

CHANGES IN THE RISK OF FINE-ROOT MORTALITY WITH AGE: A CASE STUDY IN PEACH, *PRUNUS PERSICA* (ROSACEAE)¹

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Previous studies suggest that younger roots are more vulnerable to mortality than older roots. We analyzed minirhizotron data using a mixed-age, proportional hazards regression approach to determine whether the risk of mortality (or “hazard”) was higher for younger roots than for older roots in a West Virginia peach orchard. While root age apparently had a strong effect on the hazard when considered alone, this effect was largely due to different rates of mortality among roots of different orders, diameters, and depths. Roots with dependent laterals (higher order roots) had a lower hazard than first-order roots in 1996 and 1997. Greater root diameter was also associated with a decreased hazard in both 1996 and 1997. In both years, there was a significant decrease in the hazard with depth. When considered alone, age appeared to be a strong predictor of risk: a 1-d increase in initial root age was associated with a 1.26–2.62% decrease in the hazard. However, when diameter, order, and depth were incorporated into the model, the effect of root age disappeared or was greatly reduced. Baseline hazard function plots revealed that the timing of high-risk periods was generally related to seasonal factors rather than individual root age.

Key words: minirhizotron; peach; *Prunus persica*; root age; root demography; root mortality; Rosaceae.

The production of fine roots (<1 mm in diameter) can constitute a considerable fraction of yearly net primary productivity (Caldwell and Camp, 1974; Grier et al., 1981; Sanantonio and Grace, 1987), while the mortality of fine roots represents an important input of carbon and nitrogen to the soil (Vogt, Grier, and Vogt, 1986; Joslin and Henderson, 1987; Jackson, Mooney, and Schulze, 1997). Nonetheless, the inaccessibility of the root system and the difficulty of measuring simultaneous root production and mortality have historically limited our ability to study fine root turnover. While nondestructive root system observations using minirhizotrons (root observation tubes) have provided data on the timing and extent of fine root turnover in a number of ecosystems (Aerts et al., 1989; Hendrick and Pregitzer, 1992; Reid, Sorenson, and Petrie, 1993; Hanson, Aifen, and Andren, 1995; Majdi and Kangas, 1997; Fitter et al., 1998; Ruess, Hendrick, and Bryant, 1998; Wells and Eissenstat, 2001), the underlying processes that drive fine-root mortality are still poorly understood. In some specific cases, such as the sloughing of “rain roots” in *Agave* (Huang and Nobel, 1992) or the loss of roots to *Phytophthora* infection in citrus (Kosola, Eissenstat, and Graham, 1995), the proximate

cause of fine-root mortality is clear. However, in most systems, we have not yet identified the factors responsible for fine-root mortality, nor do we know when and for which portions of the root system periods of high root mortality are likely to occur.

There is some evidence to suggest that the risk of mortality changes with root age. Higher mortality among younger roots (or early in the life of a root cohort) has been reported in an Alaskan taiga forest (Ruess, Hendrick, and Bryant, 1998) and a New Zealand kiwi orchard (Reid, Sorenson, and Petrie, 1993). A number of log-scale survivorship curves show higher mortality rates among younger roots in a British grassland (Fitter et al., 1998), and Hendrick and Pregitzer (1993) noted that older, over-wintered roots exhibited lower mortality rates in the spring than did newly produced roots.

Survivorship curves that indicate high levels of mortality among young individuals and lower rates among older individuals are referred to as “Type III” and are characteristic of organisms that produce large numbers of relatively vulnerable offspring (Pearl, 1928; Deevey, 1947; Gotelli, 1995). If fine root survivorship does exhibit a Type III pattern, then the factors driving high mortality among young, presumably active roots deserve further investigation.

Nonetheless, it is important to approach apparent age effects on root mortality cautiously. Heterogeneity within the fine root population may create the appearance of age-related decreases in mortality rate when none exist (Allison, 1995). If the fine root population contains subgroups that differ in their risk of mortality, then a survivorship curve plotted for the root population as a whole will show an initially high mortality rate due mainly to deaths in the more vulnerable groups, followed by a decrease in mortality rate as the population becomes enriched in members of lower risk groups. This will occur even when the risk of mortality within each subgroup is constant with time. If the existence of heterogeneity within the fine-

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root system is not recognized, we may erroneously conclude that younger roots are at greater risk than older roots.

The existence of subgroups within the fine-root population that die at different rates has been demonstrated in a number of species. Root demographic data have revealed marked differences in survivorship among fine roots produced at different times during the year (Head, 1966; Cheng, Coleman, and Box, 1990; Hendrick and Pregitzer, 1992, 1993; Fitter et al., 1998; Ruess, Hendrick, and Bryant, 1999), at different sites (Hendrick and Pregitzer, 1993; Fitter et al., 1998), and at different depths (Hendrick and Pregitzer, 1992; Schoettle and Fahey, 1994; Arnone et al., 2000). There is also evidence that roots of different diameter, order, and color within a single root system show contrasting patterns of mortality (Reid, Sorenson, and Petrie, 1993; Tingey et al., 1997; Gill et al., 1999; Wells and Eissenstat, 2001).

We used minirhizotron observations to examine patterns of fine root production and mortality in a 15-yr-old peach orchard over two growing seasons. Our goals were threefold: (1) to determine whether the hazard appeared to be higher for younger roots, (2) to identify additional root characteristics that were associated with differences in the hazard, and (3) to determine whether a significant effect of age on the hazard was still present when we incorporated additional aspects of root heterogeneity into our model. We also examined the timing of high-risk periods during the growing season to determine whether they were more closely related to root age or to seasonal factors. By quantifying changes in the risk of mortality with root age, we provide a conceptual basis for further investigations into the causes and controls of fine-root turnover.

MATERIALS AND METHODS

Study site and minirhizotron installation—The experiment was conducted at the United States Department of Agriculture—Agricultural Research Service Appalachian Fruit Research Station in Kearneysville, West Virginia, USA using six 15-yr-old Loring peach trees on Halford rootstock. The trees were planted in Hagerstown silt loam soil, a fine mixed Mesic Typic Hapludalf. In April 1996, six clear butyrate observation tubes (minirhizotrons) were installed beneath each tree at an angle of 30° from the vertical. The tubes were placed ~0.7 m from the trunk and at least 0.8 m from one another. They were 70 cm in length (60 cm was viewable), 6 cm in outer diameter, and were scribed with a single vertical transect of 30 1.8 × 1.2 cm windows. Bottoms of the tubes were sealed with acrylic plugs. Light penetration and radiant heating were prevented by wrapping the tops of the tubes in black electrical tape, sealing them with rubber stoppers, and covering them with white aluminum cans. Two minirhizotrons from each tree (12 minirhizotrons total) were used in the present experiment.

Root videotaping and image processing—Roots that grew against the surface of the minirhizotron tubes were videotaped at 2–4 wk intervals during the 1996–1997 and 1997–1998 growing seasons using a miniaturized camera system and portable videocassette recorder (Bartz Technology, Santa Barbara, California, USA). Videotaping was suspended during the winter, as prior observations indicated that there was little root activity during this time. Frames from the videotapes were digitized and archived using a Macintosh 7500 computer with Apple Video Player and Graphic Converter software (Wells and Eissenstat, 2001). Images of individual roots as they appeared on successive dates were reviewed, and information on root life span and life history was collected for ~450 roots.

Data recorded for each root included the date of appearance, date of death, depth, diameter, and order. Death was defined as either (a) disappearance or (b) a blackened and shriveled appearance. Roots that turned brown but appeared intact were not counted as dead. Root diameter was measured to the nearest tenth of a millimeter on the first date that a root appeared using

RooTracker software (Dave Tremmel, Duke University Phytotron, Durham, North Carolina, USA). None of the roots that we observed increased in diameter with time, although in some cases roots decreased in diameter due to sloughing of epidermal and cortical tissue. Root order was assigned according to a topological classification scheme in which roots with no visible dependent laterals were considered to be order 1, roots with a single visible set of dependent laterals were considered to be order 2, and so on. Due to the limited viewing area of the minirhizotron windows, some roots may have had lateral roots that we could not observe. We henceforth underscore the uncertainty of our order assignments by referring to roots with no visible laterals as “apparent first-order roots” and roots with dependent laterals as “higher-order roots.”

Statistical analysis—Mean diameters of higher-order roots and apparent first-order roots were compared using the Mann-Whitney *U* test (SPSS 8.0, SPSS, Chicago, Illinois, USA). The effect of root age on the risk of root mortality was evaluated using a mixed-age group Cox proportional hazards regression approach (Cox, 1972; Allison, 1995; Wells and Eissenstat, 2001; see background, below) performed with SAS PROC PHREG (SAS, 1997). Proportional hazards regression cannot be used to statistically evaluate changes in the hazard with time because the baseline hazard cancels out during the process of generating parameter estimates (Allison, 1995). Therefore, the effect of age on the hazard cannot be estimated for cohorts of same-aged roots for whom age and calendar time are inseparable. Instead, we chose to follow the survival of mixed-aged groups of roots beginning at particular times during the growing season and to include initial root age as a covariate in the proportional hazards model. This approach allowed us to compare the hazards of different root age classes that were experiencing identical seasonal and environmental conditions. In 1996, we analyzed survival data from the standing crops of roots present in mid-summer (5 August 1996) and early fall (12 September 1996), and in 1997, we used the standing crops present in late spring (30 May 1997) and midsummer (31 July 1997). These groups were chosen in order to incorporate a broad range of root ages and to encompass as much of the growing season as possible. In our initial mixed-age group analysis, root age was the only covariate included in the proportional hazards model.

The effects of order, diameter, depth (0–25 cm or 26–50 cm), and tree of origin on the hazard of root mortality were estimated in separate proportional hazards analyses for each year. Roots from all cohorts in a given year were combined for this analysis in order to ensure that there were adequate numbers of roots in all categories. The data were stratified by cohort to control for the effect of production date on the hazard of mortality. The mixed-age group analyses were then repeated, including as additional covariates the fine-root characteristics that had emerged as significant predictors of root survivorship in the analyses of pooled data from each year.

Nonparametric estimates of the baseline hazard function for different age classes in the 5 August 1996 and 30 May 1997 groups were produced using the baseline statement of PROC PHREG and the SMOOTH kernel-smoothing macro described in Allison (1995) with a bandwidth of 30 d. Initial root age was used as a stratifying variable, and the resulting graphs show smoothed hazard function estimates for each age group, which were evaluated at the means of all model covariates. The smoothed curves are truncated because the macro does not produce estimates for times within one bandwidth of the first and last observations.

Proportional hazards regression: background—The concept of the “hazard” is central to survival analysis. In the context of the present work, it describes the instantaneous rate of change in the probability of root mortality, conditioned on the root having survived to time *t* (Cantor, 1997). The hazard can be thought of as the instantaneous probability of mortality, although it is not strictly a probability and can take on values >1 (Allison, 1995). In a proportional hazards model, the hazard of an individual at time *t* is written as the product of two components, a baseline hazard function that remains unspecified and a linear function of *k* covariates that becomes the exponent (Allison, 1995): $h_i(t) = h_0(t)\exp(\beta_1 x_{i1} + \dots + \beta_k x_{ik})$.

The PROC PHREG in SAS employs the method of partial likelihood (Cox,

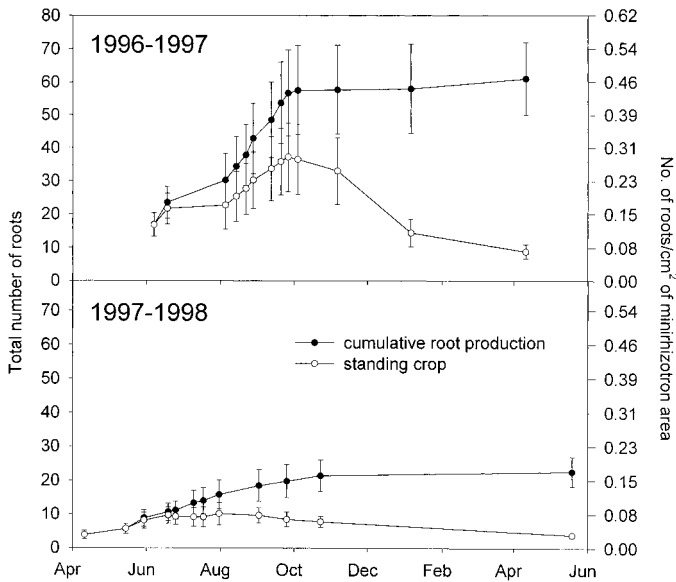


Fig. 1. Cumulative root production and root standing crop for each sampling date during the 1996–1997 and 1997–1998 seasons. Data are expressed both as the total number of roots observed per tree (left-hand axis) and as number of roots observed per square centimeter of minirhizotron frame area (right-hand axis). Roots from two minirhizotrons beneath each tree were pooled. Bars represent ± 1 SE.

1972) to estimate the β coefficient (or “parameter estimate”) associated with each covariate in the model and calculates a chi-square statistic used to test the null hypothesis that each β is equal to zero. The parameter estimates generated by proportional hazards regression have a “relative risk” interpretation such that a one unit increase in the value of covariate x_i is associated with a $100[\exp(\beta_i) - 1]$ percentage change in the hazard of mortality, controlling for the effects of other covariates. The sign of the parameter estimate indicates whether the hazard decreases (negative sign) or increases (positive sign) as the covariate value increases.

RESULTS

Fine-root production and standing root numbers—Fine-root production and mortality occurred simultaneously throughout the growing season in the peach orchard (Fig. 1). In 1996, there was a pronounced peak in root numbers in late September, followed by a rapid decline between November and January. Standing root numbers changed less dramatically over the course of the growing season in 1997 and declined only slightly during fall and winter. There appeared to be little overwinter root mortality in either year: the majority of roots present on our final fall/winter sampling date were also present on our first spring sampling date the following year.

Overall, there were many more roots produced in 1996 ($N = 316$) than in 1997 ($N = 138$), but the percentage of higher-order roots (7% in 1996 and 10% in 1997) and the average fine-root diameters (0.34 ± 0.22 mm in 1996 and 0.30 ± 0.09 mm in 1997) were similar in both years. The cumulative number of fine roots observed during the year was 1.6 (1996) to 2.0 (1997) times higher than the maximum number of roots visible on any one sampling date and 2.2 (1996) to 3.3 (1997) times higher than the average number of roots visible.

Cohort survivorship curves—Survivorship curves, plotted on a log scale to illustrate changes in mortality rate with time, revealed considerable variation in the pattern of mortality

among fine-root cohorts (Fig. 2). While a number of cohorts did appear to exhibit higher mortality rates among younger roots, many, especially those produced in the summer of 1996, showed mortality rates that were relatively constant with time. In both years, there were periods during which mortality rates increased or decreased for the majority of cohorts simultaneously. In 1996, a number of cohorts exhibited low mortality rates in early autumn, followed by increased mortality rates from November to January. In 1997, mortality rates were high for the majority of cohorts during the summer and early fall and low over winter. Cohorts produced during July exhibited particularly high mortality.

Effects of root age: initial analysis—We examined the effect of age on the hazard of root mortality for two mixed-age groups of roots in 1996 and in 1997 using a proportional hazards regression technique with age as the single model covariate (see MATERIALS AND METHODS). Root age appeared to be associated with a significant decrease in the hazard of mortality for all groups. An increase of 1 d in initial root age was associated with an estimated 1.25% decrease ($\beta = -0.0126$, $P < 0.0009$) in the hazard of mortality in the 5 August 1996 group $\{100[\exp(-0.0126) - 1] = -1.25\}$. Results from other groups were similar: the hazard appeared to decrease by 1.47% ($\beta = -0.0148$, $P < 0.0009$), 2.41% ($\beta = -0.0244$, $P < 0.0014$) and 2.62% ($\beta = -0.0266$, $P < 0.0001$) with a 1-d increase in initial root age in the 12 September 1996, 30 May 1997, and 31 July 1997 groups, respectively.

Effects of order, diameter, depth, and tree on fine-root mortality—Higher root order was associated with a significant decrease in the risk of mortality in 1996 ($P < 0.0005$; Table 1) and a marginally significant decrease in 1997 ($P < 0.0598$). Controlling for the effects of other covariates, the hazard of higher-order roots was 77% less than that of apparent first-order roots in 1996 and 51% less in 1997. Median survival times for higher-order roots (Fig. 3) were 226 d (1996) and 259 d (1997), while those of apparent first-order roots were 105 d (1996) and 95 d (1997). In 1996, the effect of order decreased with time after first appearance (for order \times time interaction, $P < 0.0282$).

Higher-order roots had a significantly greater mean diameter than apparent first-order roots in 1996 (Fig. 4; $P < 0.0006$; Mann-Whitney U test): apparent first-order roots had a mean diameter of 0.33 ± 0.042 mm, while higher-order roots had a mean diameter of 0.47 ± 0.083 mm. In 1997, the difference in diameter between apparent first-order roots (0.30 ± 0.006 mm) and higher-order roots (0.34 ± 0.027 mm) was not significant, perhaps due to the small number of higher-order roots in the sample ($N = 14$).

With root order included in the model, increasing root diameter was still associated with a significant decrease in the hazard in both 1996 ($P < 0.0001$) and 1997 ($P < 0.0438$; Table 1). This result was also reflected in the survival probabilities of apparent first-order roots from three diameter classes (Fig. 5). The median survival times of apparent first-order roots ≤ 0.25 mm in diameter were 70 d (1996) and 77 d (1997), while those of apparent first-order roots 0.25–0.5 mm in diameter were 131 d (1996) and 111 d (1997). The median survival time of apparent first-order roots > 0.5 mm in diameter was 213 d in 1996, while in 1997, none of the apparent first order roots > 0.5 mm in diameter died.

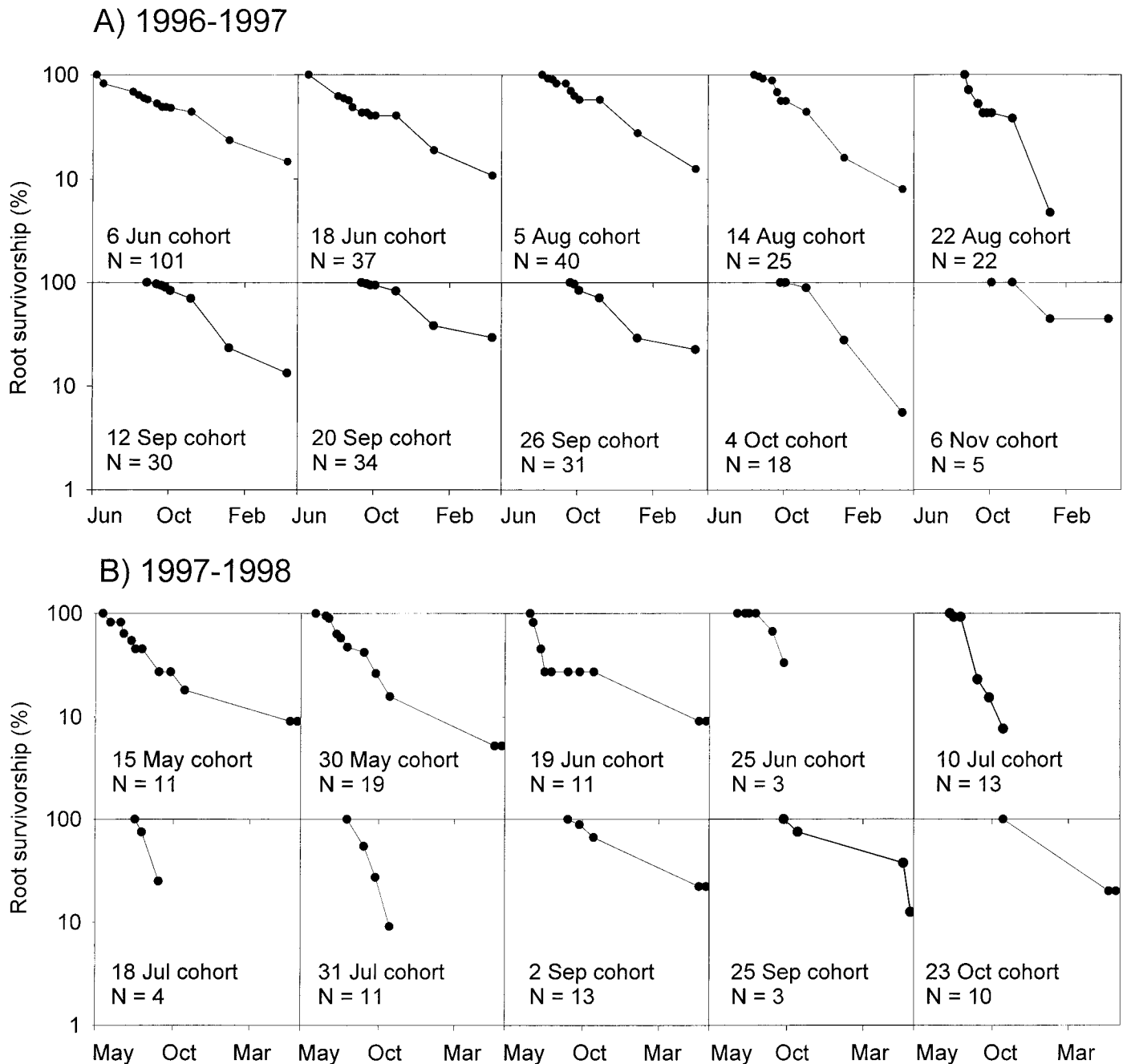


Fig. 2. Log-scale survivorship curves for individual root cohorts produced in (A) 1996–1997 and (B) 1997–1998.

In both years, there was a significant decrease in the hazard with depth ($P < 0.0505$ in 1996 and $P < 0.0009$ in 1997). There was insufficient evidence to conclude that the hazard differed among roots from individual trees ($P < 0.4234$ in 1996 and $P < 0.0915$ in 1997), although in 1997, one of the six trees showed a trend toward increased root life span relative to the other five (data not shown).

Effects of root age: second analysis—The effect of age on the hazard of four mixed-age root groups was assessed a second time, this time including order, diameter, and depth as additional model covariates. Controlling for the effects of oth-

er covariates, root age did not have a significant effect on the risk of root mortality for either of the mixed-age groups in 1996 (Table 2). In 1997, increasing root age was associated with a decrease in the hazard for the 31 July 1997 group ($P < 0.0132$), and a similar trend was observed in the 30 May 1997 group ($P < 0.0843$). However, the magnitude of the root age effect in the 31 July 1997 group was greatly reduced compared to that indicated by the single-factor model. An increase of 1 d in initial root age in the 31 July 1997 group was associated with a 2.62% decrease in the hazard in the single factor model but only a 1.04% decrease in the model that incorporated additional fine-root characteristics. The effects of

TABLE 1. Results of proportional hazards regression performed on pooled root life span data collected in 1996–1997 ($N = 348$) and 1997–1998 ($N = 138$). Interactions between covariates and time after first appearance are shown only when significant. * = $P \leq 0.05$.

Variable	df	Parameter estimate	Standard error	Wald chi-square	P > chi-square
1996–1997					
Tree	1	-0.0280	0.0349	0.6410	0.4234
Depth	1	-0.2876	0.1470	3.8252	0.0505*
Diameter	1	-1.6788	0.4166	16.2421	0.0001*
Order	1	-1.4603	0.4218	11.9890	0.0005*
Order × time	1	0.00524	0.00239	4.8136	0.0282*
1997–1998					
Tree	1	-0.1078	0.0639	2.8487	0.0915
Depth	1	-0.8363	0.2521	11.0085	0.0009*
Diameter	1	-2.8863	0.3805	4.0642	0.0438*
Order	1	-0.7161	0.3805	3.5424	0.0598

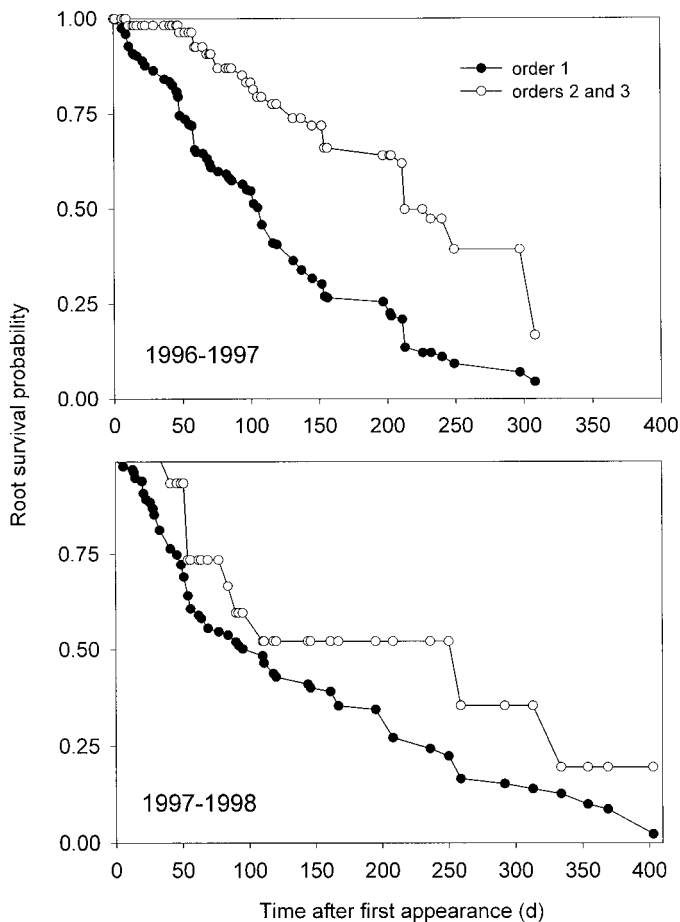


Fig. 3. Survival probabilities for first-order and higher-order roots in 1996–1997 and 1997–1998. All roots in a given year were combined and given a common start point. Survival probabilities were generated using the baseline statement of PROC PHREG in SAS with order as the stratifying variable. Median survival time is defined as the first time-point at which the survival probability is $\leq 50\%$.

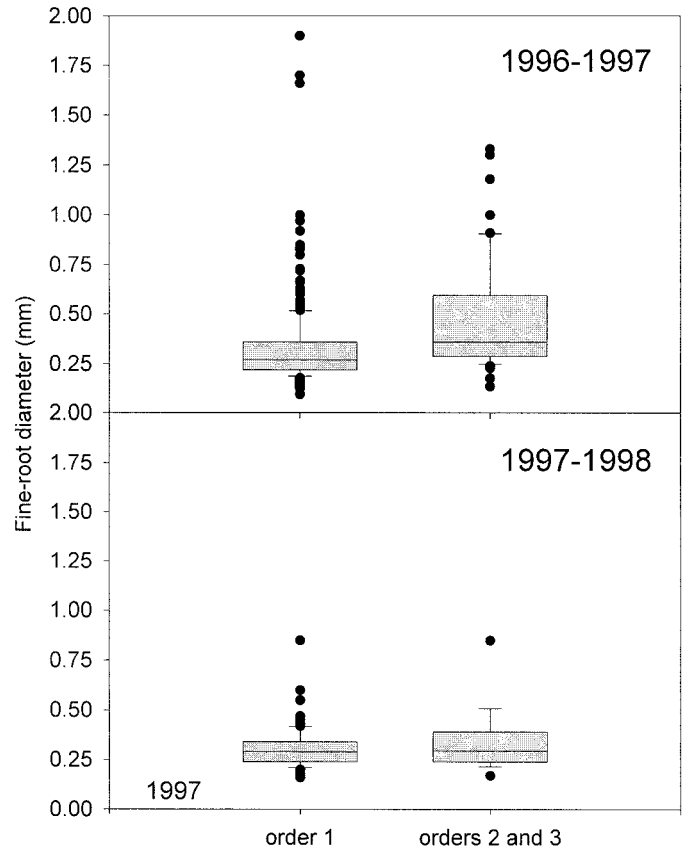


Fig. 4. Diameter distributions for first-order and higher-order roots in 1996 and 1997. The box represents diameters between the 25th and 75th percentiles, the line within the box represents the median diameter, and the circles represent outliers >1.5 box-widths from the top or bottom of the box.

order, diameter, and depth on individual mixed-age groups generally corresponded to those from the analyses of pooled data except when the number of higher-order roots or larger diameter roots was quite small (e.g., both groups in 1997).

An examination of the smoothed hazard functions for different age classes of roots reveals additional information about changes in the hazard with age and time (Fig. 6). In 1996, two distinct peaks appeared in the hazard function of the 5 August group, one in September and one in January. These peaks occurred in all age classes of roots simultaneously, although the magnitude of the peaks was somewhat higher for younger roots. In particular, roots in the youngest age class appeared to have a considerably higher hazard during the September peak than did older classes of roots (Fig. 6). We tested this possibility by constructing a dummy variable, which was coded 1 during days 40–70 of the analysis (corresponding to late September/early October) and 0 for the rest. The product of this variable and root age was included as a covariate in the model, and the interaction was shown to be significant ($\beta = -0.0247$, $P < 0.0164$), indicating that younger roots had a higher hazard than older roots during the specified interval. The true P value may of course be higher, as this interaction was constructed based on an examination of the estimated hazard functions rather than upon an a priori hypothesis (Allison, 1995).

The hazard function again rose and fell at approximately the same time for both age classes in the 30 May 1997 group (Fig.

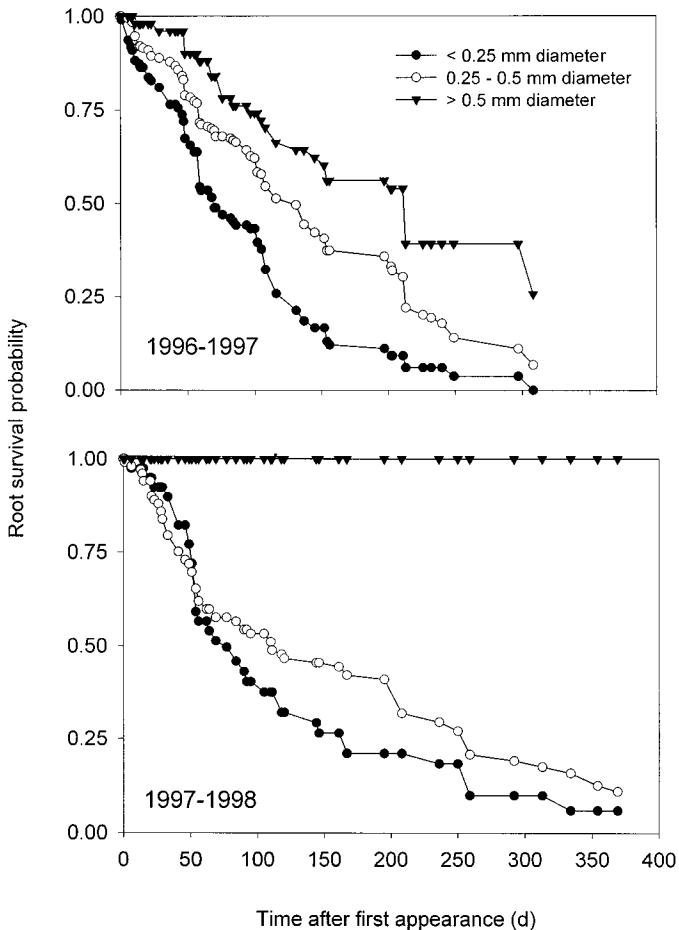


Fig. 5. Survival probabilities for first-order and higher-order roots from three diameter classes in 1996–1997 and 1997–1998. All first-order roots in a given year were combined and given a common start point. Survival probabilities were generated using the baseline statement of PROC PHREG in SAS, with diameter as the stratifying variable.

6). As in 1996, there was an early fall peak in the hazard, but in 1997, our choice of sampling intervals did not allow us to detect a second peak later in the year.

DISCUSSION

Root age and the risk of root mortality—We followed the survivorship of mixed-age groups of roots from two time points in each growing season to observe the patterns of mortality among root age classes that experienced the same progression of whole-plant phenology and were exposed to the same environmental conditions. Using mixed-age groups allowed us to separate the effects of root age from those of calendar time, a task that would not have been possible had we been following the survivorship of single-aged cohorts. When initial root age was the only covariate included in the model, its relationship to the hazard appeared to be highly significant: older roots were much less likely to die than younger roots. However, when other fine-root characteristics such as diameter and order were incorporated into the model, the effect of root age on the hazard was reduced in magnitude or disappeared entirely. This result indicates that age effects can be artifactual, reflecting underlying heterogeneity in the fine-

TABLE 2. Results of proportional hazards regression analyses performed on four mixed-age groups of roots in 1996 and 1997. Parameter estimates for the covariates depth, diameter, order, and root age are shown. * = $P \leq 0.05$.

Variable	df	Parameter estimate	SE	Wald chi-square	P > chi-square
Standing crop 5 August 1996					
Depth	1	-0.5742	0.2311	6.1722	0.0130*
Diameter	1	-1.2530	0.4697	7.1152	0.0076*
Order	1	-0.7344	0.2498	8.6426	0.0033*
Root age	1	0.0012	0.0043	0.0740	0.7857
Standing crop 12 September 1996					
Depth	1	-0.1131	0.1773	0.4071	0.5234
Diameter	1	-1.8015	0.5433	10.9959	0.0009*
Order	1	-0.5566	0.2005	7.7066	0.0055*
Root age	1	0.0005	0.0023	0.0468	0.8287
Standing crop 30 May 1997					
Depth	1	-1.1935	0.3770	10.0236	0.0015*
Diameter	1	-5.4928	2.7012	4.1350	0.0420*
Order	1	-0.6292	0.9656	0.4246	0.5147
Root age	1	-0.0135	0.0078	2.9798	0.0843
Standing crop 31 July 1997					
Depth	1	-0.4241	0.3345	1.6079	0.2048
Diameter	1	-1.9857	1.8342	1.1720	0.2790
Order	1	-0.5451	0.3977	1.8787	0.1705
Root age	1	-0.0105	0.0043	6.1360	0.0132*

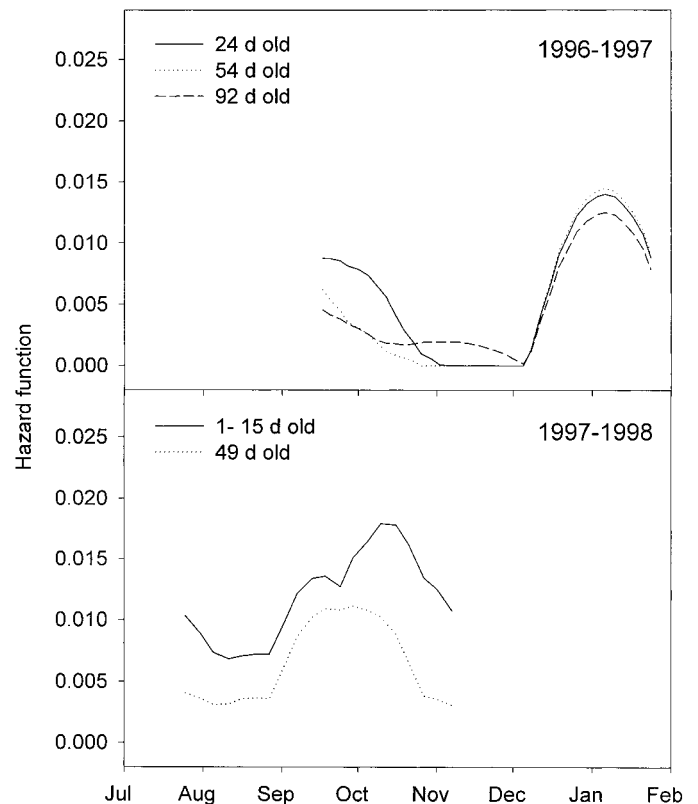


Fig. 6. Smoothed hazard function plots for roots of different ages drawn from the fine root standing crops present on (a) 5 August 1996 and (b) 30 May 1997. The estimated age of the roots in each group on the day when analyses began is shown. Hazard function estimates were generated using the baseline statement of PROC PHREG in SAS, with age class as the stratifying variable (see MATERIALS AND METHODS).

root population rather than true differences in the risk of mortality with age.

Hazard function plots of different age classes revealed that the timing of high-risk periods was generally not related to root age, although there was a transient effect of age on the hazard during the early autumn of 1996. This pattern suggests that factors acting above the level of the individual root control the timing of mortality, but that susceptibility to those factors may, at times, be a function of individual root age.

We cannot rule out the possibility that the significant effects of root age that we observed in some groups and at some points during the season resulted from additional aspects of fine-root heterogeneity that our model did not incorporate. However, anatomical and physiological changes that accompany root aging may also play a role in reducing the mortality rate of older roots. Cortical browning and senescence (McKenzie and Peterson, 1995), suberization (Scott, 1928; Head, 1966), and development of secondary vasculature (Pratt, 1990) may make roots more fibrous, less palatable to herbivores, and better able to resist the effects of drying soil. In apple, a relationship between the age-dependent process of root browning and decreased root mortality rate has been observed (Wells and Eissenstat, 2001). We did not include browning in the present study because, as a time-dependent covariate, it would have prevented us from generating estimates of the baseline hazard function (Allison, 1995). Nonetheless, many roots in our study did turn brown with age, and this process may have been associated with a decrease in the hazard of older roots.

Root morphology and the risk of mortality—The position of the root within the branched hierarchy of the root system appeared to be an important determinant of life span: apparent first-order roots had median survival times that were less than half of those of higher-order roots. This result is in agreement with that of Reid, Sorenson, and Petrie (1993) whose study showed longer life spans for higher-order roots of kiwifruit (*Actinidia deliciosa*). Furthermore, it is likely that our results underestimated the effect of root order on the hazard. The limited viewing area of the minirhizotrons did not permit us to view the full length of each root in the study, and many roots included in the “apparent first-order” category may have had laterals that we could not observe.

Differences in longevity among root orders may reflect differences in their physiological roles. While first-order roots function primarily in the uptake of water and nutrients, higher-order roots have the additional functions of transport to and from lateral roots and of new lateral root production. The loss of a higher-order root entails the loss of all lower-order roots that depend upon it, as well as the loss of the potential to produce new roots for exploration of the surrounding soil volume. It is therefore not surprising that higher-order roots were maintained for 200 d or more and that a significant portion of them appeared to function for more than one growing season. Despite their semipermanent nature, most higher-order roots in our study were quite fine, with mean diameters of 0.3–0.4 mm.

Higher-order roots tended to be of larger diameter than apparent first-order roots in 1996, although there was no significant relationship between order and diameter in 1997. Controlling for the effects of root order, root diameter was also a strong predictor of mortality in both years of our study. Smaller diameter roots may be intrinsically more vulnerable to environmental pressures such as parasitism (Graham, 1995) and

drought stress (Espeleta, Eissenstat, and Graham, 1999). Higher construction costs (Peng et al., 1993) and respiration rates (Peng et al., 1993; Pregitzer et al., 1997) of smaller-diameter fine roots may make them expensive to maintain in areas where soil depletion zones have developed (Nye and Tinker, 1977) or during times when aboveground sinks for carbon are particularly strong. Nonetheless it should be noted that difficulty in assigning root orders limits our ability to fully separate the effects of order from those of diameter.

Root depth and the risk of root mortality—The risk of mortality decreased with soil depth in 1996 and 1997. Longer root life span with depth has been also reported in Volkamer lemon (*Citrus volkameriana*; Kosola, Eissenstat, and Graham, 1995), *Bouteloua gracilis* (Gill et al., 2001), and a Swiss grassland (Arnone et al., 2000), although Hendrick and Pregitzer (1992) noted greater survivorship among shallower roots in sugar maple (*Acer saccharum*). The cause of the depth effect is not clear, but it is likely that roots at depth experience fewer fluctuations in soil temperature and water availability (Eissenstat and Yanai, 1997), two factors that have frequently been suggested to affect root life span and mortality (Huck, Hoogenboom, and Peterson, 1987; Hendrick and Pregitzer, 1993). The orchard floor surrounding the trees in our study had been cleared of weeds through the application of herbicides, and as a result, the bare soil surface was fully exposed to incoming solar radiation. The heating and drying effect of direct sunlight on the upper soil layers may have negatively impacted root function and survival, especially during summer periods of high temperature and low rainfall.

Patterns of fine-root production and mortality—Because fine-root birth and mortality occurred simultaneously in our system, the cumulative number of fine roots produced was two to three times greater than the average number of roots visible during the growing season. In 1997, rates of root production and mortality were evenly matched, and there was little accumulation of fine-root biomass despite ongoing new-root production. These results underscore the importance of nondestructive, demographic methods for quantifying fine-root production: measurements based on the root length present in sequential soil cores would have substantially underestimated fine-root production in our system, particularly in 1997.

In both years, root numbers declined in the fall and remained low over winter. Low fine-root numbers in fall and winter have been widely reported (Atkinson, 1985; Joslin and Henderson, 1987; Hansson, Aifen, and Andren, 1992; Hendrick and Pregitzer, 1992; Ruess, Hendrick, and Bryant, 1998), suggesting that significant root loss at the end of the growing season may be common in deciduous species. Hormonal signaling, redirection of carbon and nutrients to storage tissues, and extrinsic biotic and abiotic pressures are all potential mechanisms of autumn root shedding that deserve further investigation.

Effects of minirhizotron installation on root production—Recently, Joslin and Wolfe (1999) provided evidence that the soil disturbance and root damage that accompany minirhizotron installation can result in uncharacteristically high levels of root production during the season following installation. Their data show threefold greater root length production in the year following minirhizotron installation compared to that observed 3 yr afterwards. The magnitude of root production in

the third year was similar to that measured by coring of undisturbed bulk soil, suggesting that the disturbance effect was transient.

Higher root production in the year following tube installation was also observed in our study. In 1996 (the year when tubes were installed), an average of 60 new roots were observed per tree, whereas only 20 new roots per tree were observed in 1997. It is likely that higher root production in 1996 occurred in response to disturbance and damage. Our data suggest that roots produced in 1996 were morphologically and physiologically similar to those produced later in the experiment. The root population produced in 1996 had similar percentages of roots in all age, depth, diameter, and order categories compared to that produced in 1997, and the effects of these variables on root longevity were similar in both years. Nonetheless, we cannot rule out the possibility that the roots observed in the first (and perhaps the second) year of this study may not have recovered from disturbance and may not have been representative of the root population in undisturbed soil.

Implications—We found little evidence to support the hypothesis that fine roots exhibit a classic Type III pattern of survivorship. While root age had an apparently strong effect on the risk of mortality when considered alone, this effect was largely due to differential rates of mortality among roots of different order, diameter, and depth. This result underscores the importance, now widely acknowledged (Pregitzer et al., 1997, 1998; Tingey et al., 1997; Gill et al., 2001; Wells and Eissenstat, 2001) of recognizing and incorporating aspects of fine-root heterogeneity into studies of belowground plant and ecosystem function. The timing of high-risk periods was generally not related to root age: high-risk periods tended to occur for all ages of roots simultaneously. This pattern suggests that the factors that drive fine-root mortality in our system are seasonal in nature, operating above the level of the individual root. However, the degree to which individual roots respond to these factors is, at times, a function of a root age. To better understand patterns of fine-root turnover in this and other systems, we will need to identify the processes that drive fine-root loss and examine how these large-scale phenomena interact with individual root characteristics to produce the patterns of mortality that we observe in nature.

LITERATURE CITED

- AERTS, R., F. BERENDSE, N. M. KLERK, AND C. BAKKER. 1989. Root production and root turnover in two dominant species of wet heathlands. *Oecologia* 81: 374–378.
- ALLISON, P. D. 1995. Survival analysis using the SAS system: a practical guide. SAS, Cary, North Carolina, USA.
- ARNONE, J. A., J. G. ZALLER, E. SPEHN, P. A. NICKLAUS, C. E. WELLS, AND C. KORNER. 2000. Dynamics of fine root populations in a native grassland: effects of elevated atmospheric CO₂. *New Phytologist* 147: 73–85.
- ATKINSON, D., AND S. A. WILSON. 1979. The root–soil interface and its significance for fruit tree roots of different ages. In J. L. Harley and R. S. Russell [eds.], *The soil–root interface*, 259–271. Academic Press, London, UK.
- CALDWELL, M. M., AND L. B. CAMP. 1974. Belowground productivity of two cool desert communities. *Oecologia* 17: 123–130.
- CANTOR, A. B. 1997. Extending SAS survival analysis techniques for medical research. SAS, Cary, North Carolina, USA.
- CHENG, W., D. C. COLEMAN, AND J. E. BOX. 1990. Root dynamics, production and distribution in agroecosystems on the Georgia Piedmont using minirhizotrons. *Journal of Applied Ecology* 27: 592–604.
- COX, D. 1972. Regression models and life tables. *Journal of the Royal Statistical Society* 34: 187–220.
- DEEVEY, E. S. 1947. Life tables for natural populations of animals. *Quarterly Review of Biology* 22: 283–314.
- EISSENSTAT, D. M., AND R. D. YANAI. 1997. The ecology of root life span. *Advances in Ecological Research* 27: 1–60.
- ESPELETA, J. F., D. M. EISSENSTAT, AND J. H. GRAHAM. 1999. Citrus root responses to localized drying soil: a new approach to studying mycorrhizal effects on the roots of mature trees. *Plant and Soil* 206: 1–10.
- FITTER, A. H., J. D. GRAVES, G. K. SELF, T. K. BROWN, D. S. BOGIE, AND K. TAYLOR. 1998. Root production, turnover and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. *Oecologia* 114: 20–30.
- GILL, R. A., I. C. BURKE, W. K. LAURENROTH, AND G. MILCHUNAS. 2001. in press. Longevity and turnover of roots in the shortgrass steppe: influence of diameter and depth. *Plant Ecology*
- GOTELLI, N. J. 1995. A primer of ecology. Sinauer, Sunderland, Massachusetts, USA.
- GRAHAM, J. H. 1995. Root regeneration and tolerance of citrus rootstocks to root rot caused by *Phytophthora nicotianae*. *Phytopathology* 85: 111–117.
- GRIER, C. C., K. A. VOGT, M. R. KEYES, AND R. L. EDMONDS. 1981. Biomass distribution above- and below-ground in young and mature *Abies amabilis* zone ecosystems in the Washington Cascades. *Canadian Journal of Forest Research* 11: 155–167.
- HANSSON, A., Z. AIFEN, AND O. ANDREN. 1995. Fine-root production and mortality in degraded vegetation in Horqin sandy rangeland in Inner Mongolia, China. *Arid Soil Research and Reports* 9: 1–13.
- HEAD, G. C. 1966. Estimating the seasonal changes in the quantity of white unsuberized root on fruit trees. *Journal of Horticultural Science* 41: 197–206.
- HENDRICK, R. L., AND K. S. PREGITZER. 1992. The demography of fine roots in a northern hardwood forest. *Ecology* 73: 1094–1104.
- HENDRICK, R. L., AND K. S. PREGITZER. 1993. Patterns of fine root mortality in two sugar maple forests. *Nature* 361: 59–61.
- HUANG, B., AND P. S. NOBEL. 1992. Hydraulic conductivity and anatomy for lateral roots of *Agave deserti* during root growth and drought-induced abscission. *Journal of Experimental Botany* 43: 1441–1449.
- HUCK, M. G., G. HOOGENBOOM, AND C. M. PETERSON. 1987. Soybean root senescence under drought stress. In H. M. Taylor [ed.], *Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics*, 109–121. Agronomy Society of America, Madison, Wisconsin, USA.
- JACKSON, R. B., H. A. MOONEY, AND E. D. SCHULZE. 1997. A global budget for fine root biomass, surface area, and mineral nutrients. *Proceedings of the National Academy of Sciences, USA* 94: 7362–7366.
- JOSLIN, J. D., AND G. S. HENDERSON. 1987. Organic matter and nutrients associated with fine root turnover in a white oak stand. *Forest Science* 33: 330–346.
- JOSLIN, J. D., AND M. H. WOLFE. 1999. Disturbances during minirhizotron installation can affect root observation data. *Soil Society of America Journal* 63: 218–221.
- KOSOLA, K. R., D. M. EISSENSTAT, AND J. H. GRAHAM. 1995. Root demography of mature citrus trees: the influence of *Phytophthora nicotianae*. *Plant and Soil* 171: 283–288.
- MAJDI, H., AND P. KANGAS. 1997. Demography of fine roots in response to nutrient applications in a Norway spruce stand in southwestern Sweden. *Ecoscience* 4: 199–205.
- McKENZIE, B. E., AND C. A. PETERSON. 1995. Root browning in *Pinus banksiana* Lamb. and *Eucalyptus Pilularis* Sm. 1. Anatomy and permeability of the white and tannin zones. *Botanica Acta* 108: 127–137.
- NYE, P. H., AND P. B. TINKER. 1977. Solute movement in the soil–root system. Blackwell Scientific, Oxford, UK.
- PEARL, R. 1927. The growth of populations. *Quarterly Review of Biology* 2: 532–548.
- PENG, S., D. M. EISSENSTAT, J. H. GRAHAM, K. WILLIAMS, AND N. C. HODGES. 1993. Growth depression of mycorrhizal citrus at high phosphorus supply: analysis of carbon costs. *Plant Physiology* 101: 1063–1071.
- PRATT, C. 1990. Apple trees: morphology and anatomy. *Horticultural Reviews* 12: 265–305.
- PREGITZER, K. S., M. E. KUBISKE, C. K. YU, AND R. L. HENDRICK. 1997. Relationship among root branch order, carbon and nitrogen in four temperate species. *Oecologia* 111: 302–308.

- PREGITZER, K. S., M. J. LASKOWSKI, A. J. BURTON, V. C. LESSARD, AND D. R. ZAK. 1998. Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology* 18: 665–670.
- REID, J. B., I. SORENSON, AND R. A. PETRIE. 1993. Root demography in kiwifruit (*Actinidia deliciosa*). *Plant, Cell and Environment* 16: 949–957.
- ROGERS, W. S. 1929. Root studies VIII. Apple root growth in relation to rootstock, soil, seasonal and climatic factors. *Journal of Pomology and Horticultural Science* 17: 99–130.
- RUESS, R. W., R. L. HENDRICK, AND J. P. BRYANT. 1998. Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. *Ecology* 79: 2706–2720.
- SANTANTONIO, D., AND J. C. GRACE. 1987. Estimating fine root production and turnover from biomass and decomposition data: a compartment-flow model. *Canadian Journal of Forest Research* 17: 900–908.
- SAS. 1997. SAS version 6.12. SAS Institute, Cary, North Carolina, USA.
- SCHOETTLE, A. W., AND T. J. FAHEY. 1994. Foliage and fine root longevity of pines. *Ecological Bulletins* 43: 136–153.
- SCOTT, L. I. AND J. H. PRIESTLEY. 1928. The root as an absorbing organ. I. A reconsideration of the entry of water and salts in the absorbing region. *New Phytologist* 28: 125–141.
- TINGEY, D. T., D. L. PHILLIPS, M. G. JOHNSON, M. J. STORM, AND J. T. BALL. 1997. Effects of elevated CO₂ and N fertilization on fine root dynamics and fungal growth in seedling *Pinus ponderosa*. *Environmental and Experimental Botany* 37: 73–83.
- VOGT, K. A., C. C. GRIER, AND D. J. VOGT. 1986. Production, turnover and nutritional dynamics of above- and belowground detritus of world forests. *Advances in Ecological Research* 15: 303–307.
- WELLS, C. E., AND D. M. EISSENSTAT. 2001. Marked differences in survivorship among apple roots of different diameters. *Ecology* 82: 882–892.