

Root Regeneration and Tolerance of Citrus Rootstocks to Root Rot Caused by *Phytophthora nicotianae*

J. H. Graham

Professor, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred 33850. Florida Agricultural Experiment Station Journal Series No. R-04102.

This research was supported in part by the National Science Foundation (BSR911824) and the Florida Citrus Production Research Advisory Council.

I thank W. S. Castle, L. Lee Hill, and O. Lee for use of the rootstock trial sampled in this study, and N. H. Timmer, D. L. Drouillard, and M. C. Bryan for technical assistance. I also thank D. M. Eissenstat, D. J. Mitchell, L. W. Timmer, L. W. Duncan, and W. S. Castle for helpful discussions and reviews.

Accepted for publication 21 September 1994.

ABSTRACT

Graham, J. H. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. *Phytopathology* 85:111-117.

Citrus rootstock cultivars varying in tolerance to fibrous root rot were evaluated for their ability to regenerate roots in the presence of potentially damaging populations of *Phytophthora nicotianae*. In chlamydo-spore-infested soils in the greenhouse, tolerance to root rot generally was exhibited as more rapid growth of undisturbed and pruned roots of the rootstocks trifoliolate orange and Swingle citrumelo compared with Carrizo citrange, sour orange, Ridge Pineapple sweet orange, and Cleopatra mandarin. The capacity for regeneration of Volkamer lemon roots in the presence of *P. nicotianae* varied with experiments and was associated with differences in greenhouse temperature conditions. In a field trial with damaging populations of *P. nicotianae*, growth rates of regenerating

roots of trifoliolate orange and Swingle citrumelo were greater than for intolerant rootstocks, Carrizo citrange, sour orange, and Cleopatra mandarin. Regenerating roots of Volkamer lemon were infected and supported populations of *P. nicotianae* equivalent to intolerant rootstocks late in the season (October–December). Early in the season (April–June) Volkamer lemon roots apparently were tolerant because infection remained low and few propagules were detected until October. Young roots of all rootstocks supported higher levels of infection and rhizosphere populations of *P. nicotianae* than mixed-aged roots. In spite of comparable root infection, trifoliolate orange had lower pathogen populations on regenerating roots and mixed-age populations of roots than intolerant rootstocks. Tolerance to root rot may be expressed as a greater capacity to regenerate roots under certain environmental conditions (e.g., Volkamer lemon) or the limitation of conversion of infection to propagules (e.g., trifoliolate orange).

Phytophthora nicotianae Breda de Haan (syn. *P. parasitica* Dastur.) infects the root cortex and causes a decay of fibrous roots of all commercial citrus rootstocks in Florida (2,10,11). Root rot can be especially severe in infested soils of citrus nurseries (21). In orchards, damage of fibrous roots causes tree decline and yield losses (14,15,17). With mature trees, the production of new fibrous roots apparently does not keep pace with root death, and the tree is unable to maintain adequate water and mineral uptake to sustain maximum fruit production (14).

Rootstocks are referred to as tolerant rather than resistant because fibrous roots become infected under artificial inoculations (5,6,10) and in infested nursery and orchard soils (2). Tolerance was previously defined as the condition in which plants are infected but show little or no net root loss either because infected roots do not rot or because root mass density is maintained by root regeneration (10). Recent greenhouse evaluations with chlamydo-spore inoculum in soil revealed that most commercial rootstocks in Florida were intolerant to root rot caused by *P. nicotianae* (10). In contrast, trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) and its hybrid, Swingle citrumelo (*Citrus paradisi* Macf. × *P. trifoliata*), were considered tolerant. Volkamer lemon (*C. volkameriana* Pasq.) was judged as intermediate in tolerance. In greenhouse inoculations, tolerance of trifoliolate orange and Swingle citrumelo appeared to be related to the capability of these rootstocks to regenerate roots in the presence of *P. nicotianae* (10).

In Florida rootstock trials, trifoliolate orange and Swingle citrumelo supported lower soil populations of *P. nicotianae*, whereas intolerant rootstocks had higher population densities in the soil of the root zone (2), here defined as rhizosphere populations.

When population density of the pathogen exceeds 10–15 propagules per cubic centimeter of soil, fungicide treatments appear to be beneficial in orchards on intolerant rootstocks (15,17,18). Thus, rootstock is a major consideration in the interpretation of rhizosphere populations of *P. nicotianae* and the need to treat a bearing citrus orchard to control fibrous root rot (17). It is still unclear whether rootstocks that support lower populations suffer less direct root loss because their roots are resistant to infection or whether tolerant rootstocks have a greater capacity to regenerate roots, or if both mechanisms are operative.

The purpose of the greenhouse and field studies presented here was to determine whether tolerance to *Phytophthora* root rot is due to the ability of citrus rootstocks to regenerate roots after root pruning, the ability to limit infection of roots and production of propagules by *P. nicotianae*, or both.

MATERIALS AND METHODS

Greenhouse evaluations. Citrus rootstocks defined as tolerant, moderately tolerant, or intolerant to *Phytophthora* root rot, according to a previous study (10), were evaluated. The ability of rootstock seedlings to regenerate roots was measured by pruning the fibrous roots from one side of the tap root and planting the seedlings into noninfested soils or soils infested with chlamydo-spores of *P. nicotianae*. The isolate of *P. nicotianae* (R-1) and the methods of soil infestation and plant inoculation were previously described (10). Briefly, pasteurized soil was mixed with culture-produced chlamydo-spores to a density of 10–50 propagules of *P. nicotianae* per cubic centimeter of Candler fine sand soil (Typic quartzipsammments, pH 6.8, and 1.0% organic matter). Propagule levels were determined by plating triplicate 1-cm³ soil samples (five plates per sample) on a selective medium containing pimaricin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol (PARPH) that was modified as previously described (20).

Seeds of the following rootstocks were obtained from registered seed source trees of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry: trifoliolate orange, Ridge Pineapple sweet orange (*Citrus sinensis* (L.) Osbeck), Carrizo citrange (*C. sinensis* × *P. trifoliata*), Swingle citrumelo, sour orange (*C. aurantium* L.), Cleopatra mandarin (*C. reticulata* Blanco), and Volkamer lemon. Seeds were sown in 150-cm³ containers (Stuewe & Sons, Inc., Corvallis, OR) containing Promix (Premier Brands, Inc.) and seedlings were fertilized weekly with Peter's 20-10-20 Peat-lite Special (The Scotts Company, Marysville, OH). Nine-month-old seedlings of each rootstock were selected for uniformity of root system size, within and among cultivars, by inspection of roots after they were washed free of growing medium.

In the first experiment, seedlings of trifoliolate orange (tolerant), Swingle citrumelo (tolerant), Cleopatra mandarin (intolerant), and Volkamer lemon (intermediate) were root pruned and a plastic 2-mm-mesh screen was fixed to the tap root with wire ties to delineate the pruned and undisturbed side of the seedling root system at harvest. The seedlings with the attached screen were transplanted into 15-cm-diameter clay pots containing noninfested soil or soil infested with 20 ± 15 propagules of *P. nicotianae* per cubic centimeter soil. After transplant, seedlings were flooded every 5–10 days to maintain conducive conditions for pathogen activity as previously described (10). Two trials were conducted December–March 1990 and November–March 1992. Greenhouse conditions fluctuated diurnally, 23–30 C and 60–100% RH, during the trials.

At 30, 64, 92, and 120 days after transplant, eight replicate seedlings of each rootstock from infested and noninfested soil treatments were harvested. Before each harvest, 45-cm³ soil samples were taken with a no. 15 cork borer (2.4-cm diameter × 10-cm deep) from the outside edge of each pot adjacent to the pruned and undisturbed sides of the root system. Propagule density of *P. nicotianae* in each soil sample was determined by plating on PARPH medium. All fibrous roots (< 2 mm diameter) on pruned and undisturbed sides of the tap root, as delineated by the mesh screen, were removed, dried (70 C, 24 h), and weighed. Propagules of *P. nicotianae* were expressed as colony-forming units per cubic centimeter soil or per milligram root weight. The results of both trials were similar based on the effects of root pruning and rootstock; the data from the second trial (November–March 1992) are presented.

Another experiment was conducted with infested soil only (13.0 ± 4 propagules per cubic centimeter soil) and five rootstocks: Swingle citrumelo (tolerant), Volkamer lemon (intermediate), Carrizo citrange (intolerant), sour orange (intolerant), and Ridge Pineapple sweet orange (intolerant). Inoculation methods, root pruning treatments, and growing conditions were the same as in the previous experiment except greenhouse conditions were warmer, ranging from 25 to 35 C, and 10 replicate seedlings were harvested at 29, 55, 84, and 112 days. Two trials were conducted March–May 1989 and January–April 1991. Root growth and pathogen populations on the pruned and undisturbed sides of the root system were determined as described above. In addition to measurement of soil populations of *P. nicotianae* in the second trial, infection in roots was quantified by enzyme-linked immunosorbent assay (ELISA) (Root D kit, Agri-Diagnostics, Cinnaminson, NJ) of 30-mg samples of dried and finely ground fibrous roots as previously described (7,19). Infection was expressed as log nanogram *P. nicotianae* protein units per milligram dried root. The trial was repeated with similar results; the second trial (January–April 1991) is presented because ELISA analysis was included.

In the two experiments, root growth of undisturbed and pruned roots were regressed over the four harvests for each replication and the slopes of linear regressions subjected to analysis of variance using Proc REG and Proc GLM (SAS Institute, Cary, NC). Means of the slopes for rootstock and pruning treatments were compared using individual Student's *t* tests and Duncan's multiple range test (DMRT). Because there was a nonsignificant interaction with harvest date, soil populations of *P. nicotianae*,

propagules per root weight, and root infection data were averaged over the four harvests with each harvest date as a replication. Propagules per milligram root weight were analyzed after log transformation to stabilize variance. Paired Student's *t* tests or DMRTs were used to compare the effects of root pruning and rootstock.

Field evaluation. A citrus rootstock trial with 17-yr-old trees of Valencia sweet orange (*C. sinensis*) on 12 rootstocks was located near St. Cloud in Osceola County, Florida. The rootstock trial was planted on a site with several soil types typical of coastal Flatwoods areas of Florida: Pomona, Immokalee, Myakka, and St. Johns series were the most prevalent. The six rootstocks examined were sour orange, Cleopatra mandarin, Carrizo citrange (intolerant), Volkamer lemon (intermediate), trifoliolate orange (tolerant), and Swingle citrumelo (tolerant). Rootstocks were arranged in a randomized complete block design with four replications of each rootstock containing two trees. Samples were taken from the two trees in each replicate block (eight trees per rootstock).

Root containers were constructed of 2-mm-mesh plastic screen in the form of a cylinder 3 cm in diameter and 26.5 cm in length. In February 1991, the containers were inserted into holes 3.5 cm in diameter and 25 cm deep located at one-third of the distance from trunk to the canopy dripline within the zone wetted by microsprinkler irrigation. The cores of soil removed to create the holes under each tree were combined, sieved to remove roots, and soil replaced into the root containers. There were 10 containers per tree and trees were sampled at 74 (April), 130 (June), 186 (August), 260 (November), and 324 (January) days after containers were installed. At each sampling time, two root containers were extracted (disturbed soil) and two soil cores of equal dimensions were removed from adjacent undisturbed soil with a sampling tube. The cores from either disturbed or undisturbed soil were bulked together for determination of root mass density (mg/cm³ soil) and propagule density of *P. nicotianae*. Dried roots were ground to pass through a 40-mesh screen and 100-mg samples used to determine pathogen infection by ELISA as described above. Analyses and statistical evaluations of the data were as described for the greenhouse experiments.

The trees were left undisturbed during 1992 to avoid excessive root damage that might alter rootstock responses. In February 1993, the containers were reinstalled on the same trees and sampled at 83 (May), 127 (June), 197 (August), and 295 (December) days. Rootstock responses were similar to the 1991 trial, but several trees on Carrizo citrange were lost due to citrus blight. For this reason, the results of the 1991 trial are presented.

RESULTS

Greenhouse evaluations. In noninfested soil, growth rate of undisturbed roots (milligrams per day) was greatest for Volkamer lemon (26.2) and Swingle citrumelo (22.8) and least for Cleopatra mandarin (12.0) and trifoliolate orange (8.7) (Fig. 1A). Growth of fibrous roots from the tap root commenced between 30 and 64 days after pruning and the patterns of root growth among rootstocks followed those of undisturbed roots (Fig. 1A and B). Infestation of soil with *P. nicotianae* reduced ($P < 0.001$) root growth rate of undisturbed and pruned roots of all rootstocks. Growth rate of undisturbed roots and regenerating roots in the presence of the pathogen was greater for trifoliolate orange and Swingle citrumelo than for Volkamer lemon and Cleopatra mandarin (Fig. 1C and D; Table 1). For Cleopatra mandarin, fibrous root weight was no greater at 120 days than at time of inoculation (Fig. 1D).

Populations of *P. nicotianae* in soil were similar in association with undisturbed roots and young, regenerating roots (Table 1). In contrast, populations of the pathogen per root weight were higher on pruned roots irrespective of rootstock. There was no rootstock effect on soil propagules or propagules per root weight.

In the second greenhouse experiment, growth rates of pruned roots in the presence of *P. nicotianae* were higher for Volkamer lemon and Swingle citrumelo than for sweet orange, sour orange,

and Carrizo citrange, but no differences among rootstocks were observed for undisturbed roots (Table 2). *P. nicotianae* populations in soil tended to be higher on undisturbed roots than when density of roots was reduced by pruning, except for Swingle citrumelo and Volkamer lemon, which had the greatest root regeneration among rootstocks (Fig. 2, Table 2). In contrast to experiment 1, regenerating roots did not support higher populations than undisturbed roots on a root mass basis (Tables 1 and 2). As in experiment 1, few significant effects of rootstock on populations of *P. nicotianae* for pruned or undisturbed roots were detected (Tables 1 and 2).

Infection of undisturbed roots as measured by ELISA was highly variable among sampling dates (data not shown). Average *Phytophthora* protein levels of *P. nicotianae* over the 112-day sampling period for undisturbed roots and at the last harvest date for pruned roots were similar for all rootstocks except Swingle citrumelo, which had lower infection levels (Table 2).

Field evaluation. Growth rates of mixed-age roots in undisturbed soil were slightly negative for all six rootstocks from April to January (Fig. 3, Table 3). This was primarily attributable to the decrease in root mass density from April to June after the spring flush of roots and during the months of greatest rainfall

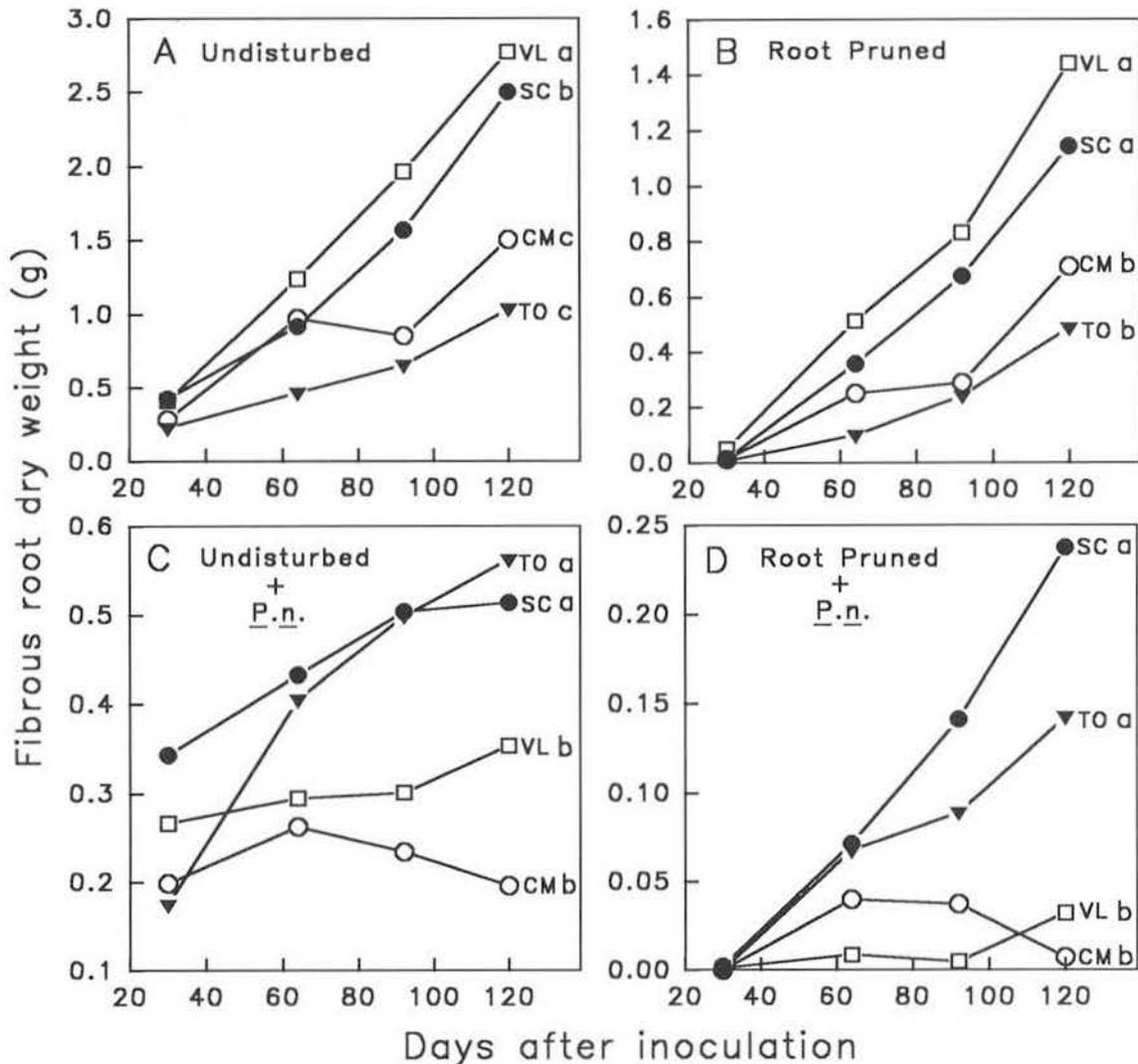


Fig. 1. Growth of undisturbed and pruned roots in the presence and absence of *Phytophthora nicotianae* for seedlings of four citrus rootstocks varying in tolerance to root rot. VL = Volkamer lemon, SC = Swingle citrumelo, CM = Cleopatra mandarin, and TO = trifoliate orange. Root growth curves followed by unlike letters have significantly different slopes (root growth rate) at $P < 0.05$ according to Duncan's multiple range test.

TABLE 1. Rate of growth of undisturbed and pruned roots in soil infested with *Phytophthora nicotianae* and the rhizosphere populations per soil volume or root mass for seedlings of four citrus rootstocks varying in tolerance to root rot (experiment 1)

Rootstock	Root growth rate ^w (mg/day)		Propagules per soil volume ^x (cfu/cm ³)		Propagules per root weight ^t (cfu/mg)	
	Undisturbed ^y	Pruned ^y	Undisturbed	Pruned	Undisturbed	Pruned
Trifoliate orange	4.6 a	1.5 a ^z	16.9 a	13.3 a	2.6 a	36.2 a ^z
Swingle citrumelo	2.3 a	2.6 a	15.8 a	11.4 a	1.6 a	9.4 a*
Volkamer lemon	1.6 b	0.3 b	25.9 a	14.2 a	1.6 a	30.5 a*
Cleopatra mandarin	0.7 b	0.2 b	23.7 a	6.6 a*	3.2 a	60.3 a*

^w Means (n = 8) in columns followed by unlike letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

^x Values are the means of four harvests at 30, 64, 92, and 120 days after inoculation.

^y Pruned refers to the ability of the rootstocks to regenerate roots after pruning the fibrous roots from one side of the tap root while undisturbed refers to the fibrous roots on the other side of the tap root left intact (see Materials and Methods).

^z * denotes pruned significantly different from undisturbed at $P \leq 0.05$ according to a paired Student's *t* test.

TABLE 2. Rate of growth of undisturbed and pruned roots in soil infested with *Phytophthora nicotianae* and the rhizosphere populations and root infection levels for seedlings of six citrus rootstocks varying in tolerance to root rot (experiment 2)

Rootstock	Root growth rate ^v (mg/day)		Propagules per soil volume ^w (cfu/cm ³)		Propagules per root weight ^w (cfu/mg)		ELISA ^w Log ng protein units/g root	
	Undisturbed ^x	Pruned ^x	Undisturbed	Pruned	Undisturbed	Pruned	Undisturbed	Pruned ^y
Swingle citrumelo	4.7 a	3.4 a	10.9 c	6.4 b	0.9 b	2.4 b	2.8 b	2.5 b
Volkamer lemon	5.9 a	4.3 a	35.3 ab	46.5 a	3.0 ab	20.1 a	3.8 a	4.6 a
Carrizo citrange	3.8 a	1.3 b	40.4 a	4.1 b ^z	12.3 a	0.8 b*	4.3 a	4.8 a
Sweet orange	2.7 a	2.0 b	17.9 bc	5.7 b*	1.5 b	1.4 b	3.7 a	4.4 a
Sour orange	3.2 a	1.6 b	16.6 bc	5.6 b*	4.1 ab	2.9 ab	4.3 a	4.3 a

^v Means (n = 10) in columns followed by unlike letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

^w Values are the means of four harvests at 29, 55, 84, and 112 days after inoculation.

^x Pruned refers to the ability of the rootstocks to regenerate roots after pruning the fibrous roots from one side of the tap root while undisturbed refers to the fibrous roots on the other side of the tap root left intact (see Materials and Methods).

^y Values for 112 day harvest only.

^z* denotes pruned significantly different from undisturbed at $P \leq 0.05$ according to a paired Student's *t* test.

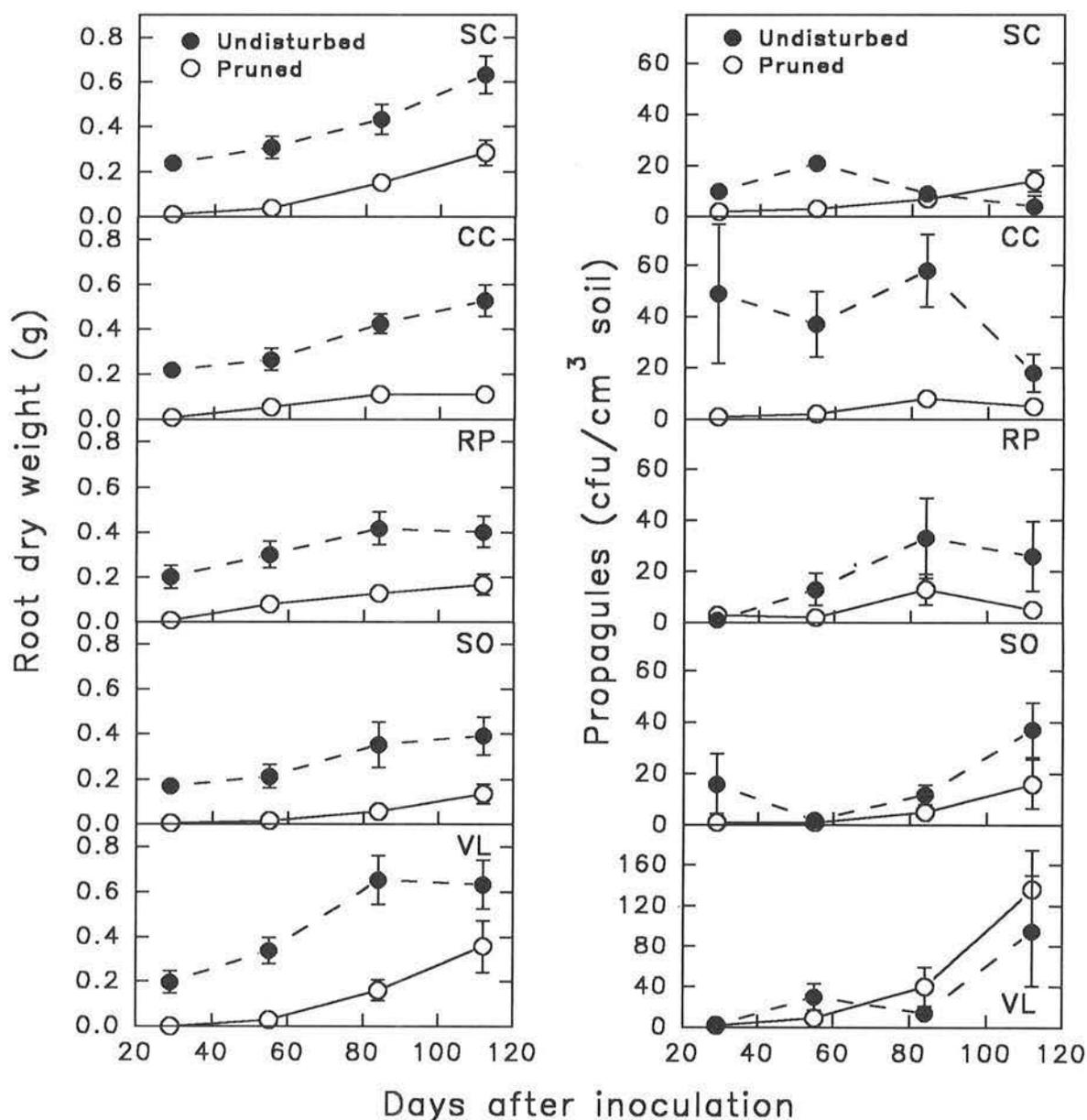


Fig. 2. Growth of undisturbed and pruned roots in the presence of *Phytophthora nicotianae* and the soil populations of the fungus for seedlings of six citrus rootstocks varying in tolerance to root rot. SC = Swingle citrumelo, CC = Carrizo citrange, RP = Ridge Pineapple sweet orange, SO = sour orange, and VL = Volkamer lemon. Points represent means of eight replications \pm one standard deviation.

(Fig. 3). The rate of decline in root mass density in undisturbed soil did not differ significantly among rootstocks (Table 3).

For all rootstocks except Cleopatra mandarin, growth rate of regenerating roots in disturbed soil was greater than the growth rate of the respective, mixed-age roots in undisturbed soil (Fig. 3,

Table 3). Trifoliolate orange and Swingle citrumelo had greater root growth rate in disturbed soil than Carrizo citrange, sour orange, and Cleopatra mandarin, whereas growth rate of regenerating roots of Volkamer lemon was intermediate between the two groups of rootstocks (Table 3). Trifoliolate orange, Swingle

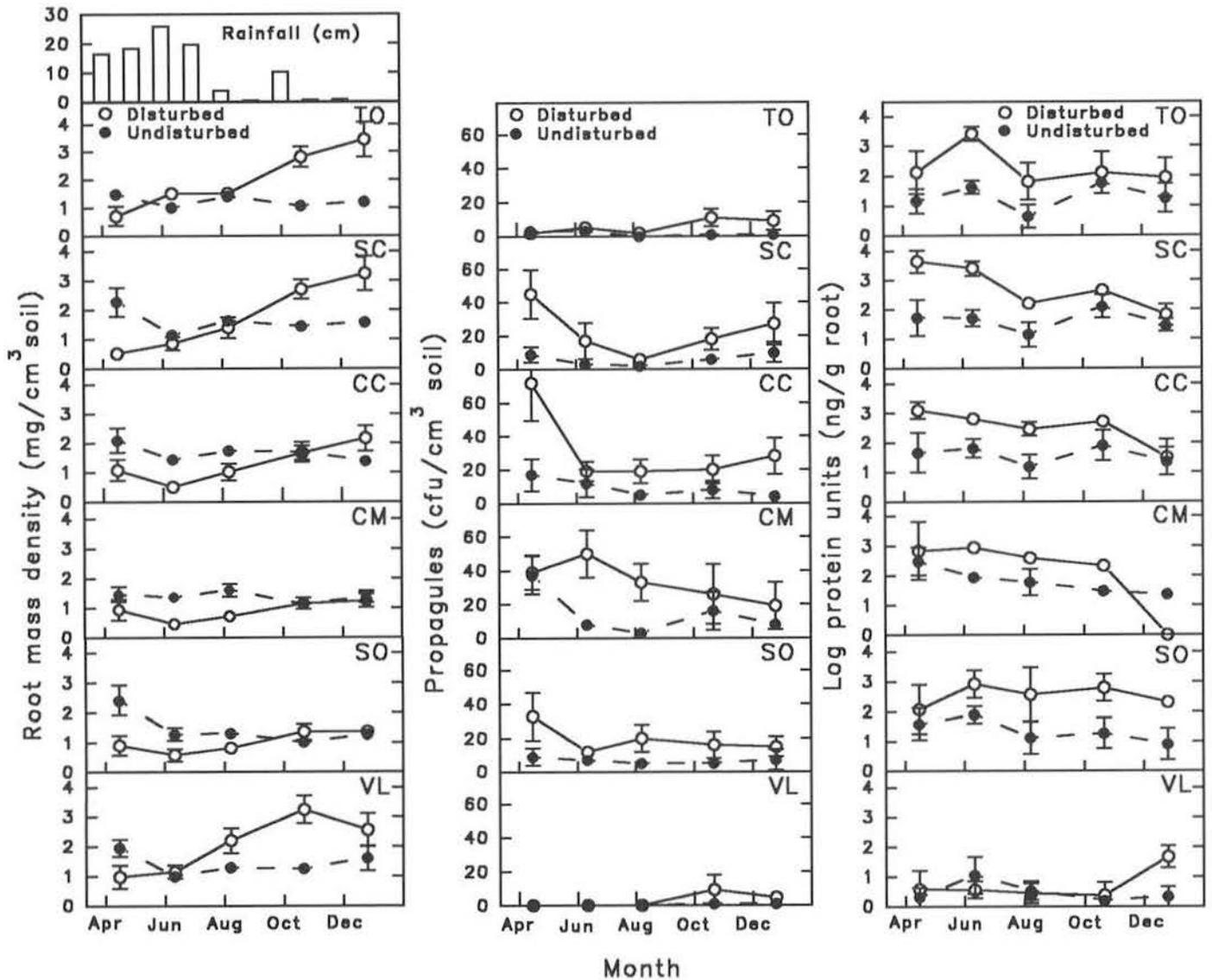


Fig. 3. Root mass density of undisturbed and regenerating disturbed roots and the rhizosphere populations of *Phytophthora nicotianae* and root infection levels for 17-yr-old Valencia sweet orange trees on six rootstocks varying in tolerance to root rot in St. Cloud, FL, from April 1991 to January 1992. TO = trifoliolate orange, SC = Swingle citrumelo, CC = Carrizo citrange, CM = Cleopatra mandarin, SO = sour orange, and VL = Volkamer lemon. Points represent means of eight replications \pm one standard deviation.

TABLE 3. Rate of growth of undisturbed and regenerating disturbed roots, and the rhizosphere populations of *Phytophthora nicotianae* and root infection levels for 17-yr-old Valencia sweet orange trees on six rootstocks in St. Cloud, FL

Rootstock	Root growth rate (mg/day) ^y		Propagules per soil volume ^x (cfu/cm ³)		Propagules per root weight ^x (cfu/mg)		ELISA ^x Log ng protein units/g root	
	Undisturbed ^y	Disturbed ^y	Undisturbed	Disturbed	Undisturbed	Disturbed	Undisturbed	Disturbed
Trifoliolate orange	-0.13 a	1.84 a ^z	1.6 bc	5.5 b ^z	0.06 bc	0.15 b	1.62 ab	2.99 a ^z
Swingle citrumelo	-0.27 a	1.98 a [*]	5.8 bc	15.4 ab [*]	0.44 ab	3.54 a	1.94 a	3.27 a [*]
Volkamer lemon	-0.10 a	1.34 ab [*]	0.3 c	2.5 b	0.01 c	0.01 b	0.89 b	1.26 b
Carrizo citrange	-0.32 a	0.92 bc [*]	9.0 ab	32.1 a [*]	0.38 ab	31.19 a [*]	2.19 a	2.85 a [*]
Sour orange	-0.66 a	0.48 bc [*]	6.6 abc	19.4 ab [*]	0.26 ab	8.13 a	1.58 ab	3.17 a [*]
Cleopatra mandarin	-0.12 a	0.37 c [*]	14.3 a	33.3 a [*]	3.39 a	36.64 a [*]	2.17 a	3.08 a [*]

^yMeans (n = 8) in columns followed by unlike letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

^xValues are the means of five bimonthly harvests from April 1991 to January 1992.

^yDisturbed refers to single-aged roots that regenerate into root containers installed under each tree while undisturbed refers to mixed-aged roots in cores of soil adjacent to the root containers (see Materials and Methods).

^z* denotes disturbed significantly different from undisturbed at $P \leq 0.05$ according to a paired Student's *t* test.

citrumelo, and Volkamer lemon did not show a reduction in mass density of regenerating roots between April and June, the months of highest rainfall, while the other rootstocks showed approximately a 50% reduction in root mass density (Fig. 3).

Rhizosphere populations of *P. nicotianae* in both disturbed and undisturbed soil tended to be the lowest on trifoliolate orange and Volkamer lemon (Table 3). Propagule levels in the soil were higher on regenerating young roots than on mixed-age roots for all rootstocks except Volkamer lemon, whose populations were low in the rhizosphere of both disturbed and undisturbed roots. Few propagules of *P. nicotianae* were recovered from this rootstock until October 1991. At that time, there was a buildup on the regenerating roots that coincided with a reduction in root mass density between October 1991 and January 1992.

Root infection by *P. nicotianae* of regenerating roots was lower on Volkamer lemon, whereas differences among the other rootstocks were not apparent (Fig. 3, Table 3). Infection was higher on regenerating roots than on mixed-age roots, except for Volkamer lemon, in which infection was low on both types of roots.

DISCUSSION

Tolerance of citrus rootstocks was identified as the capability to regenerate fibrous roots in greenhouse and field soils infested with *P. nicotianae*. The capacity of rootstocks for root regeneration in the presence of the pathogen was not necessarily related to potential rates of root growth in the absence of *P. nicotianae*. In the first greenhouse experiment, trifoliolate orange had the lowest root growth rate and root regeneration potential in the absence of *P. nicotianae* compared with other tolerant and intolerant rootstocks. In the presence of the pathogen, trifoliolate orange exhibited a greater ability to regenerate roots, which is recognized here as tolerance (10). Since the expression of tolerance by trifoliolate orange was not strongly related to root growth potential in the absence of the pathogen, tolerance may be more related to biochemical resistance as observed for other citrus tissues (1,16). Conversely, Volkamer lemon had the greatest root growth rates in the absence of *Phytophthora*, but was unable to regenerate roots in the presence of *P. nicotianae* under the cooler conditions that prevailed in the first greenhouse experiment. In the second greenhouse experiment conducted under warmer greenhouse conditions, Volkamer lemon displayed greater ability to regenerate roots. The effect of environmental conditions in the greenhouse on tolerance of Volkamer lemon was not inconsistent with the field study. Root growth of Volkamer lemon in disturbed soil was similar to that of tolerant trifoliolate orange and Swingle citrumelo until after October, when infection of young roots of Volkamer lemon increased (Fig. 3) as soil temperatures dropped (4). The complex behavior of Volkamer lemon is supported by the previous study in this field location by Agostini et al (2) who reported highly variable rhizosphere populations of *P. nicotianae* on this rootstock in a single growing season. The behavior of intolerant rootstocks Carrizo citrange, sour orange, and Cleopatra mandarin was more consistent with past greenhouse and field evaluations (2,10,17). To varying degrees, these rootstocks appeared to have less ability to grow roots and regenerate roots in the presence of *P. nicotianae* than tolerant rootstocks did.

Young regenerating roots of all citrus rootstocks generally were susceptible to infection as previously suggested in other greenhouse and field evaluations (2,7,10). Rhizosphere populations of *P. nicotianae* differed on undisturbed mixed-age roots and disturbed regenerating root systems. In pots, root pruning initially decreased populations of *P. nicotianae* in soil but propagule levels increased as root mass density increased and young roots became infected. Differences in soil and root populations among rootstocks were difficult to discern on both young, regenerating roots and undisturbed mixed-age roots of seedlings in pots where conditions were continuously conducive for pathogen activity. In the field, under periodically conducive conditions for *P. nicotianae*, regenerating roots on mature trees generally supported higher

levels of infection than mixed-age roots did. The tolerant rootstocks, trifoliolate orange and Volkamer lemon, had lower rhizosphere populations on mixed-age roots than intolerant rootstocks. On regenerating roots, the differences in populations of the pathogen between tolerant and intolerant rootstocks were less evident. This supports the previous contention that very young roots of rootstocks are susceptible to infection, irrespective of their ultimate tolerance to root rot as roots mature (7,10). Susceptibility of young citrus roots to infection by *P. nicotianae* is consistent with a root architecture study of resistant and susceptible tobacco cultivars that demonstrated greater infection by *P. nicotianae* of first-order lateral roots than of older, higher order roots (9).

In the field trial, trifoliolate orange and Volkamer lemon behaved as tolerant rootstocks supporting lower populations in soil even though root density was higher than on intolerant rootstocks. For trifoliolate orange, this apparent tolerance was not reflected in lower levels of root infection by ELISA. This is consistent with reports of resistant and susceptible varieties and species to other *Phytophthora* diseases in that no differences occurred in the initial infection of roots (3,5,9,12). Conversely, in the greenhouse, lower root infection of Swingle citrumelo roots was not consistently related to lower populations of *P. nicotianae* in the rhizosphere. In either case, the ELISA assay of *Phytophthora* protein levels does not distinguish between live and dead fungal tissue and may not be an accurate estimation of active infection by *P. nicotianae*.

The presence of lower populations of *P. nicotianae* in the rhizosphere of trifoliolate orange in spite of infection development in the root suggests that infection was less active or conversion of infection to soil propagules was inhibited by a biochemical mechanism. Previously, the phytoalexin 6,7-dimethoxycoumarin (DMC) has been detected in citrus root tissues infected by *P. citrophthora* (1,16). Production of DMC was triggered by fungal invasion of the roots growing in vitro and apparently occurred at higher levels, sooner in tolerant Troyer citrange than in intolerant sour orange and rough lemon (16). The role of DMC in tolerance of roots to infection and reproduction by *P. nicotianae* has not been investigated. Our preliminary evaluation indicated that DMC was produced at very low levels in roots of Cleopatra mandarin challenged in chlamyospore-infested soil, and was not produced until 27 days after infection; no DMC was produced in tolerant Swingle citrumelo roots (J. H. Graham, A. M. Montanari, and H. N. Nigg, unpublished data). The production of other compounds with phytoalexin activity in roots of tolerant and intolerant citrus rootstocks challenged with *P. nicotianae* is under investigation.

The tolerance of Volkamer lemon rootstock contrasted with the behavior of other rootstocks. Failure to detect *P. nicotianae* populations on Volkamer lemon from spring through fall of 1991 was associated with low infection levels on both regenerating and undisturbed roots until late fall and winter. In a previous study (8) conducted in a field trial with rootstocks similar to the present study, Volkamer lemon had the highest potential root growth rate, highest root mass density in soil, and reduced soil water content. In this related rootstock trial, Volkamer lemon supported the lowest populations of *P. nicotianae* among eight rootstocks examined (J. H. Graham and D. M. Eissenstat, unpublished data). Rapidly growing roots of Volkamer lemon may escape infection early in the season by reaching a higher root mass density sooner, depleting soil moisture, thereby creating conditions less conducive for *P. nicotianae* in the spring and summer (8). Alternatively, Volkamer lemon roots may reach maturity before more conducive environmental conditions develop with summer rainfall (7). Duncan et al (7) associated maturation of citrus roots with higher levels of phenolics and lignin in mixed-age populations of roots of rough lemon (*C. jambhiri* Lush), a rootstock genetically and phenotypically similar to Volkamer lemon. Phenolics and lignin dropped during the periods of root flushing in the spring and fall, but content increased to even higher levels within 2 mo after the root flush.

Duncan et al (7) also reported a drop in mass density of the mixed-age root populations following spring flushes of roots. In

the present study, there was a drop of up to 50% in root mass density between April and June for mixed-age roots of all the rootstocks in St. Cloud even in the case of Volkamer lemon, in which soil populations of *P. nicotianae* were not detected for several months. Root death in mixed-age root populations is affected by several host and environmental factors but also may in part be attributable to *Phytophthora* root rot. For regenerating roots, there was a drop in mass density between April and June for intolerant rootstocks, but not for tolerant rootstocks. Monthly rainfall was consistently high during the late spring and early summer. As previously shown over several seasons (7), higher soil moisture levels were favorable for pathogen activity during this period as indicated by the generally higher root infection and *P. nicotianae* population levels. During this period, tolerant rootstocks showed a net gain in root mass, whereas intolerant rootstocks apparently lost new, regenerating roots to root rot. The contribution of *P. nicotianae* to root death in the mixed-age population of roots could not be identified. Previously, very high populations of *P. nicotianae* (> 40 propagules per cubic centimeter of soil) developed on root systems of heavily freeze-damaged rough lemon rootstock trees, suggesting that *P. nicotianae* opportunistically colonized and accelerated senescence and death of stressed roots (7). In pot studies, older roots of transplanted seedlings often show the greatest visual root damage while recently regenerated roots appear to be unaffected even though they are infected. The degree to which *P. nicotianae* contributes to senescence and death of older roots requires further investigation.

The in situ root container method was very useful for study of a single-age class of regenerating roots over a single season when little natural root death would be expected. However, the role of *P. nicotianae* in turnover of mixed-age populations of roots in tolerant versus intolerant rootstocks could not be evaluated by the root censusing methods used in the present study. To study the effects of root rot on the overall age-class structure of root systems requires a detailed knowledge of life histories of roots obtained from rhizotron observations (4,13). Only then will it be possible to discern whether *P. nicotianae* significantly affects root turnover in relation to factors such as rootstock tolerance to root rot, root age, root structure, and production of phytoalexins.

LITERATURE CITED

1. Afek, U., and Szejnberg, A. 1988. Accumulation of scoparone, a phytoalexin associated with resistance of citrus to *Phytophthora citrophthora*. *Phytopathology* 78:1678-1682.
2. Agostini, J. P., Timmer, L. W., Castle, W. S., and Mitchell, D. J. 1991. Effect of citrus rootstocks on soil populations of *Phytophthora parasitica*. *Plant Dis.* 75:296-300.
3. Beagle-Ristaino, J. E., and Rissler, J. F. 1983. Histopathology of susceptible and resistant soybean roots inoculated with zoospores of *Phytophthora megasperma* f. sp. *glycinea*. *Phytopathology* 73:590-595.
4. Bevington, K. B., and Castle, W. S. 1985. Annual root growth pattern

of young citrus trees in relation to shoot growth, soil temperature and soil water content. *J. Am. Soc. Hortic. Sci.* 110:840-845.

5. Broadbent, P. 1969. Observations on the mode of infection of *Phytophthora citrophthora* in resistant and susceptible citrus roots. Pages 1207-1210 in: *Proc. First Inter. Citrus Symp.* H. D. Chapman, ed. University of California, Riverside.
6. Carpenter, J. B., and Furr, J. R. 1962. Evaluation of tolerance to root rot caused by *Phytophthora parasitica* in seedlings of citrus and related genera. *Phytopathology* 52:1277-1285.
7. Duncan, L. W., Graham, J. H., and Timmer, L. W. 1993. Seasonal patterns associated with *Tylenchulus semipenetrans* and *Phytophthora parasitica* in the citrus rhizosphere. *Phytopathology* 83:573-581.
8. Eissenstat, D. M. 1991. On the relationship between specific root length and the rate of root proliferation: A field study using citrus rootstocks. *New Phytol.* 118:63-68.
9. English, J. T., and Mitchell, D. J. 1988. Relationships between the development of root systems of tobacco and infection by *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* 78:1478-1483.
10. Graham, J. H. 1990. Evaluation of tolerance of citrus rootstocks to *Phytophthora* root rot in chlamydospore-infested soil. *Plant Dis.* 74:743-746.
11. Graham, J. H., and Timmer, L. W. 1992. *Phytophthora* diseases of citrus. Pages 250-269 in: *Plant Diseases of International Importance. Vol. III. Diseases of Fruit Crops.* J. Kumar, H. S. Chaube, U. S. Singh, and A. N. Mukhopadhyay, eds. Prentice-Hall, New Jersey.
12. Grant, B. R., and Byrt, P. N. 1974. Root temperature effects on the growth of *Phytophthora cinnamoni* in the roots of *Eucalyptus marginata* and *E. calophylla*. *Phytopathology* 74:179-184.
13. Hendrick, R. L., and Pregitzer, K. S. 1993. Patterns of fine root mortality in two sugar maple forests. *Nature* 361:59-61.
14. Lutz, A., and Menge, J. A. 1986. *Phytophthora* root rot. *Citrograph* 71:33-39.
15. Sandler, H. A., Timmer, L. W., Graham, J. H., and Zitko, S. E. 1989. Effect of fungicide applications on populations of *Phytophthora parasitica* and on feeder root densities and fruit yields of citrus trees. *Plant Dis.* 73:902-906.
16. Sulistyowati, L., Keane, P. J., and Anderson, J. W. 1990. Accumulation of the phytoalexin, 6,7-dimethoxycoumarin, in roots and stems of citrus seedlings following inoculation with *Phytophthora citrophthora*. *Physiol. Mol. Plant Pathol.* 37:451-461.
17. Timmer, L. W., Agostini, J. P., Graham, J. H., and Castle, W. S. 1991. Relationship of citrus rootstock to *Phytophthora* root rot and populations of *Phytophthora parasitica*. *Proc. Fl. State Hortic. Soc.* 104:173-178.
18. Timmer, L. W., Graham, J. H., Sandler, H. A., and Zitko, S. E. 1988. Populations of *Phytophthora parasitica* in citrus orchards and tree response to fungicide applications. *Citrus Ind.* 69(11):40-44, 54.
19. Timmer, L. W., Menge, J. A., Zitko, S. E., Pond, E., Miller, S. A., and Johnson, E. L. V. 1993. Comparison of ELISA techniques and standard isolation methods for *Phytophthora* detection in citrus orchards in Florida and California. *Plant Dis.* 77:791-796.
20. Timmer, L. W., Sandler, H. A., Graham, J. H., and Zitko, S. E. 1988. Sampling citrus orchards in Florida to estimate populations of *Phytophthora parasitica*. *Phytopathology* 78:940-944.
21. Zitko, S. E., Timmer, L. W., and Castle, W. S. 1987. Survey of Florida citrus nurseries for *Phytophthora* spp. *Proc. Fl. State Hortic. Soc.* 100:82-85.