CITRUS LEPROSIS: understanding a re-emerging threat to citrus production

By Ron Brlansky and Avijit Roy

In the 1950s, citrus leprosis was among the most important virus diseases of citrus in Florida. In the early 1960s, the disease was unintentionally eradicated, probably due to the use of new and effective acaricides and a severe winter that destroyed a large number of the weakened trees. It was assumed that the disease was caused by a virus that was transmitted by mites in the genus *Brevipalpus*. However, the virus was not determined. Apparently the population of mites carrying the virus also was eliminated, and since that time period, citrus leprosis has not been found in the United States.

In recent years, the disease has been moving steadily northward from South America to Central America and to Mexico. Leprosis was present in Brazil in 1931, never disappeared, and caused major problems there during the 1990s. It was reported in Venezuela in 1999, in Panama and Costa Rica by 2000 and in Colombia and southern Mexico in 2005–06. Leprosis is now present throughout South America and Central America in most areas where citrus is grown.

Because of this movement, research was initiated at the University of Florida's Citrus Research and Education Center (UF-CREC), in conjunction with the U.S. Department of Agriculture's Agricultural Research Service (USDA-ARS) in Beltsville and Frederick, Md., and with the USDA's Animal and Plant Health Inspection Service (USDA-APHIS) in Beltsville.

The first virus associated with citrus leprosis was shown by electron microscopy in the nucleus of infected leaves by Elliot Kitajima in 1972 in Brazil. Since that time, little was done with leprosis until the virus disease was found to be recently spreading in Brazil and Panama. In 1995 and again in 2003, a virus was found by electron microscopy in specimens from Brazil, Panama and other countries. The virus was not in the nucleus as seen previously, but instead was present in the cytoplasm, and also was larger than the virus seen previously in the nucleus. The virus genome was sequenced by scientists in both Brazil and at the UF-CREC using newly developed molecular techniques

Early citrus leprosis lesions on Valencia orange fruit.

Photo by Guillermo Leon, CORPOICA

Advanced citrus leprosis lesions on Valencia orange in Colombia caused by cytoplasmic citrus leprosis virus.

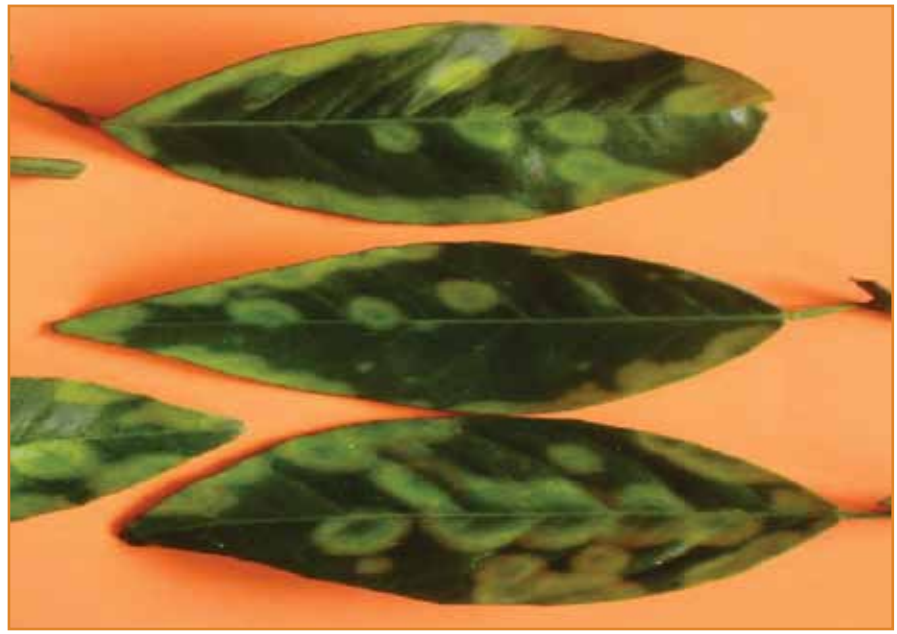

Photo by Guillermo Leon, CORPOICA

Cytoplasmic citrus leprosis lesions on leaves in Colombia.

and is now called cytoplasmic citrus leprosis virus (CiLV-C), since it is different from the virus found in the nucleus.

To address the threat of leprosis to the U.S. citrus industry, further work began in our lab in conjunction with the USDA-ARS and the USDA-APHIS to develop specific diagnostics for this cytoplasmic type of citrus leprosis virus. In addition, another project supported by the Citrus Research and Development Foundation was initiated to determine if the cytoplasmic citrus leprosis virus is transmitted with our endemic population of *Brevipalpus* mites that were previously identified as the vector of the virus. All of this work had to be done in conjunction with containment facilities of the USDA-ARS, Beltsville (John Hartung) and in Frederick (Bill Schneider and Avijit Roy) and the USDA-APHIS in Beltsville (Laurene Levy and Mark Nakhla), where it could be done safely away from citrus-growing areas. We also began work with cooperators from Colombia (Guillermo Leon of CORPOICA) and Mexico (Gabriel Otero Colina) to gain access to leprosis samples.

Immediately, we discovered that our detection systems for CiLV-C did not work with Leon's leprosis samples in Colombia, even though we earlier had seen virus particles of the same size in the cytoplasm that we had worked with previously from Panama and Brazil. By applying (next generation) sequencing techniques with nucleic acid preparations to nucleic acid preparations from leprosis infected leaves and organizing and analyzing the data, we were able to identify a new cytoplasmic virus (CiLV-C2) causing symptoms of citrus leprosis.

In addition, as our work has continued, we have identified the genome sequence of a virus from symptomatic citrus plant materials obtained from Otero in Mexico. These plants had virus particles in the nucleus like those found in Brazil in 1972. In addition, we have now identified another nuclear leprosis virus in Colombia closely related to the nuclear virus in Mexico.

Work by another researcher (Mike Melzer) in Hawaii has reported a hibiscus virus similar to our cytoplasmic citrus leprosis virus from Colombia and another hibiscus virus that produces leprosis-like symptoms in *Citrus volkameriana*.

As of this writing, there are now four to five different viruses that cause leprosis disease symptoms, and each virus is apparently transmitted by mites in the genus *Brevipalpus*. Other researchers — Ron Ochoa and Gary Bauchan at the USDA-ARS in Beltsville and Evan Braswell and Roxanne Farris at USDA-APHIS in Mission, Texas — are identifying the *Brevipalpus* mites at the species level that transmit these viruses, using special scanning electron microscopy and molecular techniques, respectively.

CONCLUSION

In conclusion, significant progress has been made on this important exotic citrus disease due to the efforts of a highly effective multi-institutional multi-discipline team. This has consisted of University of Florida researchers (Ron Brlansky, Roy, Nandlal Choudhary, Carmen Bierman and Jorge Pena), USDA-ARS scientists (Hartung, Jonathan Shao, William Schneider and Andrew Stone), USDA-APHIS scientists (Levy, Nakhla, Gang Wei and Evan Braswell) and our foreign cooperators Leon and Colina.

We have now developed specific diagnostics for the causal viruses and are determining transmission by endemic *Brevipalpus* and other mite species that may transmit these important viruses that each cause symptoms referred to as leprosis.
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