical isolate" from Argentina (Avelar et al. 2019). CLRDV falls in the genus *Polerovirus*, which are reported to be phloem limited and aphid transmitted. CLRDV is transmitted by *Aphis gossypii* (Glover), the cotton aphid, in a persistent and circulative manner (Cauquil and Vaissayre 1971, Michelotto and Busoli 2007, Mukherjee et al. 2012). Both apterous and alate morphs of *A. gossypii* transmit CLRDV.

Understanding how quickly viruses are acquired and transmitted by their vectors is necessary to identify effective strategies to reduce CLRDV incidence. Previously, Michaletto and Busoli (2007) reported virus persistence of up to 12 days by apterous (wingless) morphs and transmission in 40 sec by a single alate (winged) morph for the atypical form of CLRDV. The acquisition time for atypical CLRDV was not recorded, and the quick transmission time for alates is not consistent with electrical penetration graph studies of aphid feeding behavior that report longer time periods are required to reach the phloem. The U.S. isolates of CLRDV are also reported to be genetically distinct from isolates in South America (Ayelar et al. 2020) and may have different transmission characteristics. The goals of this research are to characterize the mode of transmission of CLRDV by *A. gossypii* in the U.S. and determine if *Bemisia tabaci* is a vector of CLRDV.

Virus persistence, acquisition, and transmission is examined using adult alate and apterous aphids that are one-two days old. To examine persistence, fourteen apterous *A. gossypii* were reared on excised leaves from a CLRDV infected plant. Experiments were conducted in a growth chamber at 30 °C with a 12/12 light cycle. The adults were transferred individually to healthy 1" cotton leaf discs every 24 hours until aphid death. Leaf discs were kept on agar to maintain turgidity. Controls included both aphid-free leaf discs and non-viruliferous aphids on leaf discs. To examine transmission in *B. tabaci*, adults, groups of ten adults were placed on ten excised leaves from a CLRDV-infected plant for four days to acquire the virus, then onto healthy cotton leaf discs for two days. In aphid and whitefly transmission experiments leaf discs were held for a total of five days after the insects were removed, then flash frozen and stored before RNA extraction. Infection of source plants and leaf discs was confirmed using nested PCR according to the methods of Sharman et al. (2015).

Results indicated that there was no transmission of CLRDV observed in studies involving *B. tabaci*. This is not completely unexpected because of the vector specificity that has been observed in insect transmission studies to-date. Results of the persistence study with apterous aphids showed that all aphids included in the study transmitted the virus on at least one day, and no aphids transmitted every day. One aphid transmitted up to 15 days, but most transmission occurred in the first 10 days. This is longer than the retention time previously reported in Brazil (Michelotto and Busoli, 2007), and confirms that CLRDV is retained by the vector for long periods of time. Experiments characterizing the inoculation and acquisition times of aphids, as well as persistence in alate aphids, are ongoing.

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