Examples of citrus leprosis.

Citrus leprosis research update

By Ron Brlansky, William Schneider, John Hartung and Avijit Roy

Citrus leprosis is one of the oldest citrus diseases, but is also one of the most important emerging citrus diseases in South America and Central America. It is apparently spreading northward toward the United States. The disease causes leaf, twig and fruit lesions with severe defoliation, girdled limbs, premature fruit drop, death of twigs and reduction in both fruit quality and yield, as well as possible tree death within three to five years.

The disease is currently not present in the United States, but it was first reported from Florida in the 1860s and resulted in serious impacts on citrus production. After 1926, the incidence of the disease in Florida began to decline. By the 1950s, citrus leprosis was present only in isolated areas on the east coast of Florida, and since 1968, surveys have not found it in Florida.

The cause of citrus leprosis has always been considered to be a virus transmitted by mite species. Virus particles were reported in 1972 in the cell nucleus, and in the cytoplasm in 1995. The viruses were similar in structure but different in size, and thus were considered to be different. However, each virus caused essentially the same symptoms on citrus, and both were transmitted by flat mites.

With the spread of citrus leprosis northward from South America into Central America and Mexico, work recently began in our lab group for the detection and diagnosis of citrus leprosis and to test transmission by endemic *Brevipalpus* (flat) mites, respectively. The research is funded by grants from the U.S. Department of Agriculture-Animal and Plant Health Inspection

Service and the Citrus Research and Development Foundation.

Research in our labs and by others has shown that citrus leprosis disease is caused by a complex of viruses, and that five different viruses currently present in South America, Central America and North America (Mexico) can cause the disease. The finding that five different viruses are associated with the disease was made possible by using modern molecular technology (next-generation sequencing) and the associated analysis. The new techniques have made the identification and characterization of these unknown viruses quicker.

The citrus leprosis-associated viruses are present in either the cytoplasm or the nucleus of the cells, which is a big difference at the cellular level. However, the symptoms produced in citrus by most of them are very similar.

The characterized citrus leprosis-associated viruses found in the cytoplasm are **citrus leprosis virus C** (CiLV-C), **citrus leprosis virus C2** (CiLV-C2) and **hibiscus green spot virus 2** (HGSV-2).

The characterized nuclear viruses are **citrus leprosis virus N** (CiLV-N) and **citrus necrotic spot virus** (CiNSV). All of these viruses cause similar leaf, twig and fruit lesions, and do not move systemically in the infected plant.

The diversity of viruses is also matched by a previously unexpected diversity in the flat mites associated with the disease. So far, two species of *Brevipalpus* (flat) mites are involved. Mites collected from CiLV-C and CiLV-N infected citrus groves in Mexico were identified as *B. yothersi* and *B. californicus*, respectively. Only *B. yothersi* was detected from CiLV-C2 and CiLV-N in citrus infected with both viruses in the Orinoco region of Colombia. It has now been found that both cytoplasmic viruses (CiLV-C and CiLV-C2) replicate in the *Brevipalpus* flat mites.

Recent sequence analysis of these cytoplasmic leprosis viruses has revealed a close relationship with virus sequences found in mosquitoes. This

suggests that the genus *Cilevirus,* to which these viruses belong, may have originally evolved in mites and that citrus may actually be a secondary host. Flat mites collected from Florida were identified as *B. yothersi*; however, transmission tests (in quarantine) with CiLV-C from Colombia were negative. Further transmission tests are required with Florida *Brevipalpus* mites since citrus leprosis has not been present since the 1950s.

As mentioned above, citrus leprosis virus was present in Florida in the 19th and 20th centuries, but rather mysteriously disappeared. Fortunately, a sweet orange specimen with leprosis symptoms was collected by University of Florida Professor Knorr in Volusia County, Fla. in 1948, and was deposited at the USDA herbarium in Beltsville, Md. for safekeeping. We used a portion of this specimen for next-generation sequencing and analysis, and were able to obtain the genomic sequence of a member of the CiLV-N group of viruses. The genome of the Florida CiLV-N virus is noticeably different from the other strains of CiLV-N that are widespread today. Presumably, the Florida strain – designated CiLV-N0 – is not extinct but survives elsewhere, and primers and assays have been developed to detect it.

Specific detection methods are now in place for diagnosis of the known viruses that cause citrus leprosies, and plans are to combine them into a multiplex assay. A recovery plan is in place with the USDA in case the disease should be introduced into the United States.

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