Review of exotic citrus diseases By Megan Dewdney and Ron Brlansky

CITRUS LEPROSIS

Citrus leprosis is an emerging citrus disease that currently causes important economic losses in many countries in South America such as Brazil, Venezuela and Colombia. It has been found in many Central American countries, most recently in Mexico, and appears to be moving closer to the United States. The causal viruses are transmitted by false spider mites in the genus Brevipalpus. Citrus leprosis was reported in Florida around 1926 and had serious negative effects on citrus production. After that time, the incidence of leprosis in Florida drastically declined, coincidental with the introduction of sulfur as an effective miticide for citrus rust mite. In 1968. L.C. Knorr reported that leprosis was present only in isolated areas on the east coast and, since then, the disease has not been found in Florida.

Historically, citrus leprosis was reported in Paraguay in 1920, and around 1930, it was considered to be the same disease as reported from Florida. Citrus leprosis was also found in Argentina, Uruguay and Brazil in the 1930s. Over time, this disease has moved northward in South America and into Central America. It was reported in Venezuela in 1991, which caused Colombia to quarantine citrus imports from Venezuela; it was confirmed in Panama in 2003, in Guatemala in 2003 and in Nicaragua



Citrus leprosis leaf and fruit drop. The large brown lesions on the fruit are caused by one of the viruses responsible for leprosis.

in 2004. The disease has recently spread into Costa Rica, Honduras and El Salvador. It was reported in Bolivia and southern Mexico in 2005 and Colombia in 2006. The *Brevipalpus* mite vectors are present throughout all areas of the Caribbean and in most citrus-producing areas of the United States. Therefore, citrus leprosis, a disease of quarantine importance, poses a major threat to all citrus industries in the Caribbean, Florida and Texas.

Trees are killed because of expanding lesions that girdle tree limbs and cause leaf and fruit drop as well as unmarketable fruit. Premature fruit



drop results in greatly reduced yields. Mites must be continually controlled. Multiple acaricide applications are expensive, and the development of tolerance to pesticides by mites may occur. To manage the disease, symptomatic tissue must be pruned from the tree at least twice a year.

Diagnosis of leprosis has been difficult; it is very poorly mechanically transmitted, poorly graft transmitted since the pathogen is not systemic, and is often poorly transmitted experimentally with mites. For years, transmission electron microscopy was the only available method for positive diagnosis. In 1972, researchers showed the presence of virus particles in the nucleus of infected citrus tissues. Later, in other studies on samples from Brazil and Panama, virions of similar size and morphology were seen in the cytoplasm and not in the nucleus.

Cellular studies of different leprosis samples indicated that there are two different types of citrus leprosis virus (nuclear and cytoplasmic) causing similar symptoms. To date, at least five different viruses have been identified as associated with leprosis symptoms with the cytoplasmic-type viruses spreading rapidly throughout Central America. All of the recent identifications of these new viruses have been possible with the advances of genome sequencing. Sequencing of these different viruses has allowed for the development of sensitive and specific molecular tests. However, it has been

noted that rapid, reliable detection methods for leprosis, especially methods that could be performed with inexpensive reagents and equipment, would facilitate diagnostic/quarantine measures in Central America and at ports of entry. Antibody-based detection systems now have been developed for many of the viruses with funding from the United States Department of Agriculture's Animal and Plant Health Inspection Service, Plant Protection Quarantine.

The taxonomy of various *Brevi*palpus mites found on citrus and associated with leprosis symptoms is currently under revision by a USDA-Agriculture Research Service group. *Brevipalpus phoenicis* was reported to be the most efficient vector of the nuclear leprosis virus (CiLV-N), and experimental transmission of the cytoplasmic leprosis virus (CiLV-C) by mite vectors also was demonstrated. With positive transmission by mite vectors, symptoms were produced in about 20 days.

Recently in Leon, Colombia, *B. yothersi* mites were shown to efficiently acquire the second type of cytoplasmic citrus leprosis virus (CiLV-C2). Only 30 minutes on symptomatic Valencia orange leaves were needed and after only a 10-minute transmission period, there was 25 percent virus transmission to healthy Valencia plants. The percentage of positive transmissions increased with transmission time periods.

Non-citrus plant species have been reported as hosts of citrus leprosis viruses. In 2005, it was reported that transmission from citrus to the noncitrus plant Solanum violaefolium was possible, but not from this plant to citrus. In 2006, another researcher reported virus transmission to common bean, and in Colombia in 2008, natural infection of Swinglea glutinosa (Tabog) was reported. Tabog is a member of the same plant family as citrus and is often used as a hedge in many citrus areas of Colombia. It is now considered as a reservoir host for the virus since transmission to citrus was also reported. Another important non-citrus plant species of the cytoplasmic leprosis virus (reported in Brazil) is the Benghal dayflower or tropical spiderwort (Commelina benghalensis). This plant species is present in Florida and is considered a noxious weed.

In conclusion, citrus leprosis is caused by a number of different viruses that are transmitted by mites in the genus *Brevipalpus*. These mites exist in all U.S. citrus-production areas and can feed and reproduce on many non-citrus host plants. The disease now exists in many countries of Central America and in Mexico. Good diagnostics exist, and studies continue on identification of the mite species and on virus transmission by these various mites.

CITRUS VARIEGATED CHLOROSIS

Citrus variegated chlorosis (CVC) is one of the main diseases that affect sweet orange in Brazil and Argentina, and has been found in Costa Rica. Sweet orange is the most susceptible cultivar, and the disease is a threat to the U.S. sweet orange industry. The disease is caused by *Xylella fastidiosa*, a bacterial pathogen. The pathogen and the diseases it causes are widespread in the Americas, but are not established elsewhere in the world.

Other pathogen strains cause

diseases, including Pierce's disease of grapes, leaf scorch of almonds, phony disease of peach, and numerous leaf scald diseases of shade trees, but none of these has been shown to cause CVC. The bacterial pathogen is found in the xylem of infected plants and is transmitted by sharpshooters that feed from the xylem vessels. Sharpshooters present in the United States are able to transmit the CVC bacterium.

In Brazil, the disease is found throughout the citrus-producing areas and is most severe in the high-temperature production regions of central and northern São Paulo. A 2010 survey estimated an infection rate in São Paulo of about 35 percent. Yield losses can be as high as 90 percent. Disease symptoms are asymmetrical chlorotic spots resembling zinc deficiency with brown necrotic spots that are usually more prominent on the lower leaf surfaces. Other symptoms include tree stunting, defoliation and





Comparison of healthy sweet orange fruit (left) with a CVC-affected fruit on the right. Note chlorotic lesions on the leaf on the CVC fruit.

canopy dieback, and small hard fruit that contain little juice. Independent studies have shown that the bacterium is not seed transmitted. The causal bacterium can be cultured in the lab, and excellent molecular and antibody detection systems are available.

PSEUDOCERCOSPORA FRUIT AND LEAF SPOT

Pseudocercospora fruit and leaf spot is a fungal disease caused by *Pseudocercospora angolensis*. It has been spreading in Sub-Saharan African countries and is found in the Comoros Islands and Yemen, but not South Africa. All citrus can be infected, but grapefruit, sweet oranges, navel oranges, pummelos and tangerines are considered highly susceptible. Lemons are moderately susceptible, and limes are the least susceptible. This disease is a major quarantine disease for market access, especially in European markets.

The lesions start with nipple-like swellings on fruit with no halo, but become large, circularly to irregularly shaped with gray centers and a promi-

nent yellow halo with age. Occasionally, lesions can coalesce to cover much of the fruit surface. Mature-fruit lesions are flat to sunken with dark brown centers. Fruitlets become mummified with severe infection. When uncontrolled, the disease has led to total crop losses in some countries including Kenya, but even when less severe, yield loss occurs. The nearly circular leaf lesions generally occur singly and are smaller than fruit lesions. The lesion centers are gray to light brown, and there is often a halo around them. With severe infection, the whole leaf may turn yellow and eventually abscise.

The life cycle of Pseudocercospora fruit and leaf spot has not been fully determined, but some basic features are understood. The disease spreads long distances through windborne spores, and abandoned groves can be a major inoculum source for managed groves. The disease has been inadvertently moved long distances via planting materials. In the grove, spores are formed on old lesions that sporulate three to five weeks after a rainy period, causing lesions on new leaves and fruit. Fruit up to 1.5–2 inches in diameter are susceptible to infection. Within a grove, the disease moves via rain splash and wind-driven rain. Infection

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Pseudocercospora fruit and leaf spot caused by the fungus *Pseudocercospora angolensis* on leaves (left) and near-mature fruit. Photos were taken in Ghana.

occurs when there is sufficient leaf wetness of at least 24 hours between 59°F and 86°F. Fruit become less susceptible with age, becoming nearly resistant at 18 weeks post-petal fall.

SWEET ORANGE SCAB

Sweet orange scab is caused by Elsinoë australis, a close relative of the E. fawcettii fungus that causes citrus scab. Sweet orange scab affects sweet oranges, tangerines, lemons and grapefruit. The disease is mainly a concern for fresh market citrus. Historically, sweet orange scab was found in the humid citrus production regions of South America. At one point, it had been questioned whether sweet orange scab was a separate disease or just an E. fawcettii scab strain that infected sweet oranges. Citrus scab most commonly infects tangerine and grapefruit cultivars, and rarely sweet orange. To answer the question, researchers used genetic tools to confirm that sweet orange scab was caused by a different fungal species and was a separate disease.

An outbreak of a disease associated with *E. australis* was identified in eastern Texas in August 2010 and the fungus has now been detected by molecular means in several citrus states.

Like citrus scab, sweet orange scab symptoms on fruit begin as raised wart-like pustules that are pink to tan. The pustules are a mixture of host and fungal tissue. Lesions are more or less raised depending on the host. On Temples, the lesions are quite raised, but on grapefruit and sweet orange, the lesions are flatter and could be mistaken for windscar. As lesions age, the color changes from tan to yellow-brown to gray and can crack. With classical sweet orange scab from Brazil, leaf symptoms were never reported, and the lack of symptoms on foliage was used as a way to differentiate sweet orange from citrus scab. Leaf lesions associated with E. australis were reported from Texas, but not confirmed elsewhere. They were described as raised, flat-topped, and reddish to dark brown. Because lesion appearance changes, depending on the time of infection, citrus scab and sweet orange scab can be very difficult to differentiate based on symptoms. The only definite way to identify them is to use PCR-based techniques that have



Sweet orange scab caused by the fungal pathogen *Elsinoë australis* on fruit in Brazil.

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Synopsis of Ongoing Field Trials of HLB Solutions and Other Tools for Disease Management



By Harold Browning

Which fruit harvest season arriving, field trials that have been underway during the growing season are winding down and the harvest data will be collected over the next several months. CRDF has focused over the past several years on moving research results to field trials, and many of these trials necessarily bridge multiple seasons. Treatments target rates of spread of *Candidatus Liberibacter asiaticus (CLAS)* via Asian citrus psyllid (ACP) control, tree growth response to nutritional treatments, or growth response to treatments targeting *CLas* titer reduction. In addition, field trials are investigating how soil and/or water conditions may impact disease progression or tree response. This summary lists some of the ongoing field trials supported by CRDF that are being evaluated as harvest approaches:

Evaluation of season-long ACP population management with pesticide materials, rates and application methods

Evaluation of efficacy of antimicrobial treatments for reduction of *CLas* population and measure phytotoxicity

♦ Measurement of commercial microbe product applications on tree health and productivity

♦ Determine the effects of thermal treatment of HLB-affected trees on growth response and *CLas* titer reduction

Evaluation of candidate HLB-tolerant rootstocks in replicated field trials

Testing the ability of single full-dose or multiple low-dose applications of plant growth regulators to reduce pre-harvest fruit drop

Integrating ACP management, high planting densities, and irrigation/ nutrition strategies into new citrus plantings

♦ Treatments to adjust bicarbonates and/or pH imbalances in soil and irrigation water

✤ Large-scale demonstration of citrus leafminer disruption through pheromone technology

Many of these field trials will continue into the 2015 season to evaluate cumulative effects of treatments. Harvest information this season will allow evaluation of the season-long value of treatments and connect tree response to productivity and fruit quality.

Many citrus growers participate as cooperators in these various field trials and are acknowledged here for the contributions they are making in hosting field experiments. The placement of these field trials across the citrus regions of Florida assists in determining regional differences in response to the treatments being tested.

Additional information on CRDF research and delivery projects can be found at citrusrdf.org

Harold Browning is Chief Operations Officer of CRDF. The foundation is charged with funding citrus research and getting the results of that research to use in the grove.



Column sponsored by the Citrus Research and Development Foundation

been developed for this purpose.

Little work on the sweet orange scab disease cycle has been done, but it is assumed to be similar to citrus scab. Conidia form in fungal structures on the lesion surface. The conidia are splash-dispersed short distances, so disease spread in a grove without overhead irrigation can be very slow. Merely one to two hours are needed for conidia to form if the temperature is 75°F to 82°F when there is free moisture on the lesions. Infection can occur in five to six hours with moisture and temperatures between 70°F and 80°F. Lesions will appear in six to seven days. Although it is not understood how the fungus overwinters when all of the fruit are removed from the tree, the disease will nonetheless occur the next season.

ALL ARE THREATS

All of these citrus diseases are serious threats to the Florida industry. If you have suspicious symptoms in your grove, contact the Florida Department of Agriculture and Consumer Services-Division of Plant Industries' Citrus Health Response Program at 800-282-5153 or your local Extension agent for an evaluation of the symptoms. You may prevent a major outbreak from occurring by making that call.

Megan Dewdney is an assistant professor of plant pathology and Ron Brlansky is a professor emeritus of plant pathology; both with the University of Florida-IFAS at the Citrus Research and Education Center in Lake Alfred.

WHAT'S SHAKIN'

The cost to hand-harvest juice oranges in 2012 ranged from \$1.90 a box for early-season oranges to more than \$2.20 a box for lateseason fruit. The grove conditions set up for mechanical harvesting trunk shaker systems reduced the combined pick and roadside costs by between 20 to 30 cents per box as compared to hand-harvesting costs. The cost savings from mechanical harvesting included gleaning services.

While citrus growers are rightfully concerned about restoring the health of their HLB-infected trees, the costs to grow and harvest citrus have been escalating significantly since 2006. The cost savings potential from mechanical harvesting technologies can help Florida growers remain economically viable. Visit http://citrusmh.ifas.ufl. edu for more information.