Bacterial Citrus Canker[1]

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INTRODUCTION: Citrus canker (CC), caused by the bacterial pathogen *Xanthomonas axonopodis* Starr & Garces emend. Vauterin, *et al*. pv. *citri* (Hasse) Dye (*Xac*) [syn. *Xanthomonas campestris* pv. *citri* (Hasse) Dye], is a serious disease of most commercial citrus varieties and some citrus relatives. The pathogen causes necrotic lesions on leaves, stems and fruit. Severe infections can cause defoliation, badly blemished fruit, premature fruit drop, twig dieback and general tree decline. Considerable regulatory effort is directed at preventing the spread of CC because it is not present in all citrus-growing regions of the world where the climate is conducive to CC development.

HISTORY: The Asian, or A-strain, of CC is thought to have originated in southeastern Asia or India (Civerolo 1984) and spread through much of Asia, to Japan, southern and central Africa, the Middle East, Australia, New Zealand, Pacific Islands, South America and the southeastern United States (CABI/CMI 1996; Schubert *et al.* 2001; Gottwald *et al.* 2002b). CC has been eradicated from South Africa, Australia, the Fiji Islands, Mozambique, New Zealand and the United States (Koizumi 1985). Active eradication/containment programs are continuing in Uruguay and Brazil.

The history of CC in Florida began presumably around 1910 (Dopson 1964; Loucks 1934) when the disease was introduced into North Florida and other Gulf States on trifoliate citrus rootstock material from Japan. The disease was previously undescribed, and it was not until 1915 that the pathogen was identified as bacterial. By 1913, South Florida citrus growers had reached a consensus that eradication efforts were warranted since all control efforts against the rapidly spreading disease proved ineffective. The pathogen had spread throughout the Gulf States and up the Atlantic coast to South Carolina by the time eradication efforts were well underway. A quarantine prohibiting the importation of all citrus plants was enacted in 1915. The newly formed State Plant Board took over the eradication program for Florida that same year. Federal assistance from the USDA - Bureau of Plant Industry commenced soon after. The last infected tree in Florida was removed in 1927. An intensive inspection program continued until 1933, when the disease was declared officially eradicated. The disease was declared eradicated from the United States in 1947. Citrus canker has been a target of regulatory detection surveys in Florida ever since.

In June 1986, CC was detected in residential citrus in Hillsborough, Pinellas, Sarasota and Manatee counties, and was also found soon after in two Manatee County commercial citrus groves and one commercial grove in Highlands County. All infected citrus were removed and all exposed trees within 125 ft (38.1 m) were either removed or cut back to brown wood (Schubert 1991). This second eradication program concluded with the last detection in January 1992. The official declaration of eradication was made two years later.

In late September 1995, CC was discovered for a third time in Florida in a residential area near the Miami International Airport. Initial surveys delimited an area of about 50 square miles containing thousands of diseased trees. An eradication effort was begun immediately. Genetic fingerprinting of the isolate of *Xac* causing CC in the Miami area revealed that the pathogen was recognizably different from the archived isolates from the 1986-92 outbreak in west-central Florida (Schubert *et al*. 1996). As of March 2001, even with an extensive eradication effort, the disease had managed to spread predominantly northward from Dade into Broward and Palm Beach counties. The logistics of regular inspections and eradication activities in the urban residential setting of Southeast Florida have proven very challenging.

In May 1997, CC was rediscovered in eastern Manatee County in both commercial groves and residential citrus. Genetic fingerprinting of the isolate of *Xac* present there closely matches that of the 1986-92 outbreak, and suggests that some holdover infections went undetected in the final two years of intensive inspection in the area.

A third concurrent outbreak of CC was discovered in June 1998 in a commercial grapefruit grove in the Immokalee area of Southwest Florida, and in a remote residential area nearby a year later. Two outbreaks of CC were detected in commercial groves in southeast Hendry County in February 1999. In June 1999, the disease was detected in a commercial grove in western Manatee County. All pathogen isolates from new locations are routinely genetically characterized to help identify the source(s) of inoculum. With the exception of the isolates in eastern Manatee County and some isolates in Hillsborough County, all other pathogen isolates in Florida are identical to the pathogen which was initially discovered in Miami in 1995. For an update on the status of the Citrus Canker Eradication Program in Florida, visit the DPI citrus canker website at <http://www.doacs.state.fl.us/canker/>.

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SYMPTOMS AND INFECTION PROCESS: All young above-ground tissues of citrus are susceptible to *Xac* (Fig. 1). The bacterial pathogen enters plant tissues through natural openings (stomates) and wounds. The earliest symptoms on leaves appear as slightly raised tiny blister-like lesions about 4-7 days after inoculation under optimum conditions, *i.e.*, a water film present and temperature between 20-30^{o}C (Koizumi 1985). Under less than optimum conditions, symptoms may take 60+ days to appear (Loucks 1934; Goto 1992). As the lesions age, they turn tan to brown, and a water-soaked margin appears surrounded by a chlorotic halo. The center of the lesion becomes raised and corky (Figs. 2, 3). Lesions are usually visible on both sides of a leaf. Eventually, the centers of leaf lesions become crater-like and may fall out, creating a shot-hole effect. Defoliation and twig dieback become a problem as the disease intensifies on a plant.

On twigs and fruit, CC symptoms are similar: raised corky lesions surrounded by an oily or water-soaked margin. No chlorosis typically surrounds twig lesions (Fig. 4), but may be present on fruit lesions (Fig. 5). Chlorosis symptoms can fade over time. It is the twig lesions on angular young shoots that provide much of the perpetuating *Xac* inoculum in areas where CC is endemic. Fruit blemishes and early fruit drop are major economic impacts of the disease.

As a general rule, *Xac* is capable of naturally infecting green citrus tissues while they are in the expansion phase of growth. Once leaves, twigs and fruit reach mature size and begin to harden off, they become more resistant to infection. Since the young growth provides the susceptible tissues, vigorously growing trees are most threatened by *Xac*. Any wound sites on young or older tissues, however, can provide the infection court for *Xac* establishment and disease development. The serpentine mines caused by the citrus leafminer (*Phyllocnistis citrella* Stainton), also Asian, first detected in 1993 in Florida (Heppner 1993), provide ample wounding on new growth to greatly amplify CC infection (Sohi and Sandhu 1968; Sinha *et al*. 1972) (Fig. 6). Wounds become naturally infected at much lower inoculum concentrations than via the stomatal route (minimum dose of approx. 10^2 cells / ml for wounds vs. approx. 10^5 cells / ml for stomates) (Goto, 1992).

Wind-driven rain is the primary short- to medium-distance dispersal mechanism for CC, similar to other bacterial diseases. A drop of rainwater can carry a maximum load of bacteria of about 10^7 to 10^8 cells. Inoculum-contaminated equipment and hands can transmit *Xac*. Long-distance spread normally occurs by movement of infected or exposed citrus plant material, but circumstantial evidence points to occasional long-distance transport by unusual storm events such as tornadoes and tropical storms.

Xac easily persists season to season in old lesions, especially in warmer climates and in lesions formed late in the growing season (Pruvost *et al*. 2002). The pathogen is not systemic in the host plant. *Xac* can remain viable as long as host cells in the vicinity of the lesion remain viable, though the bacterial titer will drop considerably. Reports on inoculum longevity outside host tissue are inconsistent. On exposed, symptomless citrus, circumstantial evidence suggests some inoculum persistence for at least several months, since root sprouts from infected trees that have been removed months prior frequently become infected themselves. *Xac* may persist for several weeks on non-host plant material, with some exceptional reports of longer persistence (about 8 months) in the root zone of certain grasses under infected trees in Japan (Goto *et al*. 1975). Once infected/exposed leaves or fruit drop to the ground, the bacterial population declines to a non-detectable level in 1-2 months because of antagonism and competition with saprophytic microorganisms (Goto 1992). Reports of survival on inanimate surfaces vary from a few hours to several months. In general, when inoculum dries on nonporous surfaces, it dies. Concentrated inoculum, such as from natural lesions or from culture, survives longer than cells diluted in a water suspension. The intact polysaccharide slime coating on the bacterial cells is thought to be essential for longer survival.

HOST RANGE: Civerolo (1984) lists a number of plants in the Rutaceae other than *Citrus* and *Poncirus* that can serve as hosts of *Xac* under experimental conditions or heavy disease pressure in nature. A comprehensive host list is presented on the DPI website (address above). Among commercial citrus varieties and rootstocks, CC is most severe on grapefruit (*C.* x *paradisi*), Key limes (*C. aurantiifolia)*, and trifoliate orange (*Poncirus trifoliata*) and their hybrids because of their high susceptibility (Table 1). All natural hosts of CC must be considered potential risks when eradication is the goal.

Table 1. Susceptibility of several commercial citrus varieties and rootstocks to *Xac*.

Highly Susceptible	Moderately Susceptible
Citrus x *paradisi* Macf., grapefruit	*C. sinensis* (L.) Osbeck, sweet orange
C. aurantiifolia (Christ.) Swingle, Key lime	*C. aurantium* L., sour orange
C. limettioides Tan., Palestine sweet lime	*C. limon* (L.) Burm. lemon
Poncirus trifoliata (L.) Raf., trifoliate citrus & hybrids	*C.* x *tangelo* J. Ingram & H.E. Moore, tangelo
Susceptible	**Resistant**
C. reticulata Blanco, mandarin, tangerine	*C. medica* L., citron
C. maxima (Burm.) Merr., pummelo	X *Citrofortunella microcarpa* (Bunge) Wijnands, calamondin
C. aurantiifolia (Christ.) Swingle, Persian or Tahiti lime	*Fortunella spp.*, kumquat

Recently, it was reported that goat weed (*Ageratum conyzoides* L.) could serve as a host of *Xac.* This plant is common in citrus orchards in India (Kalita *et al*. 1997). This represents one of only a few unsubstantiated reports of a non-Rutaceous host of *Xac*.

CONTROL: In Florida, the predicted negative economic impact of allowing CC to become endemic clearly favors eradication. The Florida approach to CC eradication has evolved over time. In the first program from 1915 to 1933, infected trees were usually doused with an incendiary fuel and burned in place. In later programs, infected trees have been burned in place or removed mechanically. The necessity of removing exposed in addition to obviously infected trees was recognized in the 1986 program, and a guideline calling for removal of all citrus within 125 ft of infected citrus was adopted based on studies of inoculum dispersal in Argentina (Stall *et al.* 1980). In the latest eradication program with citrus leafminer involvement (Gottwald *et al*. 1997; Gottwald *et al.* 2001, 2002a), even the 125 ft radius was deemed inadequate for eradication in most situations, and a general policy of removing infected trees plus exposed trees within a 1900 ft radius is now in place. Through risk assessment, exposure zones are determined commensurate with the amount of disease, age of infection, inoculum dispersal opportunities, environment, susceptibility of hosts, physical access to potential hosts for regular inspection, plus other factors. In these exposure zones, all citrus is removed.

Guidelines call for quarantine areas to extend one to two miles in all directions from infected citrus. No citrus material is allowed to move into or out of the quarantine zone unless risk-assessed. Survey and inspection crews should practice rigorous sanitation of hands, shoes, clothing or any equipment that comes in contact with citrus before moving from property to property. Quaternary ammonium disinfectants are available for use on both inanimate surfaces and for application to bare skin and clothing.

In areas of the world where CC is endemic, disease management involves use of resistant varieties, windbreaks to hinder inoculum dispersal and timely applications of copper-containing bactericides. Selective pruning of infected tissues is also utilized in citrus growing areas where labor-intensive practices prevail.

SURVEY AND DETECTION: Look for raised, corky, tan lesions with water-soaked margins and yellow halos on the citrus leaves. Similar citrus leaf pathogens—sour orange scab caused by *Elsinoe fawcettii* Bitancourt & Jenkins, melanose caused by *Diaporthe citri* F.A. Wolf, and citrus bacterial spot caused by *Xanthomonas axonopodis* Starr & Garces emend. Vauterin *et al*. pv. *citrumelo* Gabriel *et al*.—are illustrated in Figs. 7-9, respectively. The field inspector unfamiliar with CC should not rely on these photographs for a definitive distinction of these three diseases. Any suspicious plant symptoms should be brought to the attention of the DPI Pathology Section. Samples of suspect citrus should be double-bagged with the inner bag and hands disinfected with quaternary ammonium before placing in the outer bag. Attach paperwork to the outer bag indicating 'suspect citrus canker' so the sample can be immediately processed in the DPI Plant Disease Quarantine Facility in Gainesville.

LITERATURE CITED

Civerolo, E. L. 1984. Bacterial canker disease of citrus. Journal of the Rio Grande Valley Horticultural Society 37: 127-146.

Commonwealth Agricultural Bureaux International - International Mycological Institute. 1996. Distribution maps of plant diseases, *Xanthomonas campestris* pv. *citri*. Map No. 11. Edition 6. 2 p.

Dopson, R. N. 1964. The eradication of citrus canker. Plant Disease Reporter 48: 30-31.

Goto, M. 1992. Citrus canker. *In:* Plant Diseases of International Importance: Diseases of Fruit Crops (Vol. III) Ed. by J. Kumar, *et al*. Englewood Cliffs, NJ, Prentice Hall, pp. 170-208.

Goto, M., K. Ohta and N. Okabe. 1975. Studies on saprophytic survival of *Xanthomonas citri* (Hasse) Dowson. 1. Detection of the bacterium from a grass (*Zoysia japonica*). Annals of the Phytopathological Society of Japan 41: 9-14.

Gottwald, T. R., J.H. Graham and T.S. Schubert. 1997. An epidemiological analysis of the spread of citrus canker in urban Miami, Florida, and synergistic interaction with the Asian citrus leafminer. Fruits 52: 371-378.

Gottwald, T.R., G. Hughes, J.H. Graham, X. Sun and T. Riley. 2001. The citrus canker epidemic in Florida: The scientific basis of regulatory eradication policy for an invasive species. Phytopathology 91: 30-34

Gottwald, T.R., X. Sun, T. Riley, J.H. Graham, F. Ferrandino and E.L. Taylor. 2002a. Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. Phytopathology 92: 362-377.

Gottwald, T.R., J.H. Graham and T.S. Schubert. 2002b. Citrus canker: The pathogen and its impact. Online. Plant Health Progress doi:10.1094/PHP-2002-0812-01-RV. http://www.plantmanagementnework.org/pub/php/review/citruscanker/

Heppner, J. P. 1993. Citrus leafminer, *Phyllocnistis citrella*, in Florida. Tropical Lepidoptera 4: 49-64.

Kalita, P., L.C. Bora and K.N. Bhagabati. 1997. Goat weed - a host of citrus canker - (*Xanthomonas campestris* pv. *citri*). Journal of Mycology and Plant Pathology 27: 96-97.

Koizumi, M. 1985. Citrus canker: the world situation. Pp. 2-7. *In* Timmer, L.W. (ed.). Citrus canker: An international perspective. University of Florida, Lake Alfred.

Loucks, K. W. 1934. Citrus canker and its eradication from Florida. (Unpublished manuscript, Library) Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL. 111 p.

Pruvost, O., B. Boher, C. Brocherieus, M. Nicole and F. Chiroleu. 2002. Survival of *Xanthomonas axonopodis* pv. *citri* in leaf lesions under tropical environmental conditions and simulated splash dispersal of inoculum. Phytopathology 92: 336-346.

Schubert, T.S. 1991. Recent history of citrus canker eradication programs in Florida. Newsletter of the Florida Phytopathological Society 2: 1-6.

Schubert, T.S., J.W. Miller and D.W. Gabriel. 1996. Another outbreak of bacterial canker on citrus in Florida. Plant Disease 80: 1208.

Schubert, T.S., S.A. Rizvi, X. Sun, T.R. Gottwald, J.H. Graham and W.N. Dixon. 2001. Meeting the challenge of eradicating citrus canker in Florida - Again. Plant Disease 85: 340-356.

Sinha, M.K., R.C. Batra and D.K. Uppal. 1972. Role of citrus leaf-miner (*Phyllocnistis citrella* Staintan (sic)) on the prevalence and severity of citrus canker (*Xanthomonas citri* (Hasse) Dowson). Madras Agricultural Journal (Madras) 59: 240-245.

Sohi, G. S. and M. S. Sandhu. 1968. Relationship between citrus leafminer (*Phyllocnistis citrella* Stainton) injury and citrus canker (*Xanthomonas citri* (Hasse) Dowson) incidence on citrus leaves. Journal of Research Punjab Agriculture University (Ludhiana) 5: 66-69.

Stall, R. E., J. W. Miller, C. M. Marco and B. I. C. DeEchenique. 1980. Population dynamics of *Xanthomonas citri* causing cancrosis of citrus in Argentina. Proceedings of the Florida Horticultural Society 93: 10-14.

USDA-APHIS-PPQ Biological Assessment Support Staff. 1982. Citrus canker. Pests not known to occur in the United States or of limited distribution. No. 27. 12 p.

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Fig. 1. Typical CC symptoms on leaves, stems and fruit of grapefruit.

Fig. 2. Whole leaf symptoms of CC on top and bottom of grapefruit leaves (left) and key lime leaves (right).

Fig. 3. Close-up views of CC lesions on top (left) and bottom (right) of grapefruit leaf. Photo credit: Jeffrey Lotz, DPI.

Fig. 4. Twig symptoms of *Xac*. young lesions on grapefruit (left); older lesions on Key lime (right). Photo credit: Jeffrey Lotz, DPI.

Fig. 5. Fruit symptoms of CC on sweet orange (left) and grapefruit (right). Photo credit (left): Jeffrey Lotz, DPI.

Fig. 6. Leaf symptoms of CC on lemon exacerbated by citrus leafminer wounding, top (left) and bottom (right) of leaves.

Fig. 7. Sour orange scab on sour orange leaf (left), close-up view of scab lesion (center), and scab lesions on tangelo (right), caused by *Elsinoe fawcettii* Bitancourt & Jenk., for comparison to CC. Photo credit (right): V. Jane Windsor, DPI.

Fig. 8. Melanose (*Diaporthe citri*) and CC lesions on the same grapefruit leaf, bottom side. CC lesions are the larger, brown raised corky lesions on the lower left; melanose lesions are the other smaller, black raised lesions.

Fig. 9. Citrus bacterial spot (Cbs) top (left) and bottom (right) of leaf on grapefruit, caused by *Xanthomonas axonopodis* Starr & Garces emend. Vauterin, *et al*. pv. *citrumelo* Gabriel, *et al*., for comparison to CC. Cbs lesions are less pustular and corky than CC, and aggressive strains exhibit a much more pronounced watersoaked margin than CC. Cbs can perpetuate in young trees in crowded nursery environments, but disappears spontaneously when trees are outplanted. All photos by T. S. Schubert, DPI, unless otherwise credited.