Seasonal Differences in Susceptibility of Three Citrus Rootstocks to Root Lesions Caused by *Phytophthora citrophthora* and *P. parasitica*

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

ABSTRACT

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Root and shoot segments from Lisbon lemon trees established on sour orange, rough lemon, and volkamer lemon rootstocks were evaluated for seasonal changes in susceptibility to *Phytophthora citrophthora* and *P. parasitica*. Plant material was wounded, inoculated with *P. citrophthora* or *P. parasitica*, then incubated in moist chambers. Root segments of sour orange, rough lemon, and volkamer lemon inoculated with *P. citrophthora* developed shorter lesions during January–February than during July–October. Similarly, lesions on excised root tissue of sour orange inoculated with *P. parasitica* were shorter during January–February than during July–December. The mean annual lesion size on root pieces inoculated with *P. citrophthora* or *P. parasitica* was significantly greater on sour orange than on rough lemon and volkamer lemon. In contrast, the mean annual lesion size on shoot pieces of Lisbon lemon collected from trees established on sour orange, rough lemon, and volkamer lemon rootstocks and inoculated with the same pathogens did not differ. A significant linear relationship was observed between the number of days in December and January with a mean air temperature below 10 C and the length of lesions that developed during January–February on root segments of sour orange inoculated with either pathogen. Seasonal changes in susceptibility of citrus rootstocks to *P. citrophthora* and *P. parasitica* may facilitate timing of disease control measures to coincide with periods when disease development is greatest.

Species of *Phytophthora*, causal agents of gummosis and foot and root rot, are the most important fungal pathogens of citrus (12). In Arizona, a high incidence of root rot has been observed in commercial plantings of citrus. *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian and *P. parasitica* Dastur are isolated routinely from diseased root tissue as well as from rhizosphere soil (17). Root disease is favored by abundant soil moisture, conducive soil temperatures, wounds or other injuries, and the relative susceptibility of rootstock tissue to infection by the pathogens (6).

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Temperature is an environmental parameter that can exert a profound influence on sporangium formation, rootlet infection, and lesion formation on secondary root segments of citrus (16). Temperature also affects the physiological activity of the citrus host. Previous research has demonstrated the existence of seasonal changes in susceptibility of citrus shoot and bark tissue to *P. citrophthora* and *P. parasitica* (14). Knowledge concerning conditions for lesion development on citrus stem and bark tissues could aid in scheduling of fungicide applications or other disease control measures to achieve more effective control of gummosis and foot rot. We do not know if there are similar seasonal changes in the susceptibility of citrus root tissue to these pathogens.

Girton (7) reported that root growth of citrus usually ceases at soil temperatures around or below 10 C. According to Sinclair (19), the cessation of cambium and root growth is most pronounced in the cooler winter climates of California and Arizona. This study was initiated to determine possible seasonal changes in the extent of colonization of three different citrus rootstocks by *P. citrophthora* and *P. parasitica*. Concurrently, we examined the influence of the same rootstocks on susceptibility of lemon (*Citrus limon* (L.) N.L. Burm. 'Lisbon') shoot tissue to these pathogens. Air and soil temperatures were monitored to detect a potential relationship between temperature and the degree of susceptibility of citrus root tissue to *P. citrophthora* and *P. parasitica*, as suggested by Girton's work (7). A partial account of this research has been published (15).

MATERIALS AND METHODS

Excised root inoculations. Segments of roots 6–8 cm in length and 5–10 mm in diameter were collected at monthly intervals from January 1988 through December 1989 and during January and February in 1990 and 1991. Root material was gathered from lemon trees established in 1973 on rough lemon (*C. jambhiri* Lush.), sour orange (*C. aurantium* L.), and volkamer lemon (*C. volkameriana* (Pasq.) Tan.) rootstocks. Roots were collected from a group of eight trees of each tested citrus rootstock at the Yuma Agricultural Center and stored in plastic bags until inoculation later the same day.

During the middle of each month, 40 replicate root segments were collected at random from the eight trees of each *Citrus* sp., washed in tap water to remove soil, then inoculated with either *P. citrophthora* (isolate C-7-S) or *P. parasitica* (isolate C-2-C), both obtained from citrus trees in Arizona. Root sections were not surface-sterilized before inoculation. Pathogens were grown on V8 juice agar for 7 days at 24 C. Agar disks 6 mm in diameter were removed from the edge of actively growing cultures and placed directly into 6-mm-diameter wounds in the middle of excised root pieces. Phloem tissue was completely removed at the wound site to an approximate depth of 1 mm, leaving exposed cambium. Root segments were incubated for 4 days at 24 C in moist chambers, after which the lengths of resulting lesions were recorded. Noninoculated controls were prepared by placing agar disks without mycelium into wounds of excised roots.

Excised shoot inoculations. Woody segments of lemon shoots 6 cm in length and 5–10 mm in diameter were collected at monthly intervals from January through December 1989. Forty replicate shoot segments of lemon were collected at random from the eight trees on rough lemon, sour orange, or volkamer lemon rootstock, then inoculated and incubated as described for excised roots.

To confirm that lesion development resulted from infection by the appropriate species of *Phytophthora*, serial pieces of necrotic tissue 2 mm in length from root and shoot segments were plated onto 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) (11). Plates were incubated at 24 C in darkness, and the pieces of tissue were examined microscopically to identify those from which the species of *Phytophthora* emerged.

All data were subjected to analysis of variance. Duncan's multiple range test (8) was used to determine differences between seasonal development of lesions as well as differences in lesion development among tested species of citrus. All data were processed with the MSTAT-C statistical software package (18).

Effect of temperature on lesion development. To determine the possible correlation of air or soil temperature with subsequent changes in lesion development, these environmental parameters were recorded by the Arizona Meteorological Network station located at the Yuma Mesa Agricultural Center. Soil temperature was recorded at the 10-cm depth. Linear regression analysis was used to detect a potential relationship between temperature and length of lesions that developed on inoculated root segments.

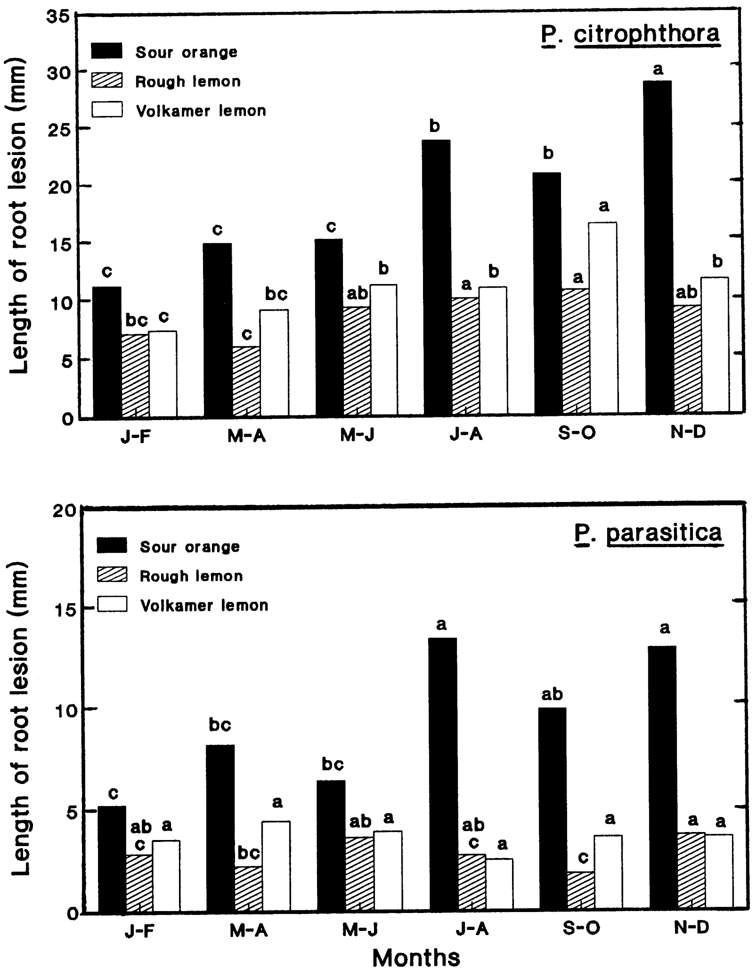

Fig. 1. Bimonthly changes in lesion development on excised root segments collected from Lisbon lemon trees on three different rootstocks and inoculated with *Phytophthora citrophthora* or *P. parasitica*. Each value is the average of 80 root segments for each 2-mo period and represents combined data from 1988 and 1989. For each rootstock-pathogen combination, values with the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

Excised root and shoot inoculations. Lesions that developed on excised root segments of sour orange, rough lemon, or volkamer lemon inoculated with *P. citrophthora* were significantly shorter during January–February than during July–August or September–October (Fig. 1). Similarly, lesions on roots of sour orange and volkamer lemon inoculated with *P. citrophthora* were smaller during January–February than during November–December.

Root pieces of sour orange inoculated with *P. parasitica* had shorter lesions during January–February than during July–August, September–October, and November–December (Fig. 1). On the other hand, lesions formed on root segments of rough lemon and volkamer lemon inoculated with *P. parasitica* in Janurary–February did not differ from those formed during any other 2-mo period.

The mean annual size of lesions on root pieces inoculated with *P. citrophthora* or *P. parasitica* was significantly greater on sour orange than than on rough lemon or volkamer lemon (Table 1). On the other hand, the mean annual size of lesions on shoot pieces of Lisbon lemon collected from trees established on sour orange, rough lemon, and volkamer lemon rootstocks and inoculated with the same pathogens did not differ.

When apparently healthy and necrotic tissues from root and shoot segments were plated onto PARP selective agar, mycelium of *Phytophthora* routinely formed on necrotic tissue but not on healthy tissue.

Effect of temperature on lesion development. There was a significant linear

Table 1. Annual mean lesion development on excised root and shoot tissues of lemon trees resulting from inoculation with *Phytophthora citrophthora* or *P. parasitica*

	Lesion length[z]	
Rootstock	*P. citrophthora*	*P. parasitica*
Root segments		
Sour orange	22 a	9 a
Rough lemon	11 b	3 b
Volkamer lemon	13 b	4 b
Shoot segments		
Sour orange	11 a	3 a
Rough lemon	11 a	4 a
Volkamer lemon	9 a	3 a

[z] Each value is the average of 20 root or shoot segments inoculated each month for 12 consecutive months. For either root or shoot segments, numbers in each column followed by the same letter differ ($P = 0.05$) according to Duncan's multiple range test.

relationship between the number of days in December–January with a mean air temperature below 10 C and the length of lesions that developed during January–February on root segments of sour orange inoculated with *P. citrophthora* or *P. parasitica* (Fig. 2). In contrast, no significant relationship between temperature and lesion development was detected on root pieces of rough lemon and volkamer lemon inoculated with either pathogen.

In a comparison of air and soil temperatures from November through March of 1988–1989, 1989–1990, and 1990–1991, the mean daily air temperature did not deviate more than 1.5 C from the mean daily soil temperature at a depth of 10 cm in the citrus production areas of Arizona. Monthly averages of air and soil temperatures were within 0.5 C of each other.

DISCUSSION

Seasonal changes in susceptibility of root tissue of sour orange and rough lemon to *P. citrophthora* and *P. parasitica* and of root tissue of volkamer lemon to *P. citrophthora* have been demonstrated. Citrus cultivars stop growing during the winter months in subtropical regions but maintain minimal levels of water transport and starch consumption (1). Dormancy in citrus trees involves the cessation of growth of roots as well as of cambium tissue of shoots and branches (4,7). In our studies, the extent of lesion development on root tissue of sour orange inoculated with *P. citrophthora* or *P. parasitica* was at minimum levels during January–February, a period of dormancy for citrus in Arizona. Likewise, the lesions on root tissue of rough lemon and volkamer lemon inoculated with *P. citrophthora* were shortest during this same period.

The degree to which dormancy is exhibited in winter is partially dependent on the species of citrus. Sour orange trees are induced more easily into dormancy by cool temperatures than are lemon trees (19). This may partially explain the significant decrease in length of lesions on root pieces of sour orange, but not of rough lemon, that occurred from November–December to January–February when inoculated with *P. citrophthora* and *P. parasitica*.

The average annual length of lesions on root pieces inoculated with either *P. citrophthora* or *P. parasitica* was significantly greater on sour orange than on rough lemon and volkamer lemon. However, the average annual length of lesions developing on shoot tissue of Lisbon lemon from trees on each rootstock did not differ, indicating that rootstock did not affect the susceptibility of lemon scion tissue to *Phytophthora* spp. When shoot tissue was inoculated with either *P. citrophthora* or *P. parasitica* (14), lesions on excised shoot pieces of sour orange trees were significantly longer than those on rough lemon and volkamer lemon. The inherent relative susceptibility of different species of citrus to lesion development appears to remain the same regardless of whether root or shoot phloem tissue is concerned.

In another comparison of disease development on shoot tissue vs. root tissue, minimum development of lesions on both shoot (14) and root tissue of sour orange inoculated with *P. citrophthora* and *P. parasitica* was during the winter. However, lesion development on shoot tissue was significantly greater than these minimum levels during March–August, whereas significantly greater lesion development from minimal winter levels on root tissues of sour orange was during July–December. Additionally, lesion development on shoot and root tissue of volkamer lemon inoculated with *P. citrophthora* was minimal during the winter, whereas significantly greater levels of disease development were recorded during June–August and May–December for shoot (14) and root tissue, respectively. In these examples, minimum disease development for both shoot and root tissue was during the winter months, whereas periods of maximum disease development for shoot and root tissue did not coincide. A partial explanation may involve the physiological status of these tissues during the year. During the winter months, when citrus cultivars are not actively growing in subtropical regions, lesion development on the "dormant" shoot and root tissue of sour orange and volkamer lemon is at minimal levels. During the period of active tree growth, on the other hand, root flushes occur after shoot flushes (13), suggesting a different physiological status for citrus shoot and root tissues at any particular time during active growth. Such temporal differences in physiological activity may partially account for the dissimilar duration of maximum disease development observed for shoot tissue and for root tissue of sour orange and volkamer lemon.

Conflicting reports exist concerning the relative susceptibility of sour orange and lemon rootstocks to Phytophthora root rot. In a recent study (9), the severity of root rot caused by *P. parasitica* was significantly greater on sour orange than on volkamer lemon rootstock, which agrees with our findings. In contrast, other researchers have found sour orange to be highly tolerant, volkamer lemon to be susceptible, and rough lemon to be very susceptible to Phytophthora root rot (1). Possible explanations for differing conclusions on relative susceptibility of sour orange, rough lemon, and volkamer lemon may involve the methods used for introducing the pathogens to the soil or root tissue, methods utilized for rating disease severity and variability in the degree of virulence of isolates used in the different studies. Also, resistance of excised phloem tissue to colonization by *Phytophthora* may be modified by changes in the physiology of the tissue brought about by physical detachment from the growing plant. In addition, direct inoculation of inner phloem tissue only evaluates resistance mechanisms operative once the pathogen has entered host tissue, bypassing defense mechanisms present in the outer phloem tissue of the root. 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 (20) reported significant variation in susceptibility to colonization by *P. citrophthora* among

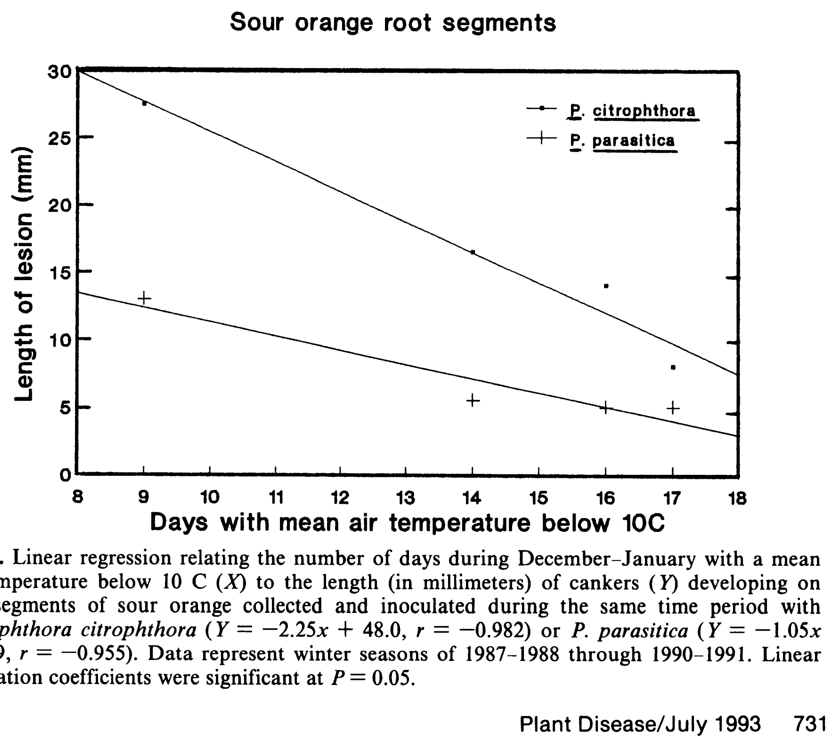

Fig. 2. Linear regression relating the number of days during December–January with a mean air temperature below 10 C (X) to the length (in millimeters) of cankers (Y) developing on root segments of sour orange collected and inoculated during the same time period with *Phytophthora citrophthora* ($Y = -2.25x + 48.0$, $r = -0.982$) or *P. parasitica* ($Y = -1.05x + 21.9$, $r = -0.955$). Data represent winter seasons of 1987–1988 through 1990–1991. Linear correlation coefficients were significant at $P = 0.05$.

selections of sour orange seedlings. In shoot inoculation tests of seedling plants, the extent of colonization of one selection of sour orange was significantly greater than that of a selection of rough lemon.

For citrus, the length of the winter dormant period is related to the air temperature during the winter months (19). According to Cooper and Peynado (5), an increase in the number of days during the winter in which the daily air temperature fell below 10 C induced greater tree dormancy and maintained it for a longer period. Our findings with sour orange are in agreement with these earlier studies and demonstrate a significant linear relationship between the number of days in December–January with a mean air temperature below 10 C and the length of lesions that developed on excised roots inoculated with *P. citrophthora* and *P. parasitica*.

Since the environment of the root is soil and not air, perhaps we should compare root or tree dormancy to soil temperature rather than air temperature. In Arizona, the mean daily air temperature did not deviate more than 1.5 C from the mean daily soil temperature at a depth of 10 cm in citrus production areas. Monthly averages of air and soil temperatures were within 0.5 C of each other. Similarities of mean daily air and soil temperatures also were reported in citrus orchards in California, Florida, and Texas (2,3,10). The similarity between air and soil temperatures suggests that the number of days in which either parameter is below 10 C could be a potentially useful indicator of the possible susceptibility of sour orange root tissue to colonization by *P. citrophthora* or *P. parasitica* during January–February.

The seasonally low susceptibility of sour orange to *P. citrophthora* and *P. parasitica* during January–February, as well as the low susceptibility of rough lemon and volkamer lemon to *P. citrophthora* during the same period, coincides with the occurrence of temperatures highly restrictive to sporulation of both pathogens (16). The simultaneous occurrence of low susceptibility to colonization by *P. citrophthora* and *P. parasitica* with reduced sporulation of the pathogens suggests that fungicides or other disease control measures may not be as necessary or effective during winter dormancy as during other times of the year when both rootstock susceptibility and temperature are more conducive to disease development.

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LITERATURE CITED

1. Anonymous. 1991. Integrated Pest Management for Citrus. Publ. 3303. University of California Division of Agriculture and Natural Resources, Oakland.
2. Bliss, D. E. 1944. Air and soil temperature in a California orchard. Soil Sci. 58:259-274.
3. Bliss, D. E., Moore, D. C., and Bream, C. E. 1942. Air and soil temperatures in a California date garden. Soil Sci. 55:55-64.
4. Cooper, W. C. 1959. Cold hardiness in citrus as related to dormancy. Proc. Fla. State Hortic. Soc. 72:61-66.
5. Cooper, W. C., and Peynado, A. 1959. Winter temperature of three citrus areas as related to dormancy and freeze injury of citrus trees. Proc. Am. Soc. Hortic. Sci. 74:333-347.
6. Fawcett, H. S. 1936. Citrus Diseases and Their Control. McGraw-Hill, New York.
7. Girton, R. E. 1927. The growth of citrus seedlings as influenced by environmental factors. Univ. Calif. Publ. Agric. Sci. 5:83-117.
8. Gomez, K. A., and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley & Sons, New York.
9. Graham, J. H. 1990. Evaluation of tolerance of citrus rootstocks to Phytophthora root rot in chlamydospore-infested soil. Plant Dis. 74:743-746.
10. Halma, F. F., and Compton, C. 1936. Growth of citrus trees. Proc. Am. Soc. Hortic. Sci. 34:80-83.
11. Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.
12. Klotz, L. J. 1978. Fungal, bacterial and nonparasitic diseases and injuries originating in the seedbed, and nursery orchard. Pages 1-66 in: The Citrus Industry. Vol. 4, Crop Protection. W. Reuther, E. C. Calavan, and G. F. Carman, eds. University of California Agricultural Sciences Publications, Richmond.
13. Lutz, A., and Menge, J. 1986. Seasonal growth of citrus feeder roots and shoots and rhizosphere population fluctuations of *Phytophthora parasitica*. (Abstr.) Phytopathology 76:1093-1094.
14. Matheron, M. E., and Matejka, J. C. 1989. Temporal changes in susceptibility of citrus phloem tissue to colonization by *Phytophthora citrophthora* and *P. parasitica*. Plant Dis. 73:408-411.
15. Matheron, M. E., and Matejka, J. C. 1990. Seasonal variation in susceptibility of citrus root tissue to *Phytophthora citrophthora* and *P. parasitica*. (Abstr.) Phytopathology 80:891.
16. Matheron, M. E., and Matejka, J. C. 1992. Effects of temperature on sporulation and growth of *Phytophthora citrophthora* and *P. parasitica* and development of foot and root rot on citrus. Plant Dis. 76:1103-1109.
17. Matheron, M., Matejka, J., and Bacon, D. 1988. Distribution of two species of *Phytophthora* within the citrus acreage in Arizona. Pages 19-20 in: 1988 Citrus Report. Univ. Ariz. Coll. Agric. Ser. P-76.
18. MSTAT Development Team. 1988. MSTAT-C, a Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments. Michigan State University, East Lansing.
19. Sinclair, W. B. 1984. The Biochemistry and Physiology of the Lemon and Other Citrus Fruits. Publ. 3306. University of California Division of Agriculture and Natural Resources, Oakland.
20. Tuzcu, O., Cinar, A., Goksedef, M. O., Ozsan, M., and Bicici, M. 1984. Resistance of citrus rootstocks to *Phytophthora citrophthora* during winter dormancy. Plant Dis. 68:502-505.