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Root demography of mature citrus trees: the influence of *Phytophthora nicotianae*

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Abstract

The amount of root mortality caused by root pathogens such as *Phytophthora nicotianae* (syn. *Phytophthora parasitica*) has typically been inferred from the net change in root length density in sequential soil cores. Because such measurements give information only on net changes in root populations, the actual rate of root turnover is often underestimated. We used minirhizotrons to track the fate of a large number of individual fine roots of mature field-grown citrus trees over a 6-month period. This method enabled us to examine the effect of *P. nicotianae* population levels on fine-root mortality. Seasonal and genotypic variation in patterns of citrus fine root mortality were associated with variation in population levels of *P. nicotianae*. Fine root lifespans were shorter when populations of *P. nicotianae* were high. Fine roots of the *Phytophthora*-susceptible rootstock, rough lemon (*Citrus jamibhiri*), had shorter median lifespans and supported larger populations of *P. nicotianae* than the fine roots of the more tolerant rootstock, Volkamer lemon (*Citrus volkameriana*). Rates of root mortality were either relatively constant for roots of all ages, or increased with age; the latter pattern was most pronounced for Volkamer lemon roots. Differences in the age-dependence of root mortality may, therefore, play a role in genotypic differences in tolerance of *Phytophthora* root rot by these two rootstocks.

Introduction

Despite widespread interest in root initiation, growth, and death (i.e. root turnover) and the effects of root pathogens on the rate of root turnover, technical difficulties have limited our ability to observe pathogen effects on root growth and mortality. This is especially true for mature field-grown trees. Studies of root pathogen effects on rates of tree root turnover have typically employed windowed pot techniques for seedlings (e.g. Blaker and MacDonald, 1986) or, for mature trees, soil coring methods (e.g. Duncan et al., 1993; see Smucker, 1993 for review). Sequential measurements of roots in soil cores can give information only on the net change in root populations over the sampling interval, and, consequently, often underestimate the actual rate of root production and root death. Differences in root density in the presence of a pathogen may be the result of differences in root mortality, differences in the rate of new root production, or a combination of both factors. Methods of following the fate of individual roots are required to separate these two aspects of root turnover.

There are large, seasonally variable populations of root-damaging soil organisms such as *Phytophthora nicotianae* van Breda de Haan - syn. *Phytophthora parasitica* Dastur and burrowing nematodes in many Florida citrus groves (DuCharme, 1967; Duncan et al., 1993; Timmer et al., 1989). If a given pathogen is one of the causes of root mortality, we would expect to observe different rates of fine root mortality in resistant and susceptible rootstock species exposed to the pathogen, and would also expect to observe synchrony between changes in pathogen populations and changes

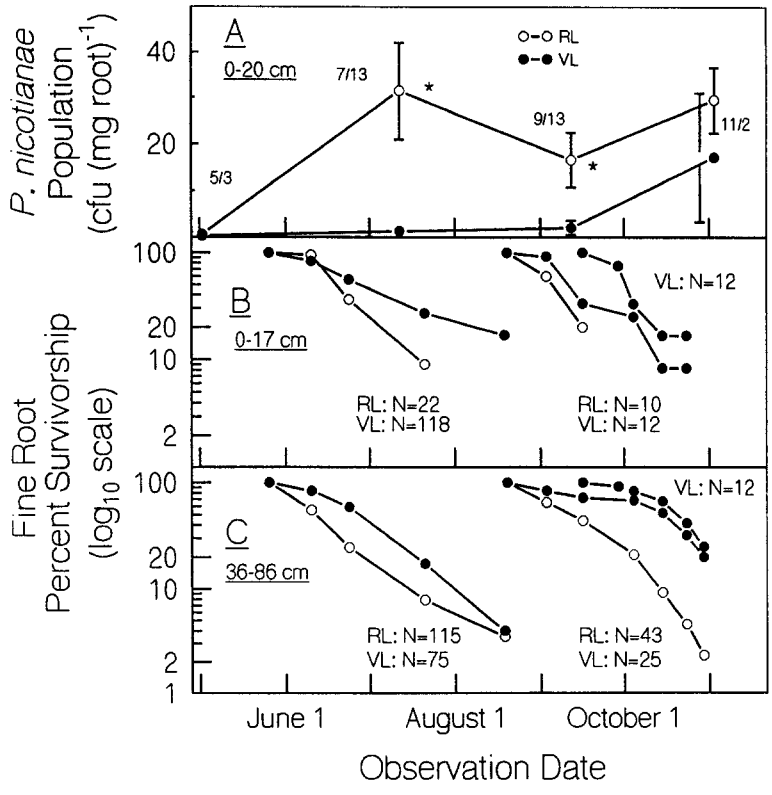

Fig. 1. **A)** Populations of *Phytophthora nicotianae* (colony forming units (cfu) per mg root) in soil cores from 0 – 20 cm depth. Samples taken from May 3, 1993 to Nov. 2, 1993; N = 8. Starred values at each date are significantly different ($p \leq 0.05$); ANOVA done on log-transformed data. Error bars are ± standard error of the mean; error bars not shown fall within the symbol. RL = Rough lemon; VL = Volkamer lemon. **B)** Percent of the initial population surviving at each observation date (survivorship) for cohorts of rough lemon fine roots (open symbols) and Volkamer lemon fine roots (closed symbols) from 0 to 17 cm depth from the soil surface. The earliest cohort of roots appeared between May 5 and June 10, 1993; root age estimates are ± 33 days. The August cohort of roots appeared between August 4 and September 1, 1993; root age estimates are ± 17 – 28 days. The September cohort of roots appeared between September 1 and September 30; root age estimates are ± 17 – 20 days. Note the logarithmic scale of the ordinate. **C)** Survivorship curves for cohorts of rough lemon fine roots (open symbols) and Volkamer fine lemon roots (closed symbols) from 36 to 86 cm depth from the soil surface. Cohort appearance dates as above.

in root mortality. We test these assumptions here for the pathogen *P. nicotianae*.

We examined root demographic patterns in field-grown rough lemon (*Citrus jambhiri* Lush.) and Volkamer lemon (*Citrus volkameriana* Pasq.). These two rootstocks are very similar in fine root morphology (Eissenstat, 1991), but rough lemon is less tolerant of *Phytophthora* root rot than Volkamer lemon, both as seedlings and as rootstocks for mature trees (Graham, 1993, 1994). In this study, we used minirhizotron methods to track the fate of a large number of individual fine roots over time. This allowed us to apply the tools of demographic analysis to study patterns of mortality of tree roots in the field and their relationship to root age, rootstock genotype, and environmental factors (e.g. Hendrick and Pregitzer, 1992, 1993). We monitored *P. nicotianae* populations regularly during the period of root observation. Seasonal, genotypic, and depth-related variation in patterns of root mortality were associated with temporal and spatial patterns of variation in population levels of *P. nicotianae*.

Materials and methods

Field data collection

Minirhizotron tubes (clear butyrate plastic tubes, 55 mm OD) were installed January, 1992 in a 17-year-old citrus rootstock trial in central Florida, USA (for site description see Eissenstat, 1991). In the rootstock trial, Valencia orange (*Citrus sinensis* (L.) Osbeck) scions were grafted onto 14 different rootstock genotypes. We report here observations of the roots of two of these

Table 1. Estimated cohort age (days) at 25%, 50% and 75% mortality. Cohort age values from linear interpolation between observed data points of survivorship curves. RL = Rough lemon, VL = Volkamer lemon

Depth (cm)	Rootstock	May cohort age (days) % mortality			August cohort age (days) % mortality			September cohort age (days) % mortality		
		25%	50%	75%	25%	50%	75%	25%	50%	75%
0 – 17	RL	27	30	57	9	20	37			
	VL	26	51	78	21	33	51	13	16	24
36 – 86	RL	11	23	45	10	33	49			
	VL	27	51	68	33	57	69	32	35	44

Table 2. Phytophthora nicotianae populations as a function of depth; soil cores collected Nov. 2 1993, Avon Park site (N = 8 for each rootstock). RL = Rough lemon; VL = Volkamer lemon. Root weights are for fine roots (< 2 mm diameter). Values with different letters are significantly different by depth for that rootstock ($p \leq 0.05$); starred values are significantly different between rootstocks at that depth ($p \leq 0.05$). ANOVA was carried out on log-transformed data to correct for skewed distribution

Depth (cm)	Root mass density $mg_{root}\ cm^{-3}$		*Phytophthora* progagules cm^{-3}		*Phytophthora* propagules mg_{root}^{-1}	
	RL	VL	RL	VL	RL	VL
0 – 20	1.0a	1.6*a	21.3a	18.6*a	29.5a	17.2*a
20 – 40	0.4b	0.7*b	21.5a	14.8*a	103.5a	37.1*a
40 – 60	0.2c	0.3c	8.0b	6.5*a	44.8a	38.2a

rootstock genotypes - rough lemon (*Citrus jambhiri*) and Volkamer lemon (*Citrus volkameriana*).

Minirhizotron tubes were buried to a depth of about 1 m, with the tops inclined radially away from the trunk at 30° from the vertical. Tubes were installed about two-thirds the distance from the bole to the canopy dripline for each tree observed. Roots growing on the upper surface of the tubes were videotaped with a minirhizotron camera (Bartz Technology, Santa Barbara, CA, USA) at regular intervals for eight trees of each genotype. Videotapes were taken at monthly intervals from May to October, and at weekly intervals from October to December.

Population sizes of *P. nicotianae* were estimated from soil cores (19.6 cm deep, 7.5 cm diam.) collected under the canopy from trees adjacent to each of the 8 minirhizotron trees (2 cores per tree). Because the extensive coring necessary may have had some effect on root growth, we did not collect cores from the minirhizotron trees. Soil cores from 20 – 40 cm and 40 – 60 cm were also collected November 2, 1993 for analysis of *P. nicotianae* populations at increasing soil depth. Cores were sieved through a 3 mm screen; all fine roots (< 2 mm diameter) were collected from the screen, dried (70°C, 24h), and weighed. Subsamples of the sieved soil were diluted in water agar and plated on selective media for estimates of *Phytophthora* propagule numbers, as described in Timmer et al. (1988). *Phytophthora* populations were expressed as colony forming units per cm^3 soil or as colony forming units per mg root mass.

Demographic analysis

We collected mortality data on individual roots in three cohorts during the spring and fall flush of new root production. We monitored changes in the status of roots growing on the minirhizotron tubes by directly comparing consecutive videotapes. New roots that appeared on the minirhizotron tubes between May 5 and June 10,

1993 were considered to be in the same age class, and are referred to as the "May" cohort (rough lemon, n = 137; Volkamer lemon, n = 193, where n = sum of new roots from all tubes for each genotype). The "August" cohort appeared between August 4 and September 1, 1993 (rough lemon, n = 53; Volkamer lemon, n = 37). The "September" cohort appeared between September 1 and September 30, 1993; only Volkamer lemon produced enough roots (n = 24) during this interval to be considered in demographic analysis. Roots were scored as dead either when they took on the transparent, water-soaked appearance symptomatic of roots infected with *P. nicotianae* (Graham, 1993), or when disintegration of the root tissue was observed. Similar symptoms could potentially be caused by other root damaging organisms or by environmental conditions leading to necrosis.

The uncertainty of our root age estimates depends upon the observation intervals for both root appearance and root mortality. Because some roots appeared on the minirhizotron tubes after the first observation but died before the second observation, the starting date for determining root age was defined as halfway between the first and second observation. The date of mortality was taken to be halfway between the observed date of mortality and the previous observation date.

Because the soil environment varies with depth, we separated cohorts into two depth classes – a shallow cohort from 0–to–17-cm depth, and a deep cohort from 36–to–86-cm depth. The shallow cohort matched the depth for the repeated soil core samples for *Phytophthora* population estimates. The deep cohort started at a depth where previous observations indicated that the soil remained consistently moist. Due to a lack of environmental information, roots of the intermediate depth class (18–to–35-cm depth) were not considered in this study.

Survivorship data (percent of the initial population surviving to each observation date) was plotted on a semilog graph (Figs. 1B, C) in order to reveal patterns of mortality rate dependence on age; the slope of the log-transformed survivorship curve at any given age equals the rate of mortality (Pianka, 1988). Differences between the survivorship distributions of rough lemon and Volkamer lemon roots were analyzed by the Kolmogorov-Smirnov test (Sokal and Rohlf, 1981), as suggested by Pyke and Thompson (1986). The median fine root lifespan (time required for 50% cohort mortality) and quartiles were estimated from survivorship curves by linear interpolation between observations. The median is a more representative measure of central tendency in skewed distributions than the mean (Sokal and Rohlf, 1981).

Results and discussion

Phytophthora *populations*

Populations of *P. nicotianae* in the soil samples from rough lemon were generally greater than in soil samples from Volkamer lemon (Fig. 1A). Populations were equally low on each genotype at the sampling date in May 1993, but increased more rapidly on rough lemon than on the more *Phytophthora*-tolerant Volkamer lemon, and remained significantly higher except for the last sampling date (November 2, 1993). These results are consistent with previous findings of partial tolerance of Volkamer lemon to *Phytophthora* root rot. In greenhouse evaluations of seedlings and in field evaluations of mature trees (Agostini et al., 1991; Graham, 1993, 1995), less tolerant rootstocks supported higher *Phytophthora* populations than more tolerant rootstocks.

Root lifespans

Compared to Volkamer lemon, the high *Phytophthora* populations supported by the more susceptible rootstock, rough lemon, are associated with shorter median root lifespans (Figs. 1B, C, Table 1). Seasonal and depth-related differences in *Phytophthora* populations are also associated with variation in root lifespans. Median lifespans are shortest for rough lemon in the shallow (0 – 17 cm) August cohort (Fig. 1B, Table 1), and are shortest for Volkamer lemon in the shallow September cohort (Fig. 1B, Table 1). *Phytophthora* populations were relatively high on both genotypes at this time (Fig. 1A) and at this depth (Table 2, soil volume basis).

These observations indicate that *Phytophthora* root rot is a contributing factor in fine root mortality in our field site. Support for this is found in greenhouse observations of seedling roots treated with the fungicide metylaxyl, commonly used to control *Phytophthora* root rot. When *Phytophthora* was controlled, median root lifespans were greater than 90 days (Kosola and Eissenstat, 1994), in contrast to the estimated median root lifespans of 16 to 57 days seen here (Table 1).

Other root-damaging organisms were present in our study site, and may have also contributed to root mortality. The citrus burrowing nematode (*Radopholus cit-*

rophilus) is present throughout the grove (Duncan et al., 1994); populations of the burrowing nematode typically increase in the fall (DuCharme, 1967). The likelihood of nematode-*Phytophthora* interactions driving increased August and September cohort mortality is, however, reduced by the pattern of depth distribution of the citrus burrowing nematode; the burrowing nematode is most commonly found deeper in the soil profile (0.6 to 1.5 m, Du Charme, 1967; Duncan et al., 1994). Median root lifespans in the August and September cohorts are shortest for shallower roots (0–to–17 cm; Table 1), the opposite of the pattern expected if the burrowing nematode caused the increased mortality in August and September.

Age-dependent mortality

There are many potential mechanisms of tolerance to *Phytophthora*; for example, several previous studies (e.g. Blaker and MacDonald, 1986; Graham, 1990, 1995) have shown that *Phytophthora*-tolerant citrus rootstocks produce new roots more rapidly than less tolerant rootstocks. While we do not have data on the rate of new root production, our data suggest that different patterns of age-dependent root mortality may also be involved in tolerance to *Phytophthora* root rot. Rough lemon (*Phytophthora*-susceptible) and Volkamer lemon (*Phytophthora*-tolerant) differed not only in median root lifespan but also in the dependence of mortality on root age, as shown by the different shape of the survivorship curves for each rootstock (Figs. 1B, C).

The survivorship curves for Volkamer lemon and rough lemon fine roots were significantly different ($p \leq 0.05$, Kolmogorov-Smirnov test) for both May and August cohorts in the 36–86 cm depth class. After Volkamer lemon roots reached the age of about one month, the rate of mortality increased in both May and August cohorts (Fig. 1C) to a rate comparable to that seen in rough lemon. This finding suggests that, under the conditions found deeper in the soil, young Volkamer lemon roots were more resistant to *Phytophthora* infection than either rough lemon roots or older Volkamer lemon roots. *Phytophthora* lesions have been found to develop at the same rate in excised rough lemon and Volkamer lemon roots (Matheron and Matejka, 1993); it seems likely, then, that the observed differences between age-dependent mortality in rough lemon and Volkamer lemon are due to differences in the rate of infection, rather than to differences in the rate of symptom development.

This study clearly points towards the role of *Phytophthora* root rot in fine root mortality of mature field-grown citrus trees. The association of variation in fine root lifespan of mature trees with pathogen populations is, to our knowledge, a new finding. Minirhizotron techniques combined with demographic analysis can contribute to plant root pathology by allowing repeated, non-destructive observations in the field. In this study, these techniques allowed us to discern genotypic differences in age-dependent mortality which may be related to genotypic differences in tolerance of *Phytophthora* root rot. Such information on age-specific mortality cannot be obtained by standard soil-coring methods. One limitation of applying the minirhizotron technique to root pathology is the difficulty in sampling the observed roots for positive identification of the organisms associated with root mortality. Despite this limitation, these windows into the belowground environment provide valuable insight into the factors influencing root infection, mortality, and decay.

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