Histological Comparison of Fibrous Root Infection of Disease-Tolerant and Susceptible Citrus Hosts by *Phytophthora nicotianae* and *P. palmivora*

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ABSTRACT

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Phytophthora nicotianae and *P. palmivora* infect and cause rot of fibrous roots of susceptible and tolerant citrus rootstocks in Florida orchards. The infection and colonization by the two *Phytophthora* spp. of a susceptible citrus host, sour orange (*Citrus aurantium*), and a tolerant host, trifoliolate orange (*Poncirus trifoliata*), were compared using light and electron microscopy. Penetration by both *Phytophthora* spp. occurred within 1 h after inoculation, regardless of the host species. No differences were observed in mode of penetration of the hypodermis or the hosts' response to infection. After 24 h, *P. palmivora* had a significantly higher colonization of cortical cells in susceptible sour orange than in tolerant trifoliolate orange. Intracellular hyphae of both *Phytophthora* spp. were observed in the cortex

of sour orange, and cortical cells adjacent to intercellular hyphae of *P. palmivora* were disrupted. In contrast, the cortical cells of sour orange and trifoliolate orange adjacent to *P. nicotianae* hyphae and the cortical cells of trifoliolate orange adjacent to *P. palmivora* were still intact. After 48 h, the cortical cells of both hosts adjacent to either *Phytophthora* spp. were disrupted. After 48 and 72 h, *P. palmivora* hyphae colonized the cortex of sour orange more extensively than the cortex of trifoliolate orange; *P. palmivora* also colonized both hosts more extensively than *P. nicotianae*. A higher rate of electrolyte leakage among host-pathogen combinations reflected the combined effects of greater cell disruption by *P. palmivora* than by *P. nicotianae*, and the higher concentration of electrolytes in healthy roots of trifoliolate orange than of sour orange. Although cellular responses unique to the tolerant host were not observed, reduced hyphal colonization by both pathogens in the cortex of trifoliolate orange compared with sour orange is evidence for a putative resistance factor(s) in the trifoliolate orange roots that inhibits the growth of *Phytophthora* spp.

In Florida citrus orchards, *Phytophthora nicotianae* Breda de Haan (synonym = *P. parasitica* Dastur) causes a fibrous root rot on tolerant as well as susceptible rootstocks (13,20). Although the epidemiology of the pathogen and losses caused by this disease have been evaluated extensively (9,13,20,29), these studies provide only indirect information on differences in the infection progress in roots of tolerant and susceptible hosts. Comparisons of several citrus rootstocks in the greenhouse and field demonstrated that infection of young roots occurs very rapidly and at the same rate on susceptible and tolerant hosts, but that root rot damage and fungal reproduction are limited subsequently on tolerant hosts (13, 20). Citrus rootstocks are generally referred to as tolerant rather than resistant, because fibrous roots become infected. Graham (12) defined tolerance, as applies to citrus rootstocks, as the condition in which plants are infected but show little or no net root loss. Tolerance was furthermore identified as the capability to regenerate roots to a greater extent in the presence of the pathogen than in the absence compared with susceptible rootstocks (13). In this study of citrus rootstocks, tolerance is used in this context.

In the late 1980s and 1990s, *P. palmivora* (E. J. Butler) E. J. Butler was identified and found to be highly pathogenic on the root system in Florida citrus orchards (44, J. H. Graham, unpublished data). In a greenhouse study, Zitko and Timmer (44) showed that *P. palmivora* was a more aggressive and damaging pathogen than *P. nicotianae* on certain *Citrus* spp. Although the competitive

nature of the two pathogens coinfecting the roots of susceptible citrus rootstocks was evaluated, differential infection of susceptible and tolerant hosts was not considered.

Ultrastructural studies of other pathosystems involving the interaction of *Phytophthora* spp. with resistant and susceptible hosts indicate that encysted zoospores rapidly form germ tubes that penetrate through the periclinal wall or, in the majority of cases, through the middle lamella of the anticlinal walls of root epidermal cells (16,26,37). Alterations of the middle-lamella matrices without major deformation of penetrated primary cell walls support the idea that root invasion by some *Phytophthora* spp. is achieved primarily by enzymatic means rather than by mechanical means (3,36). After initial penetration events, differences in cellular responses depend upon the host's susceptibility or resistance to the pathogen (6,11, 38). For example, Cahill et al. (6) showed that lignification of cell walls, deposition of phenolics, and formation of callosic papillae were more common in incompatible systems involving *P. cinnamomi* than in compatible systems.

We sought to determine if trifoliolate orange (*Poncirus trifoliata* L. selection 50-7), currently defined as highly tolerant, displays resistance reactions such as hypersensitivity, necrosis of the fungal hyphae during the penetration process, reduced rate of fungal ingress and cell disruption, or possibly other responses in contrast with the susceptible rootstock, sour orange (*Citrus aurantium* L.). Early infection processes of *P. nicotianae* and *P. palmivora* on susceptible sour orange and tolerant trifoliolate orange roots were studied using light and electron microscopy for up to 168 h after inoculation. As an independent evaluation of the host-pathogen interactions, such as cellular disruption and hypersensitivity (if observed), the rate of electrolyte leakage from zoospore-inoculated root tips was measured during the initial stages of infection. We

expected that insights into the infection process and the host's response at the cellular level would lead to a better understanding of the mechanisms involved in tolerance and resistance of citrus rootstocks to *Phytophthora* diseases and, eventually, new ideas for plant protection.

MATERIALS AND METHODS

Media and soil preparation. Strains of *P. nicotianae* and *P. palmivora* used in this study were isolated from citrus rhizosphere soil (23). They were maintained on clarified V-8 juice (Campbell Soup Co., Camden, NJ) agar at 18°C in the dark (30).

Candler fine sand (uncoated, hyperthermic, Typic Quartzipsamments), collected near Davenport, FL, was pasteurized by microwaving 1 kg of moist soil (approximately 7% wt/wt moisture) for 4 min in an 800-watt microwave oven (10,41). The soil was cooled to room temperature prior to use.

Zoospore production. Zoospores of *P. nicotianae* and *P. palmivora* were produced by a modified procedure of Mitchell and Kannwischer-Mitchell (23). Four 5-mm disks from V-8 agar cultures of *P. nicotianae* or *P. palmivora* were dispensed into each of three petri plates containing 15 ml of sterile, half-strength V-8 broth. Cultures were grown for 3 days in the dark in an incubator at 25°C. The liquid medium was aseptically removed with a pipette, and the mycelium was washed three times with sterile, distilled water. Mycelium was resuspended in sterile, distilled water and placed under direct, continuous, artificial light for 3 days at room temperature to induce sporangium formation. After chilling for 30 min at 4°C, the cultures were placed at room temperature until sporangia released zoospores. The zoospores were carefully separated from the mycelium by slowly pouring the suspension into a beaker, and an equal volume of sterile, distilled water was added to the zoospore suspension. The active zoospores were counted by pipetting 1 μ l onto a glass slide and observing them under a microscope.

Plant inoculations. Five-week-old seedlings of sour orange and trifoliolate orange were carefully removed from Metro-Mix 500 growth medium (The Scotts Co., Marysville, OH). Seedlings with

white, healthy root tips were laid on top of a layer of pasteurized soil in a large petri dish (150 by 15 mm). Small Parafilm (American National Can, Neenah, WI) wells were made by molding the Parafilm in a depression. These wells were placed under the root tips. Sterile water was pipetted into the wells to cover approximately 10 mm of the root tip. A spore suspension containing approximately 1,000 active zoospores was pipetted into each well. The remaining root system, which was not inoculated, was covered with pasteurized soil. The petri plates were covered and placed in an incubator at 27°C in the dark.

Light and electron microscopy. After 1, 2, 4, 24, 48, 72, and 168 h, inoculated roots were excised from the root system just above the point where the zoospore solution covered the root. Three root tips were sampled for each time period, for each host, and for each *Phytophthora* sp. The samples were fixed in 3% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.2 overnight at 4°C and then washed twice in the same buffer. The samples were treated in 2% OsO₄ for 3 h at room temperature. After washing twice in the buffer, the samples were dehydrated in a graded series of acetone (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%) for 10 min in each wash. The samples were washed three times in 100% acetone. The root samples were infiltrated with Spurr's resin (8) by maintaining them for 1 h at room temperature in a mixture of 30% resin and 70% acetone, for 4 h in a mixture of 50% resin and 50% acetone, and then overnight in a mixture of 70% resin and 30% acetone. After an additional 8 h in 100% resin, the samples were placed in molds, covered with fresh plastic, and placed in an oven at 70°C overnight.

Thick sections (1 μ m) were cut with a glass knife mounted in an ultra-microtome (LKB Instruments, Bromma, Sweden). The sections were mounted on a glass slide by heating, stained with a solution of methylene blue-azure A, and counter-stained with a solution of basic fuchsin for light microscopy (18). Ultrathin sections (8) were cut with a glass knife using the ultra-microtome, mounted on a 200-mesh, Formvar-coated copper grid, and stained with uranyl acetate (32) and lead citrate (27). Ultrathin sections were observed with a Philips 201 transmission electron microscope (Philips Scientific, Eindhoven, the Netherlands).

Fig. 1. Prepenetration and initial penetration stages through the periclinal walls of the hypodermis of roots of tolerant trifoliolate orange (*Poncirus trifoliata*) 4 h after inoculation with *Phytophthora nicotianae* zoospores. Bar = 20 μ m, and g = germ tube.

Fig. 2. Electron micrograph of a germ tube from an encysted zoospore of *Phytophthora palmivora* penetrating a hypodermal cell of susceptible sour orange (*Citrus aurantium*) 1 h after inoculation of roots with motile zoospores. Bar = 2 μ m, g = germ tube, and h = host cell.

Colonization of roots was measured by observing thick cross-sections of stained roots at approximately 5 mm from the root tip. Three sections from each of three different roots were examined. Colonization was rated on a scale of 0 to 9. A rating of 0 was given for 0 hyphae per section (hps), 1 for 1 to 10 hps, 2 for 11 to 25 hps, 3 for 26 to 40 hps, 4 for 41 to 50 hps, 5 for 51 to 75 hps, 6 for 76 to 90 hps, 7 for 91 to 125 hps, 8 for 126 to 175 hps, and 9 for more than 175 hps. The experiment was conducted two times with similar results. The results of one experiment are presented. Data from each experiment were subjected to the general linear model (GLM) procedure for analysis of variance (SAS Institute, Cary, NC), and mean separations of colonization ratings at each sampling time were made using least significant differences and Student's paired *t* tests.

Electrolyte leakage. Experiments were set up following a modified procedure of Zilberstein and Pinkas (42). Healthy, white root tips of 5-week-old sour orange (susceptible) and trifoliolate orange 50-7 (tolerant) seedlings were cut 15 mm from the root tip. The root tips were washed three times in sterile, double-distilled water to remove any residual electrolytes. Ten root tips each of sour orange or trifoliolate orange were placed in a single petri plate (60 by 15 mm) containing 10 ml of sterile, double-distilled water. One thousand zoospores of either *P. nicotianae* or *P. palmivora* were added to each of five petri plates containing root tips. Controls were also prepared with no zoospore inoculations. The root tips were placed in an incubator at 27°C, and the conductivity of the

solution ($\mu\text{S}/0.1$ g of fresh tissue) was measured every 12 h up to 108 h using an ElectroMark analyzer (Markson Science, Inc., Mara, CA). The conductivity of the noninoculated control was subtracted from the conductivity of the inoculated treatment to give the final conductivity value for that time period. After the last measurement, the root tips were surface-sterilized for 10 s in 70% ethanol, rinsed twice in sterile, deionized water, and plated on a medium selective for the isolation of *Phytophthora* spp. to confirm infection (pimaricin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol [PARPH]) (23).

Electrolyte content of healthy roots of each host was evaluated similarly. Ten healthy, white-tipped roots of sour orange or trifoliolate orange were cut into 15-mm sections, weighed, and placed in each of three test tubes. The root tips were washed three times in sterile, double-distilled water. After the final rinse, 10 ml of double-distilled water was added. The suspension was transferred to a tissue grinder and the roots were macerated until no visible segments were observed. Electrical conductivity of the suspension was measured immediately.

The experiments with inoculated and noninoculated roots were repeated with similar results, and the results of one of the experiments are reported. Data from each experiment were subjected to the GLM procedure for analysis of variance (SAS Institute) and mean separations of electrolyte leakage at each sampling period made using least significant differences.

RESULTS

Light and electron microscopy. Zoospores of either *P. nicotianae* or *P. palmivora* germinated and penetrated the hypodermal cells of both susceptible sour orange and tolerant trifoliolate orange roots within 1 h after inoculation. Germ tubes from encysted zoospores appeared swollen at the site of cell wall contact (Fig. 1). Penetration through the periclinal wall of the hypodermal cell or through the middle lamella of the anticlinal wall was observed in all treatments and did not vary between host or *Phytophthora* species. After initial penetration of the hosts' cell walls, the swelling of the germ tube persisted (Fig. 2). Zoospores were also attracted and germinated at sites of natural wounds on the root (Fig. 3). Germ tubes penetrated these sites after 2 h.

At 24 h after inoculation, hyphae of *P. nicotianae* and *P. palmivora* colonized the cortical cells as deeply as the third layer (of a total of 8 to 10 cell layers) in trifoliolate orange. The cortex of sour orange was penetrated by *P. nicotianae* more deeply than that of trifoliolate orange. Hyphae of *P. palmivora* were observed to colonize the stele of sour orange after 24 h. In trifoliolate orange, hyphae of *P. nicotianae* and *P. palmivora* were confined to the intercellular spaces. *P. palmivora* had a significantly higher colonization rating in sour orange than the colonization ratings for either *P. palmivora* or *P. nicotianae* on trifoliolate orange (Table 1). The percentage of hy-

Fig. 3. Attraction and germination of encysted zoospores of *Phytophthora palmivora* at a natural wound site on a root of tolerant trifoliolate orange (*Poncirus trifoliata*) 2 h after inoculation. c = encysted zoospore, g = germ tube, h = host cell, and w = wound site. **A**, Light micrograph showing accumulation and encystment of zoospores at the wound site. Bar = 100 μm . **B**, Electron micrograph of a germ tube from an encysted zoospore entering a wound site. Bar = 2 μm .

TABLE 1. Colonization of the root cortex by hyphae of *Phytophthora* spp. at 24, 48, and 72 h after zoospore inoculation of susceptible sour orange (*Citrus aurantium*) and tolerant trifoliolate orange (*Poncirus trifoliata*)

<i>Phytophthora</i> sp.	Host species	Colonization rating ^x		
		24 h ^y	48 h	72 h
<i>P. palmivora</i>	Sour orange	2.8 a ^z	8.2 a	7.9 a
<i>P. nicotianae</i>	Sour orange	1.5 ab	4.2 b	6.4 b
<i>P. palmivora</i>	Trifoliolate orange	1.0 b	4.7 b	6.7 b
<i>P. nicotianae</i>	Trifoliolate orange	0.4 b	3.0 b	4.5 c

^x Average rating of three sections from each of three different roots based upon the following scale: 0 = 0 hyphae per section (hps); 1 = 1 to 10 hps; 2 = 11 to 25 hps; 3 = 26 to 40 hps; 4 = 41 to 50 hps; 5 = 51 to 75 hps; 6 = 76 to 90 hps; 7 = 91 to 125 hps; 8 = 126 to 175 hps; and 9 = >175 hps.

^y Time sampled after inoculation with zoospores of *Phytophthora* spp.

^z Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to least significant differences.

phae that were intracellular in the cortex was higher at 24 and 72 h in the *P. palmivora*-sour orange pathosystem than in the other pathosystems (Table 2). At 48 h, the percentage of intracellular hyphae was lower for *P. nicotianae* in trifoliate orange than in sour orange.

At 24 h after inoculation, cortical cells of sour orange, adjacent to the intercellular *P. palmivora* hyphae, were disrupted (Fig. 4A). The plasma membrane had collapsed and no organelles were observed in the cell lumen. The middle lamella was more pronounced and stained darker with OsO₄. At the same period after inoculation, the cortical cells of sour orange and trifoliate orange adjacent to the intercellular *P. nicotianae* hyphae and the cortical cells of trifoliate orange adjacent to the intercellular *P. palmivora* hyphae were intact (Fig. 4B).

At 48 h from inoculation, hyphae had grown into the stele in all treatments. Cortical cells were disrupted adjacent to hyphae of *P. nicotianae* and *P. palmivora* in the intercellular spaces at 48 h after inoculation and later time intervals (data not shown) (40). This was observed for both hosts.

Intra- and intercellular colonization of roots for both pathosystems was even more extensive at 72 h (Tables 1 and 2) and 168 h after inoculation. The colonization ratings for both *Phytophthora* spp. examined separately were significantly higher ($P \leq 0.01$) in sour orange than in trifoliate orange after 72 h (Table 1).

At 24 and 72 h after inoculation, colonization of the cortex of sour orange by the two *Phytophthora* spp. (data combined) was significantly greater than the colonization of trifoliate orange (Fig. 5). There were higher percentages of intracellular hyphae in sour orange than in trifoliate orange at all times measured. *P. palmivora* had a higher colonization rating than *P. nicotianae* after 48 and 72 h when ratings for both hosts were combined. *P. palmivora* had a significantly higher percentage of intracellular hyphae at 24 and 72 h after inoculation.

Electrolyte leakage. There was no electrolyte leakage detected from the infected roots until 12 h after inoculation in any of the treatments (Fig. 6). After 24 h, only the *P. palmivora*-sour orange pathosystem had a significantly higher conductivity than the non-inoculated controls (data not shown). No measurable electrolyte leakage occurred in trifoliate orange inoculated with *P. nicotianae* until after 48 h. Rate of electrolyte leakage from trifoliate orange roots infected with *P. palmivora* was significantly greater than from all other host-pathogen combinations at 72 h. After 108 h, the electrolyte leakage of sour orange or trifoliate orange inoculated with *P. palmivora* was significantly higher than the same hosts inoculated with *P. nicotianae*. At the completion of the study, there was no significant difference in the colonization frequency of the root tips as detected on PARPH selective medium ($P \leq 0.05$). Average electrical conductivity per 0.1 g of roots was greater for macerated, noninoculated root tips of trifoliate orange (182 μ S) than for tips of sour orange (148 μ S; $P \leq 0.05$).

TABLE 2. Percentage of total number of hyphae of *Phytophthora* spp. in the root cortex that was intracellular at 24, 48, and 72 h after zoospore inoculation of susceptible sour orange (*Citrus aurantium*) and tolerant trifoliate orange (*Poncirus trifoliata*)

<i>Phytophthora</i> sp.	Host species	Intracellular hyphae (%) ^a		
		24 h ^b	48 h	72 h
<i>P. palmivora</i>	Sour orange	14.2 a ^z	21.7 a	44.0 a
<i>P. nicotianae</i>	Sour orange	0.9 b	24.4 a	27.9 b
<i>P. palmivora</i>	Trifoliate orange	0.0 b	15.1 ab	18.1 b
<i>P. nicotianae</i>	Trifoliate orange	0.0 b	8.5 b	18.3 b

^a Mean of the percentage of total number of hyphae observed that were intracellular.

^b Time sampled after inoculation with zoospores of *Phytophthora* spp.

^z Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to least significant differences.

DISCUSSION

Zoospores of *P. nicotianae* and *P. palmivora* encysted, germinated, and penetrated the hypodermal cells of tolerant and susceptible citrus hosts during the same time period and in the same manner. The penetration of roots by both *Phytophthora* spp. occurred within 2 h, confirming observations of other *Phytophthora* pathosystems (6,7,15,16,38). Zoospores were attracted to natural wounds on roots of tolerant and susceptible rootstocks alike, where

Fig. 4. Electron micrographs of intercellular hyphae of *Phytophthora palmivora* and the effect on adjacent cortical cells of susceptible sour orange (*Citrus aurantium*) and tolerant trifoliate orange (*Poncirus trifoliata*) 24 h after inoculation of roots. Bar = 2 μ m, c = cortical cell, h = hypha, ml = middle lamella, pm = plasma membrane, and w = host wall. A, *Phytophthora palmivora* disrupts the plasma membrane of sour orange. B, Disruption of the plasma membrane absent for *Phytophthora palmivora* on trifoliate orange.

they readily germinated and entered through these sites. Broadbent (5) also observed zoospores of *P. citrophthora* to be attracted similarly toward root wounds in resistant and susceptible citrus hosts. Other fungal species have been observed to penetrate through points where lateral roots emerge or through natural openings (24,28).

In the early stages of infection of young root tips, the mode of penetration of the hypodermis and cellular response of sour orange and trifoliate orange were indistinguishable. This nonspecific interaction of *P. nicotianae* and *P. palmivora* with young roots of susceptible and resistant hosts is in common with other pathosystems involving *Phytophthora* spp. (2,5,6,37,38). For example, no differences in early responses of susceptible and resistant citrus hosts infected for *P. citrophthora* were noted (5).

Based on cytological observations, it was not possible to conclude whether the mode penetration of citrus root cells by the *Phy-*

tophthora spp. was enzymatic or mechanical. Swollen germ tubes observed at the point of penetration may represent an appressorium-like structure, such as that observed on roots of bentgrass (*Agrostis palustris*) inoculated with zoospores of *Pythium aphanidermatum* (21). Swollen germ tubes were also observed for *P. cinnamomi* (6) and *P. megasperma* pathosystems (2). Lazarovits et al. (22) and Broadbent (5) called these structures appressoria. However, Beagle-Ristaino and Rissler (2) were reluctant to refer to them as appressoria, because adherence to the host cell wall was not investigated. If these structures are appressoria, penetration of the host by mechanical action should be indicated. Electron microscopy of citrus roots did not reveal an obvious disruption in the cell wall at the point of penetration. This might, as other workers have concluded (3,17), indicate that enzymatic activity rather than the mechanical action of an appressorium was responsible for penetration.

Fig. 5. Inter- and intracellular colonization of *Phytophthora nicotianae* or *P. palmivora* hyphae into the cortex of susceptible sour orange (*Citrus aurantium*) and tolerant trifoliate orange (*Poncirus trifoliata*) over time. **A**, Colonization rating for *Phytophthora* spp. in the cortex of sour orange or trifoliate orange. **B**, Colonization rating for *P. nicotianae* or *P. palmivora* in the two hosts. **C**, Percentage of total hyphae of *Phytophthora* spp. that were intracellular in the cortex of sour orange or trifoliate orange. **D**, Percentage of total *P. nicotianae* or *P. palmivora* hyphae that were intracellular in the two hosts. An asterisk (*) denotes significant difference ($P \leq 0.05$) for that specific time interval according to Student's paired *t* test.

Penetration of citrus roots occurred between anticlinal walls or through periclinal walls of the hypodermis for both citrus hosts and did not differ between *Phytophthora* spp. Stössel et al. (34) observed that penetration of soybean (*Glycine max*) between anticlinal walls or through periclinal walls by two races of *Phytophthora sojae* (synonym = *P. megasperma* var. *sojae*) was a characteristic of each race and was not influenced by the host compatibility of the interaction. In another study, *P. sojae* (synonym = *P. megasperma* f. sp. *glycinea*) penetrated the anticlinal walls of soybean roots 94% of the time (2).

Cellular response to penetration, 1 to 4 h after inoculation, did not differ among the citrus pathosystems. When initial penetration occurred, the host cell plasma membrane was disrupted, but the adjacent cells did not appear to be affected. Ward et al. (38) found that in incompatible interactions of *P. sojae* on soybean, all epidermal cells in contact with hyphae were dead within 2 to 3 h following inoculation, and that a majority of cells associated with, and in the vicinity of, hyphae were dead. Compatible interactions showed less cell damage initially, even when the cells were penetrated. Slusher et al. (31), however, observed no evidence of soybean cell necrosis in advance of *P. sojae* hyphae.

When young citrus roots of tolerant and susceptible rootstocks were evaluated in the greenhouse or the orchard for fungal infection and root rot, few differences were noted (12,13). However, as roots aged, differences in root regeneration and reproduction of the fungus in the rhizosphere developed between tolerant and susceptible rootstocks (9,13). In the current study, regardless of the *Phytophthora* species, susceptible sour orange had a higher colonization rating and percentage of intracellular hyphae in the cortex than tolerant trifoliolate orange at most observation times up to 168 h after inoculation of roots. Comparing compatible and incompatible races of *P. sojae* on soybean hypocotyls, Stössel et al. (33) determined that the compatible race produced intracellular haustoria more frequently than did the incompatible race. Our findings support those of Graham (13), who suggested that resistance is ex-

pressed in trifoliolate orange as reduction in root rot and conversion of infection to propagules of *P. nicotianae* as roots matured. The current study of root infection at the cellular level is also consistent with the finding of Zitko and Timmer (43) that *P. palmivora* is a more aggressive pathogen than *P. nicotianae* on susceptible citrus hosts. *P. palmivora* colonized and disrupted cortical cells of tolerant and susceptible hosts more rapidly after initial infection than *P. nicotianae*, although the rate of development of cellular damage in trifoliolate orange was much lower than in sour orange.

Differential colonization and cellular reactions to *P. nicotianae* and *P. palmivora* on sour orange and trifoliolate orange are supported by the electrolyte leakage from root tips. Detectable amounts of electrolytes were observed after 24 h only in roots of sour orange infected with *P. palmivora*. Coincidentally, this was the pathosystem for which disruption of the cells adjacent to the intercellular hyphae was observed at 24 h after inoculation. Other studies involving *Phytophthora* spp. showed analogous results within the same time frame. Hanchey and Wheeler (15) observed severe cellular disruption of susceptible tobacco roots as early as 3 h after inoculation with *P. nicotianae*. Infected cells showed a separation of the plasmalemma from the cell wall, rudimentary dictyosomes, dilated endoplasmic reticulum, and a decreased electron density of the cytoplasm. Protoplasmic shrinkage was also observed in eucalypts 24 h after infection by *P. cinnamomi* (37), in corn infected by *P. cinnamomi* (39), in soybean infected by *P. sojae* (38), and in susceptible potato cultivars infected by *P. infestans* (17). In the current study, all host-pathogen systems showed cell disruption after 48 h. However, in roots of trifoliolate orange infected with *P. nicotianae*, no electrolytes were detected at this time after inoculation. This might be explained by the lower colonization rating in this pathosystem and the lower percentage of intracellular hyphae.

Most cortical cells of both hosts in the vicinity of hyphae collapsed by 72 h after inoculation with *P. nicotianae* or *P. palmivora*. The roots of trifoliolate orange colonized by *P. palmivora* had a higher electrolyte leakage after 72 h than trifoliolate orange colonized by *P. nicotianae*, probably because *P. palmivora* had a higher colonization rating. The higher conductivity observed in the *P. palmivora*-trifoliolate orange system compared with that in either host colonized by *P. nicotianae* was attributable to a higher concentration of electrolytes in roots of trifoliolate orange than those of sour orange. Electrolyte leakage from sour orange roots infected with *P. palmivora* was greater than the leakage from roots of sour orange or trifoliolate orange colonized by *P. nicotianae*, because colonization was higher in the *P. palmivora*-sour orange pathosystem. Likewise, roots of sour orange colonized by *P. nicotianae* had a higher rate of electrolyte leakage than roots of trifoliolate orange infected with this pathogen. Zilberstein and Pinkas (42) used electrolyte leakage to screen avocado rootstocks for resistance to *Phytophthora* root rot. However, such a test does not appear to be promising for identification of resistance or tolerance in citrus rootstocks because of differences in cell electrolyte concentrations among hosts.

The disruption of the cells adjacent to the intercellular hyphae suggests the production of a toxin(s) by the pathogens. Necrosis of host tissue has been observed for other *Phytophthora* pathosystems (6,37,38), and toxins have been isolated in culture from several *Phytophthora* spp. (1,4,14,19,25,35). However, necrosis in advance of contact with hyphae was not observed in soybean rootlets infected with *P. sojae* (31) nor in our study. Collapse only of cells immediately adjacent to hyphae in both the tolerant and susceptible citrus hosts suggested that this was not a hypersensitivity response. Cell collapse adjacent to hyphae was also noted by Tippet et al. (37) for infection of eucalypt roots by *P. cinnamomi*.

This study established no differences in the initial infection of roots of susceptible and tolerant hosts. The differential response was after penetration of the hypodermis and during colonization of cortical cells. Although cellular responses unique to a resistant host, such as callose formation or hypersensitivity, were not ob-

Fig. 6. Electrical conductivity (μS per 0.1 g of fresh root weight) of suspensions of root tips of susceptible sour orange (*Citrus aurantium*) or tolerant trifoliolate orange (*Poncirus trifoliata*) inoculated with motile zoospores of *Phytophthora nicotianae* or *Phytophthora palmivora*. Data are means of 10 replications. Points at a specific time interval followed by the same letter are not significantly different ($P \leq 0.05$) according to least significant differences.

served in the cortex, there was lower inter- and intracellular colonization of trifoliate orange compared with sour orange by both pathogens. This leads to the conclusion that a putative resistance factor(s) in the trifoliate orange roots inhibited the growth of *Phytophthora* spp. Further studies are needed to identify the factor(s) and establish whether it is constitutive in root tissues or is expressed either locally or systemically after infection. This information may contribute to improved root rot-resistance in citrus through breeding, including transformation of genes for the factor(s) into current rootstocks.

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