Epidemiology of a Citrus Leaf-Spot Disease in Colima, Mexico

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ABSTRACT

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A leaf- and twig-spot disease (LSD) known as "bacteriosis" is a suspected form of citrus canker (*Xanthomonas campestris* pv. *citri*) that occurs primarily on Mexican lime (ML) trees (*Citrus aurantifolia*) along the central-Pacific coast of Mexico. Seasonal incidence and severity of presumptive LSD symptoms in two groves increased during the dry, cool months (November–May) and decreased during the warm, rainy season (June–October). Presumptive LSD symptoms were observed on all 15 citrus cultivars that were evaluated for natural infection in the field. Observed host susceptibility rankings showed *C. limettioides*, *C. aurantifolia*, *C. macrophylla*, and *C. latifolia* to be most susceptible to LSD, and *C. sinensis* 'Valencia', *C. taiwanica*, and *C. reticulata* to be least susceptible. *C. sinensis* 'Washington navel', *C. grandis*, *C. aurantium*, and *C. paradisi* were intermediate. Presumptive LSD symptoms could not be confirmed on varieties of *Poncirus trifoliata*. Natural symptom incidence of LSD on non-ML hosts decreased with increasing distance from ML blocks. No definitive *X. c.* pv. *citri* isolates were recovered during the course of these experiments, and epidemiological and etiological factors of LSD are unlike those of known forms of citrus canker.

Additional key words: citrus disease, foliar disease.

A leaf- and twig-spot disease (LSD) commonly known as citrus "bacteriosis" was first encountered on Mexican lime (ML) trees (*Citrus aurantifolia*) near Tecomán, Colima, Mexico, late in 1981. Lesions visually resemble those of citrus canker, caused by *Xanthomonas campestris* pv. *citri* (18). LSD is apparently limited to coastal citrus-growing areas in the central-Pacific Mexican states of Jalisco, Colima, Michoacan, Guerrero, and Oaxaca (18). This region has a semi-arid tropical climate, with warm, rainy summers and cool, dry winters. Occasional isolation of bacteria presumptively identified as *X. campestris* or *X. c.* pv. *citri* from LSD pustules has been reported (3,5,15,18,19); however, the identification, pathogenicity, and/or consistent association of these isolates with LSD has not been confirmed. In light of the lack of evidence of bacterial involvement and recent reports (2,10,12) implicating *Alternaria* sp. in the disease, it has been suggested (10,24) that the common name "bacteriosis" be changed to "leaf spot" until the complete disease etiology is clarified. The objectives of this study were to describe annual symptom development patterns of LSD in relation to meteorological data, to determine the relative susceptibility of certain citrus hosts, and to isolate *X. c.* pv. *citri* in consistent association with LSD lesions. Brief reports of some of these data have been published (8,9,11,26,27).

MATERIALS AND METHODS

Seasonal symptom development. Symptom development dynamics of LSD were monitored in two experiments over a two-year period in three commercial ML groves near Tecomán, Colima, Mexico. A heavily infected grove (Microondas; 4-yr-old trees on *C. macrophylla* rootstock) and two moderately infected groves (Michelena; 4-yr-old trees on sour orange [*C. aurantium*] rootstock and Ibañez 4-yr-old trees on *C. macrophylla* rootstock) were used. In the first experiment, five ML seedlings from a nursery in a disease-free area were defoliated, fertilized, and incubated for 15 days to initiate vegetative shoots. These seedlings were taken to the Ibanez grove and incubated between rows of LSD-infected trees for 14 days. Trees were then taken to a screenhouse 1 km from the nearest ML grove and percent leaves infected and lesions per infected leaf were counted 15 days later. Disease ratings on different trees were taken every 15 days from September 1983 to September 1984. In a subsequent experiment, five randomly selected trees in the Microondas and Michelena groves were sampled about every 14 days from May 1984 to September 1985. Sampling was done by examining two randomly chosen, fully expanded leaves from each of two of the youngest shoots in each quadrant of the trees. Leaves were visually assayed for LSD using the same parameters described above. Disease data were compared with air temperature, rainfall, and dew data obtained from Mexican Secretary of Agriculture and Hydraulic Resources weather station in Tecomán, 7–10 km from the experimental groves.

Host range. Four replications each of 15 cultivars (Table 1) of citrus rootstock and scion nursery trees growing in plastic containers were placed under the drip line of mature, LSD-infected ML trees in the Microondas and Michelena groves. All nursery trees were manually defoliated and fertilized at the beginning of each experiment and were subsequently irrigated as needed. Nursery trees were incubated until shoots of all cultivars were mature. Foliage of receptor trees was then harvested, and LSD lesions were visually identified and counted. A severity index (leaves infected × lesions/infected leaf) composited from the four experiments at two sites was used to estimate susceptibility of each variety tested. Potted tree experiments were repeated in February-March, April-May, June-July, and August-September 1985.

Disease gradient study. Leaves from field-grown Persian lime (*C. latifolia*) trees located in rows 10, 35, or 60 m from an infected ML block in a commercial grove were randomly sampled for LSD lesions in April 1985. Leaves were sampled and LSD lesions were counted in a manner identical to that in the symptom development study, except that three trees at each distance were used. A similar study was done in the Michelena grove in July 1985. Leaves of sour orange (*C. aurantium*) from single trees in a mixed-cultivar block located at distances of 0, 7, 17, 30, and 170 m from an infected ML

block were sampled and assayed, as were those of two trees each of Valencia orange (*C. sinensis*) located 8, 27, 100, and 200 m away; and grapefruit (*C. paradisi*) located 40 and 60 m away.

Isolation of *X. c.* pv. *citri*. Isolation of *Xanthomonas* spp. was attempted from citrus leaves, stems, and fruit on a weekly basis. Isolations also were made from other horticultural and weed plants associated with citrus groves near Tecomán, including leaves and fruit of banana (*Musa* ssp.) and mango (*Mangifera indica*), and foliage of johnsongrass (*Sorghum halepense*), bitter cucumber (*Momordica charantia*), and pigweed (*Amaranthus* spp.). Bacteriological growth media (20), including nutrient, King's medium B, liquid medium 523, yeast extract-dextrose-$CaCO_2$ (YDC), potato dextrose agar, and SMB as well as two media for fastidious prokaryotes (4,16) were used. Isolations were made with and without surface disinfestation of tissue samples. A light microscope was used to examine lesions of LSD and healthy tissue samples for bacterial streaming in water. In addition, antigen extracts of presumptive LSD lesions were subjected to gel immunodiffusion serodiagnostic assay using antiserum to extracts of LSD lesion tissue (24,25).

RESULTS

Seasonal symptom development. The incidence of LSD increased during the dry season (November–May) rather than during the rainy season (June–October) during the experiment with nursery trees (Fig. 1). Percent leaves infected was highest (65%) during the period of least rain, most frequent dew, and lowest mean air temperature. Conversely, disease symptoms were fewest (1%) during the period of most rain, least dew, and highest mean air temperature. Disease incidence (percent leaves infected) and severity (lesions per infected leaf) increased during the period of vegetative growth flushes throughout the dry season during the experiment with mature trees (Fig. 2). The 1983-1984 experiment was designed to have receptor trees with succulent, LSD-susceptible leaves in the grove throughout the year. However, in the 1984-1985 experiment using grove trees, no growth flushes occurred during the rainy season; thus, biweekly observations were taken from mature, previously infected leaves. Foliar symptoms reached maximum incidence (99% infected leaves) and severity (4.5 lesions/infected leaf) before the onset of the rainy season, remained high during the rainy summer on the mature leaves (no growth flushes), then dropped to minimum levels of 5% infected leaves and 1 lesion/infected leaf after the October (rainy season) flush. Although rain is infrequent during the months of November to June, dew occurs almost nightly during this period. Conversely, little dew occurs during the warmer, rainy season. Therefore, free water is present on leaves during at least part of the day nearly all year. Because the climate in the Tecomán valley is tropical, no marked seasonal temperature changes occur. Seasonal differences in minimum temperature are greater than differences in maximum temperature (Figs. 1 and 2).

TABLE 1. Leaf-spot disease severity index[a] of citrus cultivars tested

Common name	Scientific name	Severity index
Palestine sweet lime	*Citrus limettioides* Tan.	1.38
Mexican lime	*C. aurantifolia* (Christm.) Swing.	1.16
Alemow	*C. macrophylla* Wester	1.09
Persian lime	*C. latifolia* Tan.	0.97
Washington navel orange	*C. sinensis* (L.)	0.55
Pummelo	C. grandis (L.) Osbeck	0.45
Sour orange	*C. aurantium* L.	0.37
Grapefruit	*C. paradisi* Macf.	0.35
Cleopatra Mandarin	*C. reticulata* Blanco	0.15
Nansho daidai	*C. taiwanica* Tan. & Shimada	0.12
Valencia common orange	*C. sinensis* (L.) Osbeck	0.05
LSD	0.53	
Analysis of variance		
Cultivar	0.01[b]	
Block	0.01	

[a] Percent leaves infected × No. lesions/infected leaf (composite of four experiments at two sites).
[b] Level of significance.

Host range. All of the citrus cultivars showed presumptive LSD symptoms during the course of the experiments (Table 1). *C. limettioides*, *C. aurantifolia*, *C. macrophylla*, and *C. latifolia* were most susceptible to LSD, with mean disease index values of 1.38, 1.16, 1.09, and 0.97, respectively, over the four experiments. *C. sinensis* 'Washington navel', *C. grandis*, *C. aurantium*, and *C. paradisi* showed intermediate susceptibility with mean values of 0.53, 0.45, 0.37, and 0.35, respectively. *C. reticulata*, *C. taiwanica*, and *C. sinensis* 'Valencia' had disease index values of 0.15, 0.12, and 0.05, respectively. Cultivars and hybrids of *Poncirus trifoliata* including citrumelo, Troyer and Carrizo citrange, and Rubidoux trifoliate orange were not rated due to inability to confirm foliar symptoms as being those of LSD by *X. c.* pv. *citri* isolation or serodiagnostic assay. A significant block effect ($P < 0.01$) was found over the course of the four experiments. The highest disease index values of the experimental cultivars occurred during the first experiment (February-March), and the lowest values were obtained for the last experiment (August-September).

Disease gradient study. At the time ratings on the Persian lime trees were made, 96% of foliage in the adjacent ML block was infected with LSD. Percent infection on Persian lime leaves dropped from 96 to 67% (Fig. 3) when distance from the ML block increased from 10 to 60 m. Number of lesions per infected leaf likewise decreased from 12.1 to 4.8. Analysis of variance showed that these reductions were significant ($P < 0.05$) by linear and quadratic regression. Values of R^2 ranged from 0.509 to 1.0. Disease incidence in the ML block adjacent to the other tested cultivars was 98% at the time of rating. Percent leaves infected and lesions per infected leaf of sour orange, Valencia orange, and grapefruit all decreased with increasing distance from the ML block. Disease decreases on Valencia orange were significant ($P < 0.05$; $R^2 = 0.509$ to 0.964) by linear and quadratic regression. Decreases on sour orange ($R^2 = 0.518$–0.761) and grapefruit ($R^2 = 0.290$) were not statistically significant. Field susceptibility of the four non-ML cultivars agreed with data from the host range study with potted trees. Persian lime had the highest percentage of infected leaves, Valencia orange had the lowest, and sour orange and grapefruit were intermediate. Presumptive LSD symptoms on non-ML hosts were observed as far as 170 m from the nearest infected ML block. However, percent infected leaves on non-ML host trees closest to ML blocks were 35–108% higher than trees next in proximity to infected ML trees (Fig. 3).

Isolation of *X. c.* pv. *citri*. No definitive *X. c.* pv. *citri* isolates were recovered during the course of the study. Fewer than 10 isolates that were similar to the 'T20' strain of *X. campestris* (18,19) and judged to be nonpathogens (Stapleton and Garza-Lopez, *unpublished*) were recovered from ML at the Ibañez grove. Xanthomonad colony morphology was most easily differentiated

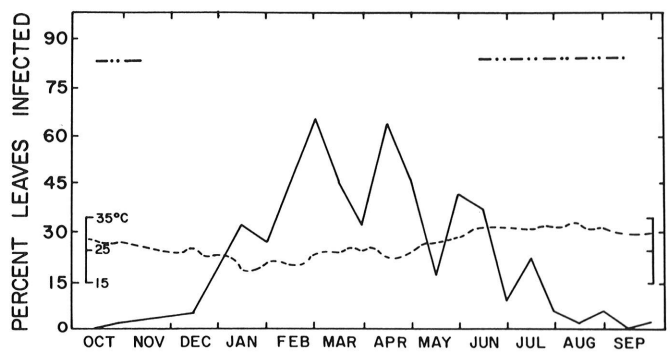

Fig. 1. Relationships of citrus leaf-spot disease index and weather data on naturally infected, succulent Mexican lime seedlings. Percent leaves infected (—); mean air temperature (- - -); seasonal rain period (—··—). Weather data taken from SARH meterological station near Tecomán, Colima, Mexico, 1983-1984.

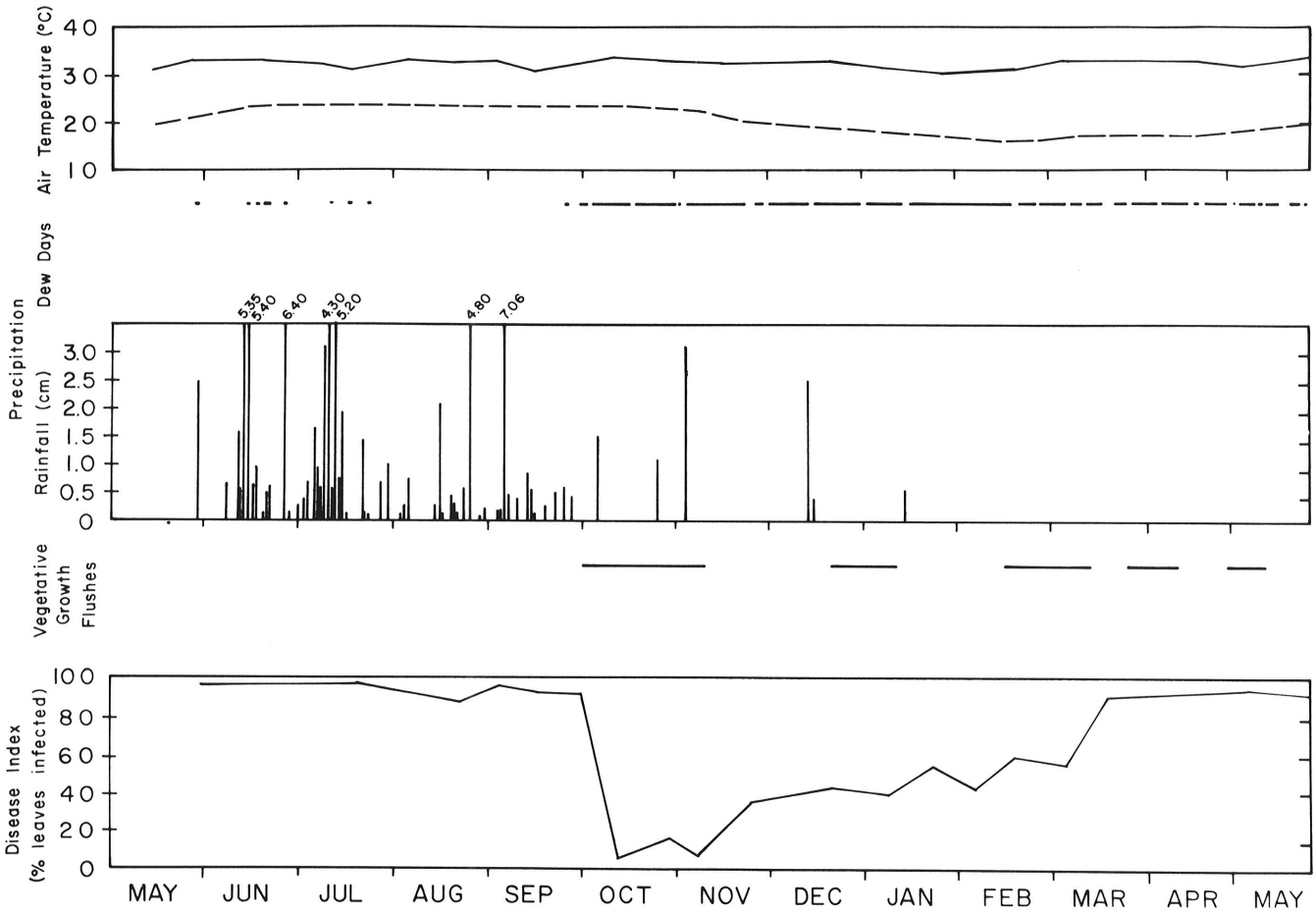

Fig. 2. Relationships of citrus leaf-spot disease index, weather data, and vegetative growth flushes of naturally infected, mature Mexican lime trees. Average maximum air temperature (—); average minimum air temperature (---). Daily dew occurrence was determined at 0800 hours. Weather data taken from SARH meteorological station near Tecoman, Colima, Mexico, 1984–1985.

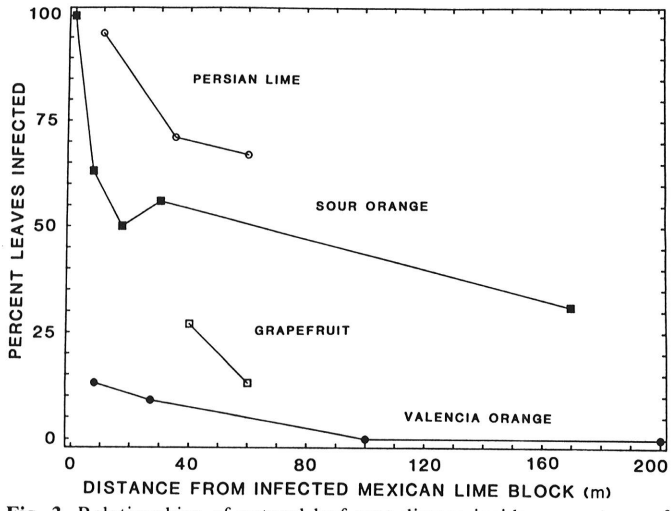

Fig. 3. Relationships of natural leaf-spot disease incidence on trees of field-grown citrus cultivars, and distance from infected Mexican lime blocks in Tecomán, Colima, Mexico, 1985.

from that of other bacteria on YDC. No successful isolations were made on SMB, or on either of the media selective for fastidious prokaryotes. No typical *Xanthomonas* spp. were isolated from any of the noncitrus plants tested. An estimated 1,000 isolations, usually of several pustules each, were made during these experiments. No bacterial streaming from healthy tissues or LSD lesions in water was observed. Lesion extracts from *Citrus* cultivars reacted positively to antiserum prepared against LSD lesion extracts from ML leaves (24,25).

DISCUSSION

Results of this study indicated that LSD symptoms increased during the winter and spring months (dry season) and were least prevalent after the late-summer (rainy season) flush. This is unlike the seasonal occurrence of lesions of known forms of citrus canker, which is greatest during periods of heavy rainfall and high air temperature (14,22). Foliar symptom dynamics similar to those presented here also were observed in 1985-1986 (J. Garza-Lopez, *unpublished*). Symptoms of LSD develop on ML leaves from 5 to more than 30 days old (17).

The host range study showed that a wide range of citrus scion and rootstock cultivars are susceptible to LSD. Another study (9) reported generally similar results. Many cultivars, however, may be unaffected if isolated from LSD-infected ML trees, as shown by the disease gradient study. This suggests that ML is the primary host and a main inoculum reservoir of LSD in Colima. Results indicated that *C. limettioides* and *C. macrophylla* also may be important sources of LSD inoculum. Although *C. limettioides* is not commonly cultivated in citrus-growing areas affected by LSD, *C. macrophylla* frequently is used as a rootstock for ML. Alternative rootstock cultivars should be developed to avoid potential LSD outbreaks during nursery production of *C. macrophylla*, or following rootstock sucker growth in ML groves. Work has been initiated on rootstock/scion combinations as related to LSD (8), which may be used as a basis for improvement of host resistance and other management strategies. The decreases in symptom severity over the four host range experiments (February-September) coincide with reductions in natural infection of ML over the same time period (Fig. 1). The reaction of *Citrus* cultivars, but not the cultivars of *P. trifoliata*, with the antiserum from LSD lesion tissue of ML suggests that the antigenic determinants are

primarily products of host-pathogen interaction (25).

As a suspected form of citrus canker regulated by quarantine restrictions, LSD is considered a threat to citriculture in Mexico and the United States. However, observations of a number of etiological and epidemiological factors, including the lack of association with or isolation of *X. c.* pv. *citri*, the comparatively mild symptoms of the disease, and the increased development of symptoms during the dry season indicated that LSD is significantly different from known forms of citrus canker (14,21,22).

Several other factors regarding xanthomonad ecology complicate diagnosis of suspected canker outbreaks. Pathovars of *X. campestris* were reported to reside epiphytically on nonhosts (28). It was suggested that the pathogen of the "nursery strain" of citrus canker occurring in Florida is a variant of *X. c.* pv. *alfalfae* or *phaseoli*. And fastidious Cancrosis-B strains of *X. c.* pv. *citri* were encountered in Argentina (1).

Reports on LSD, previously named citrus "bacteriosis" (3,6,13,17–19,23–25), have discussed the difficulty of isolating *X. c.* pv. *citri* or other pathogenic bacteria in assocation with the disease and have suggested the possible involvement of other causal agents. The identification of the *X. campestris* isolates reported by Delgado-Sanchez and Ruiz-Loaiza (5) has not been confirmed; moreover, the 'T20-T24' isolates of Rodriguez et al (18,19), which caused lesions resembling those of LSD when inoculated into ML leaves under screenhouse conditions in Tecomán, failed to induce similar symptoms in greenhouse and detached ML leaf inoculations in Beltsville, MD (25). In addition, Hartung and Civerolo (13) reported that only one isolate of *X. c.* pv. *citri* was obtained from LSD lesions from Colima. The inconsistency of microscopic and serologic association of *X. c.* pv. *citri* with disease lesions (24,25), and the provocati of similar symptoms by *Alternaria* sp. (2,10,12,25) suggest that LSD is primarily a fungal disease. This leaf-spot disease cannot be considered to be a form of citrus canker unless compelling evidence of consistent *X. c.* pv. *citri* involvement is shown.

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