Temporal Changes in Susceptibility of Citrus Phloem Tissue to Colonization by *Phytophthora citrophthora* and *P. parasitica*

M. E. MATHERON, Extension Plant Pathologist, and J. C. MATEJKA, Research Assistant, Department of Plant Pathology, University of Arizona, Yuma Agricultural Center, Yuma 85364

ABSTRACT

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Four rootstocks and two commercial cultivars of citrus were evaluated for temporal variation in susceptibility to colonization by *Phytophthora citrophthora* and *P. parasitica*. For 24 consecutive months, terminal shoots were collected from field-grown trees of *Citrus macrophylla*, *C. jambhiri*, *C. aurantium*, and *C. volkameriana*. They were then wounded, inoculated with *P. citrophthora* or *P. parasitica*, and incubated in moist chambers. Shoot pieces and bark strips of lemon (*C. limon* 'Lisbon') and tangelo (*C. reticulata* × *C. paradisi* 'Orlando') were inoculated and incubated in moist chambers at monthly intervals for 24 consecutive months. Shoot pieces of *C. jambhiri*, *C. aurantium*, and *C. volkameriana* inoculated with *P. citrophthora* or *P. parasitica* developed smaller lesions during December–February than during June–August. Colonization of excised shoot tissue of *C. macrophylla* by *P. citrophthora* was less during December–February than June–August, whereas colonization of the same plant tissue by *P. parasitica* was less during December–February than March–May. Minimum development of lesions occurred during November–December on bark strips from Lisbon lemon and Orlando tangelo. These findings demonstrate temporal fluctuations in the colonization of citrus rootstock and scion tissues by *P. citrophthora* and *P. parasitica*.

Phytophthora spp. cause the most important fungal diseases of citrus (11) and, in Arizona, Phytophthora gummosis is widespread. *P. citophthora* (R. & E. Sm.) Leonian and *P. parasitica* Dastur are consistently recovered from infected bark tissue from declining citrus trees. Active growth of trunk lesions occurs during May and June, declines somewhat during the hot summer months of July and August, then resumes during September and October. Destruction of bark tissues leads to increasing yield loss and eventual tree death.

Control of Phytophthora gummosis of citrus has been enhanced by the availability of the systemic fungicides fosetyl-Al (Aliette) and metalaxyl (Ridomil) (4,5,12,17,20). After a single trunk application of either fungicide, bark tissue from treated citrus trees was inhibitory to growth of *P. citrophthora* and *P. parasitica* for at least 117 days (14). However, to achieve optimum disease control, fungicides should be in place when colonization of the host and development of disease are most likely to occur.

Previous research has demonstrated the existence of seasonal variation in the susceptibility of deciduous trees, such as apple (2,6,9,19) and walnut (15), to colonization by *Phytophthora* spp. The purpose of this research was to determine seasonal susceptibility of citrus to infection by *P. citrophthora* and *P. parasitica*. A knowledge of seasonal changes in susceptibility of citrus phloem tissue to invasion by *Phytophthora* spp. could enhance disease control by indicating optimum periods for application of fungicides or other disease control measures. A preliminary report of this work has been published previously (13).

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MATERIALS AND METHODS

Excised shoot inoculations. At monthly intervals from May 1985 through April 1987, woody sections approximately 8 cm long were collected from shoots of the most recent growing season. Shoots were collected from a group of four trees of each tested *Citrus* sp. at the Yuma Agricultural Center and were stored in plastic bags until inoculation later in the day. Plant scion material was gathered from trees established in 1972 on *C. macrophylla* Wester rootstock and included *C. macrophylla* (alemow), *C. jambhiri* Lush. (rough lemon), *C. aurantium* L. (sour orange), and *C. volkameriana* (Pasq.) Tan.

Twenty replicate shoot sections, five from each of four trees of each *Citrus* sp., were inoculated at the beginning of each month with either *P. citrophthora* (isolate C-7-S) from a citrus tree in Mesa, AZ, or *P. parasitica* (isolate C-2-C) from a citrus tree in Yuma, AZ. Pathogens were grown on V-8 juice agar for 7 days at 21 C. Agar disks (6 mm diameter) were removed from the edge of actively growing cultures and were placed directly into 6-mm-diameter wounds in the middle of excised shoot sections. Bark and phloem tissues were completely removed at the wound site to an approximate depth of 1 mm, leaving exposed cambium. Shoot pieces were then incubated for 7 days at 21 C in moist chambers, after which the lengths of resulting lesions were recorded. Shoot sections were not surface-sterilized before inoculation. Noninoculated controls were prepared by placing agar disks without mycelium into wounds of excised shoots.

Concurrent bark strip and excised shoot inoculations. Two sources of phloem tissue, from shoots and from trunks of trees, were used in these citrus studies. Every month from January 1986 to December 1987, 20 replicate bark strips and shoot sections from tangelo (*C. reticulata* Blanco × *C. paradisi* Macf. 'Orlando') and lemon (*C. limon* (L.) Burm. 'Lisbon') trees were inoculated with *P. citrophthora* or *P. parasitica* and evaluated for disease severity. Vertical strips of bark (7 cm long × 1.5 cm wide) as well as sections of shoots (7 cm long), four from each of five trees, were collected from a high-density planting of Orlando tangelo established in 1968 and Lisbon lemon trees established in 1978, both on *C. macrophylla* rootstock. Bark strips and shoot pieces were stored in plastic bags until inoculation later in the day. *P. citrophthora* (isolate C-7-S) and *P. parasitica* (isolate C-2-C) were grown on V-8 agar for 6 days at 24 C. Agar disks (6 mm diameter) were removed from the edge of actively growing cultures and were placed in the center of the bark strips on the inner phloem tissue and into 6-mm-diameter wounds in the middle of excised shoot sections. Bark strips and shoot sections that were not surface-sterilized were then incubated in moist chambers for 4 days at 24 C. The extent of colonization was determined by measuring the length of cankers that developed at the inoculation sites.

P. citrophthora and *P. parasitica* were reisolated monthly from colonized bark tissue to help maintain virulence of the pathogens. To confirm that canker development resulted from infection by the appropriate *Phytophthora* sp., necrotic tissue from shoot sections and bark strips was plated into a selective medium containing 17 g of cornmeal agar, 5 mg of pimaricin, 200 mg of ampicillin, 10 mg of rifampicin, and 100 mg of pentachloronitrobenzene per

liter of water (PARP) (10). Plates were incubated at 24 C in the dark and were examined microscopically to compare the original length of the visible lesion with the length of tissue from which the *Phytophthora* sp. emerged. Duncan's multiple range test was used to determine differences between seasonal development of lesions as well as differences in lesion development among tested *Citrus* spp. Student's *t* test was used to determine a significant difference in the degree of lesion development caused by each of the two species of *Phytophthora*.

RESULTS

Excised shoot inoculations. Over a 2-yr period, colonization of excised shoot pieces of *C. macrophylla*, *C. jambhiri*, *C. aurantium*, and *C. volkameriana* by *P. citrophthora* was significantly less during December–February than during June–August (Table 1). There was no difference in lesion development from March to May through September to November on shoot pieces of *C. macrophylla*, *C. jambhiri*, and *C. aurantium* inoculated with the same pathogen.

Lesions that developed on shoot pieces of *C. jambhiri*, *C. aurantium*, and *C. volkameriana* inoculated with *P. parasitica* were smaller during December–February than during June–August, whereas lesion size on tissue of *C. macrophylla* was smaller during December–February than during March–May (Table 1). Lesion development was similar from March to May through September to November on shoot pieces of *C. macrophylla*, *C. jambhiri*, and *C. volkameriana* inoculated with *P. parasitica*.

The size of lesions on shoot tissue inoculated with *P. citrophthora* was significantly greater on *C. aurantium* than lesions developing on *C. macrophylla*, *C. jambhiri*, and *C. volkameriana* (Table 2). Cankers developing on shoot pieces inoculated with *P. parasitica* were larger on *C. aurantium* and *C. macrophylla* than those developing on *C. jambhiri* and *C. volkameriana*.

Analysis of the data for lesion development on excised shoot tissue from *C. macrophylla*, *C. jambhiri*, *C. aurantium*, and *C. volkameriana* for the entire 2-yr study revealed that inoculation with *P. citrophthora* or *P. parasitica* produced lesions with a mean length of 14 and 7 mm, respectively. According to Student's *t* test, this difference is highly significant ($P = 0.004$).

Concurrent bark strip and excised shoot inoculations. From the results of a 2-yr study, the least lesion development on bark tissue from Lisbon lemon colonized by *P. citrophthora* or *P. parasitica* occurred during November–December, whereas minimum development of cankers on excised shoot tissue colonized by the same pathogens occurred during January–February (Fig. 1A,B). Canker development was greatest on shoot sections inoculated with *P. citrophthora* during May–June, whereas maximum development of lesions on bark tissue of Lisbon lemon occurred during March–April (Fig. 1A). Canker development on excised shoots inoculated with *P. parasitica* was significantly greater from May to June through September to October than from November to December and January to February, whereas the largest cankers on strips of bark tissue occurred from January to February through September to October (Fig. 1B).

Length of cankers on bark tissue collected from Orlando tangelo trees and inoculated with *P. citrophthora* and *P. parasitica* was smallest during November–December, whereas minimum development of lesions on shoot tissue by these pathogens took place during November–December and January–February (Fig. 1C,D). The largest cankers were produced during May–June on shoot tissue of Orlando tangelo inoculated with *P. citrophthora* or *P. parasitica*. Maximum lesion development on bark tissue inoculated with *P. citrophthora* (Fig. 1C) and *P. parasitica* (Fig. 1D) occurred from March to April through September to October and from July to August through September to October, respectively.

When apparently healthy and necrotic tissues from shoot pieces and bark strips were plated onto PARP selective agar medium, mycelium of *Phytophthora* routinely developed from the necrotic tissue but not from the healthy tissue.

For the entire 2-yr study, the average length of lesions on excised shoot tissue from Lisbon lemon and Orlando tangelo inoculated with either *P. citrophthora* or *P. parasitica* was 13 and 6 mm, respectively; whereas lesions on bark strips inoculated with the same pathogens were 30 and 22 mm in length, respectively. The isolate of *P. citrophthora* caused significantly larger lesions than *P. parasitica* on excised shoot pieces as well as bark tissue. Both *P. citrophthora* and *P. parasitica* caused larger lesions on excised bark tissue than on excised shoot pieces. According to Student's *t* test, all of these differences were highly significant ($P < 0.001$).

DISCUSSION

This research demonstrates seasonal fluctuations in the colonization of citrus rootstock and scion tissues by *P. citrophthora* and *P. parasitica*. In subtropical climates, citrus cultivars stop growing during the winter but maintain a minimum level of starch consumption and water transport (1). The extent of colonization of bark and shoot tissue from citrus was at minimum levels during this time. Maximum invasion of tissue by the same pathogens usually occurred from March through October or November and was dependent upon the species of *Citrus*. In Arizona, the main flush of growth and the production of flowers in *Citrus* spp. occur in late February and March; additional minor growth flushes occur throughout the growing season until October. Apparently, the degree to which shoot and bark tissues can be affected by *P. citrophthora* and *P. parasitica* is related to this growth pattern.

Rossetti (18) found that citrus plants with young, developing shoots had larger

Table 1. Seasonal changes in lesion development on excised shoot sections of four *Citrus* spp. resulting from inoculation with *Phytophthora citrophthora* or *P. parasitica*

Test pathogen and time period	Length of lesion[z] (mm) resulting from inoculation of: *C. macrophylla*	*C. jambhiri*	*C. aurantium*	*C. volkameriana*
P. citrophthora				
December–February	5 c	8 b	13 c	8 b
March–May	14 ab	11 ab	30 ab	10 b
June–August	19 a	14 a	33 a	16 a
September–November	11 abc	11 ab	20 abc	12 ab
P. parasitica				
December–February	4 b	3 c	3 c	2 c
March–May	12 a	5 abc	15 ab	4 abc
June–August	11 ab	6 ab	17 a	7 a
September–November	10 ab	7 a	9 bc	6 ab

[z]Each value is the average of 120 replicates and represents data collected monthly for 24 consecutive months. For each pathogen, numbers within each column followed by the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Comparative lesion development on excised shoot sections of four *Citrus* spp. resulting from inoculation with *Phytophthora citrophthora* or *P. parasitica*

Citrus sp.	Length of lesion[z] (mm) resulting from inoculation with: *P. citrophthora*	*P. parasitica*
C. aurantium	24 a	9 a
C. jambhiri	11 b	6 b
C. macrophylla	12 b	9 a
C. volkameriana	11 b	5 b

[z]Each value represents data collected monthly for 24 consecutive months. Numbers within each column followed by the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

lesions than those with no growth flush. Broadbent (3) cites an experiment where sweet orange trees were half-pruned and inoculated with *P. citrophthora* on both the pruned and unpruned sides. Lesions were larger on the unpruned side of inoculated trees. When she grafted bark of the susceptible *C. jambhiri* into resistant seedlings of *Poncirus trifoliata* (L.) Raf., or vice versa, resistance of the bark of *Poncirus trifoliata* to *Phytophthora citrophthora* was changed to moderately susceptible in one of three experiments when the only variable was the stage of growth flush. Because both pruning and growth flushes can affect carbohydrate levels, perhaps the degree of colonization of bark and stem tissues by *P. citrophthora* and *P. parasitica* is influenced by their total carbohydrate content, as postulated by Grainger (7,8).

Temporal fluctuations in the extent of colonization of citrus shoot and bark tissues by *P. citrophthora* and *P. parasitica* differ somewhat from seasonal changes in the degree of colonization of deciduous trees by *Phytophthora* spp. Development of lesions on excised apple shoots (2,6,9,16,19) by *P. cactorum* (Lebert & Cohn) Schroet. and *P. cambivora* (Petri) Buisman and walnut stems (15) by *P. citricola* Sawada was usually lowest from November through February, which is comparable to our findings with stem sections of citrus. On the other hand, excised citrus shoot tissue had an extended period of increased susceptibility to invasion by *P. citrophthora* and *P. parasitica* (Tables 1 and 2), whereas the extent of colonization of walnut shoot pieces by *P. citricola* and apple shoot sections by *P. cactorum* and *P. cambivora* increased to a pronounced peak during May, June, or July, then decreased to low susceptibility by late autumn. The reaction of shoot tissue from apple also was influenced by the species of *Phytophthora* used as an inoculant. Maximum development of cankers on apple shoot tissue inoculated with *P. cactorum* occurred during late spring and summer (9), whereas two peaks of activity, one in summer and one in winter, occurred with *P. megasperma* Drechsler and *P. cryptogea* Pethybr. & Lafferty (9), and one peak of winter activity was found with *P. syringae* (Klebahn) Klebahn (19).

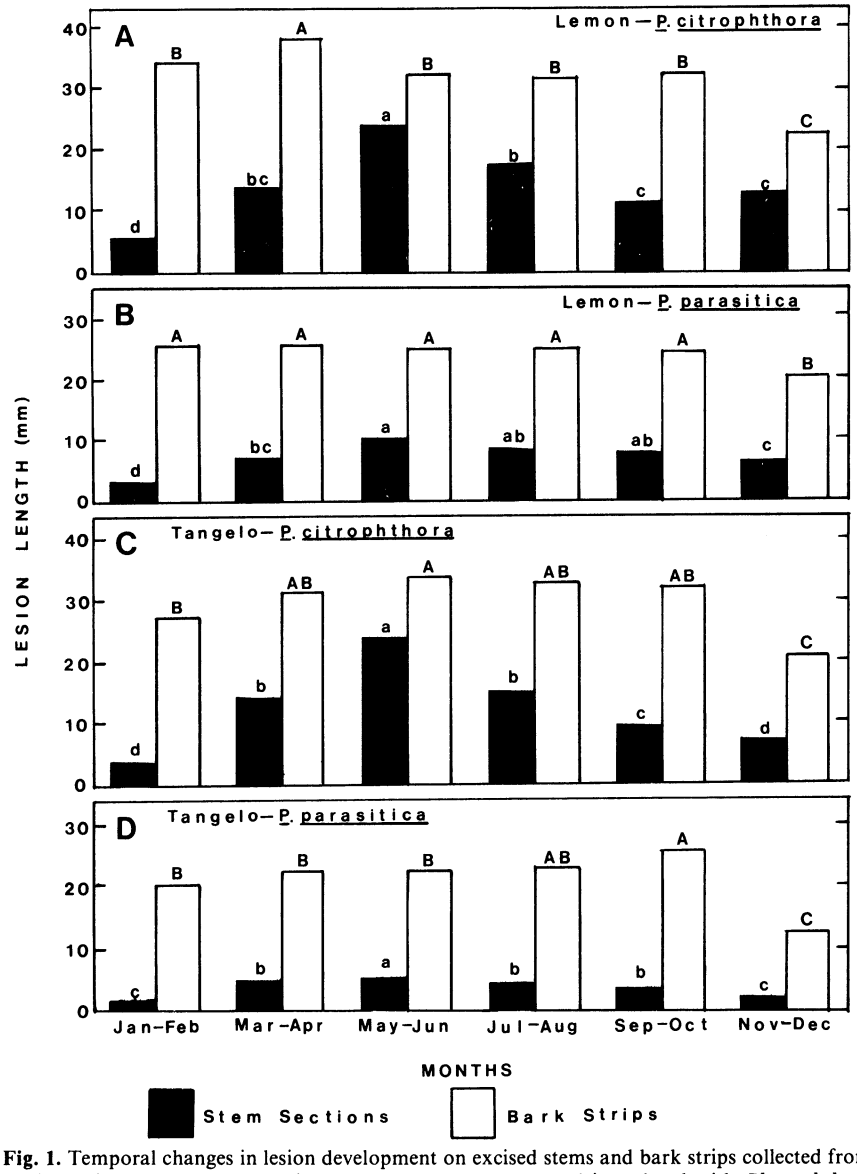

Fig. 1. Temporal changes in lesion development on excised stems and bark strips collected from cultivar Lisbon lemon and cultivar Orlando tangelo trees and inoculated with *Phytophthora citrophthora* or *P. parasitica*. Each value is the average of 80 replicates for 2 mo and represents combined data from 1986 and 1987. Plant material collected from **(A)** Lisbon lemon and inoculated with *P. citrophthora*; **(B)** Lisbon lemon inoculated with *P. parasitica*; **(C)** Orlando tangelo inoculated with *P. citrophthora*; and **(D)** Orlando tangelo inoculated with *P. parasitica*. For A, B, C, or D, lesion lengths for stem sections or for bark strips with the same lowercase or uppercase letters, respectively, do not differ ($P = 0.05$) using Duncan's multiple range test.

Disease severity, as measured by the length of canker on excised phloem tissue inoculated with *P. citrophthora* and *P. parasitica*, does not always correspond to the relative susceptibility of citrus rootstocks and scion cultivars to gummosis in the grove. For example, lesions on excised stems of *C. aurantium* were significantly greater than those on *C. jambhiri*, although field studies show that *C. aurantium* is resistant, whereas *C. jambhiri* is susceptible, to attack by *P. citrophthora* and *P. parasitica* (1). Resistance of excised phloem tissue to colonization by *Phytophthora* may be altered by changes in the physiology of the tissue brought about by physical detachment from the growing plant. Also, direct inoculation of inner phloem tissue only evaluates resistance mechanisms there, once the pathogen has entered host tissue, having bypassed defense mechanisms in tree bark tissue. Finally, different selections of several *Citrus* spp. and citrus hybrids can exhibit markedly different levels of resistance to colonization by *Phytophthora* spp. Tuzcu et al (21) reported significant variation in susceptibility to colonization by *P. citrophthora* among selections of sour orange seedlings. In stem inoculation tests of seedling plants, the extent of colonization of one selection of sour orange was significantly greater than the selection of *C. jambhiri*.

Two sources of phloem tissue, from shoots and from trunks of trees, were used in these studies. Consistently larger lesions developed on bark strips than on shoot sections when inoculated with *P. citrophthora* or *P. parasitica*. This difference in size of cankers produced with the bark strip in comparison to the shoot tissue test is of minor importance in the determination of temporal fluctuations in colonization of citrus phloem tissue by *Phytophthora*. Of more value is the concurrent indication by both tests of a period of minimum colonization of citrus phloem tissue. The bark strip test may be more relevant than the excised shoot test to the susceptibility of trunks of citrus trees in the grove to colonization by *P. citrophthora* and *P. parasitica*, because phloem tissue from tree trunks, not from shoots, is the site of invasion by

the pathogen.

P. citrophthora consistently produced larger cankers than *P. parasitica* on excised shoot and bark tissue of *Citrus* spp. Because only one isolate of each pathogen was used in this study, a comparison of the relative virulence of each *Phytophthora* sp. cannot be adequately addressed. Also, incubation temperatures of 21–24 C used in these tests would be more optimal for growth of *P. citrophthora* than for *P. parasitica*.

Considerable lesion development was evident on phloem tissue collected from tree trunks of lemon and tangelo during November–December, the period of time when the least growth of lesions occurred. Under field conditions, trunk lesions are inactive from November through March. The average ambient air temperature in citrus groves in Arizona from November through March (14 C) may partially explain differences between natural development of lesions in citrus groves and lesion development on excised phloem tissue incubated at 21–24 C.

By itself, apparent temporal variation in susceptibility of phloem tissue to attack by *P. citrophthora* and *P. parasitica* does not offer the possibility of choosing optimum times for application of fungicides for disease control. However, the effect of lower ambient air temperatures in late autumn, winter, and early spring, combined with seasonal variation in susceptibility to disease, may lead to more effective timing of fungicide applications.

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