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MARKED DIFFERENCES IN SURVIVORSHIP AMONG APPLE ROOTS OF DIFFERENT DIAMETERS

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Abstract. Fine roots are responsible for a substantial fraction of terrestrial net primary productivity, and a better understanding of fine root production and turnover is crucial to improving global carbon and nutrient cycling models. In most studies, roots less than 1 or 2 mm in diameter (''fine roots'') have been treated as structurally and physiologically identical individuals. We used minirhizotron data from 16-yr-old apple (*Malus domestica*) trees to investigate differences in life span and life history among fine roots whose diameters differed by tenths of a millimeter. We also introduced the use of Cox proportional hazards regression models to assess the effects of multiple covariates on root mortality.

Overwinter survivorship differed markedly among diameter classes in both years: 3–12% for roots <0.3 mm in diameter, 30% for roots 0.3–0.5 mm in diameter, and 55–60% for roots 0.5–1.1 mm in diameter. Diameter was also shown to be negatively related to the risk of root mortality using a proportional hazards regression ($P < 0.0001$ in 1994–1995, $P < 0.030$ in 1995–1996). Smaller diameter fine roots were more likely to be found in densely proliferated patches, while the majority of larger diameter fine roots were found alone or with a single neighboring root. Number of neighbors was shown to be positively correlated ($P < 0.0001$) with the risk of root mortality in 1994–1995. Pigmentation had a marginally significant ($P < 0.053$ in 1994–1995, $P < 0.078$ in 1995–1996) negative effect on the risk of root mortality. Tetrazolium staining of brown fine roots harvested in March of 1996 indicated that 90% were viable. The majority of roots which survived from October to May in both years were >0.5 mm in diameter and brown, and these roots gave rise to new white laterals during the spring root flush. Based on the considerable differences in morphology and life history within the class of roots <1 mm in diameter, we advocate the use of a functional definition for the fine root whenever possible.

Key words: *apple roots; fine roots;* Malus; *minirhizotron; root demography; root diameter; root life span; survival analysis.*

INTRODUCTION

In most studies of the root system, all roots less than 1 or 2 mm in diameter are treated as a single class of structurally and physiologically identical individuals (e.g., Kosola et al. 1995). However, there is evidence to suggest that differences in form and function exist within this single size class of roots. Recent work by Pregitzer and others (Pregitzer et al. 1997, 1998) has shown differences in C/N ratio and respiration rate among roots of different diameters within the fine root system of sugar maple, indicating higher levels of metabolic activity in the highest order, smallest diameter roots.

Fine roots play an important role in both whole-plant carbon budgets and ecosystem-level carbon and nutrient cycling. The growth and maintenance of fine roots have been estimated to consume 50% of daily photosynthate in vegetatively growing crop plants (Lambers 1987), while as much as 67% of annual net primary production can be allocated to fine roots in some forest ecosystems (Grier et al. 1981, Santantonio and Grace 1987). The mortality and decomposition of fine roots has been shown to return nitrogen to the soil in amounts equaling or exceeding that from leaf litterfall (Vogt et al. 1986). Life spans of individual fine roots have been estimated to range from range from 14–28 d in apple (Atkinson 1985) to 125–340 d in sugar maple (Hendrick and Pregitzer 1992, 1993). With a low C/N ratio and relatively rapid rate of turnover, live fine root tissue constitutes a dynamic carbon pool equal in magnitude to 5% or more of total atmospheric carbon (Jackson et al. 1997).

We used minirhizotron data from 16-yr-old apple (*Malus domestica*) trees to evaluate the hypothesis that all fine roots <1 mm in diameter have similar patterns of survivorship during autumn and winter regardless of their diameter. While it is intuitively obvious that large diameter woody structural roots will have longer life spans than small feeder roots, differences in survivorship have not previously been shown for fine roots whose diameters differ by only tenths of a millimeter.

In addition, we introduced the use of a Cox proportional hazards regression technique that permits the analysis of the effects of multiple covariates on root longevity. We investigated the relationships among di-

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ameter, pigmentation, and number of neighboring roots and assessed the effects of these covariates on the risk of root mortality. We propose that understanding the differences in life history and physiology among classes of roots within a single root system will not only lead to a more functional definition of the ''fine root,'' but will also improve the design and interpretation of root system experiments.

Methods

Study site

The study was conducted at the Russell E. Larson Agricultural Research Center in Rock Springs, Pennsylvania, using six 20-yr-old ''Red Chief Delicious'' trees on M.26 rootstocks. Mean daily ambient temperature data for this site were provided by the Pennsylvania State University weather station. The soil at Rock Springs belongs to the Hagerstown series and is characterized by a 20-cm surface layer of dark brown silt loam and a 93-cm layer of reddish brown silty clay subsoil. The soil is moderately permeable and has a high available water capacity.

Eight clear butyrate observation tubes (minirhizotrons) were placed in the ground around each of the six trees at an angle of 30° from the vertical in July of 1994 (48 tubes total). The outer four tubes around each tree were 0.5 m in length and installed 1.3 m from the base of the trunk. The inner four tubes around each tree were 1 m in length and installed 0.67 m from the base of the trunk. The tubes were 6 cm in diameter and were etched with three vertical rows of thirty 1.8 × 1.2 cm windows (90 windows total per tube). The 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.

Videotaping and image processing

A miniaturized camera system and portable VCR (BTC-2 Minirhizotron Camera System, Bartz Technology, Santa Barbara, California, USA) were used to record video images of roots that had grown against the surface of the minirhizotron tubes at biweekly or monthly intervals. The videotapes were played back in the lab using a Sony Hi-8 video deck and a Macintosh 7500 computer, and images of all minirhizotron windows that contained roots were digitized, compressed, and stored. Image capture was performed using Apple Video Player software; images were compressed and stored in PICT format with Graphic Converter shareware (Lemke Software 1998).

Images of individual roots as they appeared on sequential video sampling dates were reviewed and used to construct a database of life history information for over 600 individual roots. For each root we noted the date of appearance, the date of death, the root diameter, the number of neighboring roots, and the degree of pigmentation. Roots were classified as dead when they disappeared or when they became blackened and shriveled. Root diameter was measured on the first date that a root appeared using Root Tracker software (David Tremmel, Duke University Phytotron, Durham, South Carolina, USA). Number of neighbors was defined as the number of other roots sharing a minirhizotron frame with an individual on the first day it appeared. Most information was recorded directly into a spreadsheet after examination of the images. This method allowed us to bypass the use of a separate image analysis program for all but the diameter measurements and resulted in considerable time savings.

Classification of cohorts

Root videotaping began in late October and was continued at approximately monthly intervals until bud break in May of the following year. In both years, the roots that were present on our first video date in October (26 October in 1994 and 30 October in 1995) were classified as belonging to ''cohort 1.'' These roots were considered to represent the fall standing crop of roots, although their date of production could not be estimated with certainty. The majority of cohort 1 roots were likely produced during the fall root flush which occurs in mid- to late September in our orchard (C. Wells, *personal observation*). Roots produced between the first and second (11 November in 1994 and 22 November in 1995) and between the second and third (25 November in 1994 and 15 January in 1996) video dates in each year were classified as belonging to cohorts 2 and 3, respectively. No new root production was observed between late November and April in either year. Analyses involving diameter classes, numbers of neighbors and pigmentation were performed using data from cohort 1 only, as later cohorts did not contain enough roots to permit division into separate classes.

The number of days between the date of first appearance and the date of 50% mortality is reported as the median survival time for each cohort. Roots in cohorts 2 and 3 were produced in the two weeks prior to their date of appearance in our videos, and therefore median survival times are estimates of median life span for these cohorts. The production dates of roots in cohort 1 were not known with certainty, and therefore the median survival times for these cohorts underestimate median life span and are not strictly comparable to the median survival times for cohorts 2 and 3.

Tetrazolium staining

Triphenyl tetrazolium chloride (TTC) vital staining was performed in March 1996 to assess the viability of pigmented roots that had survived over winter (Smith 1951, Steponkus and Lanphear 1967). Because there was no significant production of new fine roots by this date, we assumed that the roots that we har-

TABLE 1. Mean root diameter (±1 SD) and survivorship data for three cohorts of fall apple roots in 1994–1995 and 1995–1996.

Cohort (*n*)	Diameter (mm)	Mortality (d)†			Final survivorship (%)‡
		25%	50%	75%	
1994–1995					
1 (404)	0.32 ± 0.26	22	48	118	16
2 (37)	0.30 ± 0.16	12	36	105	16
3 (15)	0.30 ± 0.18	13	36	66	13
1995–1996					
1 (152)	0.33 ± 0.22	45	114	167	20
2 (34)	0.28 ± 0.14	45	84	119	6
3 (16)	0.27 ± 0.16	22	55	81	0

† Number of days until 25%, 50%, and 75% mortality were calculated by linear interpolation between observed data points of survivorship curves.

‡ Percentage of roots in each cohort that survived to May of the following year.

vested had survived from the previous fall. Fine root material (<1 mm diameter) was obtained from six soil cores, 20 cm deep and 10 cm in diameter, taken 0.67 m from the base of six trees on the same trellis row as those used in the minirhizotron experiment. Roots were immediately washed free of soil, and five 2–4 cm long segments of brown root were taken from each core for use in the TTC assay (30 root segments total). Each segment was placed in a test tube containing 2.0 mL of TCC phosphate buffer (300 mg 2,3,5-triphenyl tetrazolium chloride in 100 mL 0.05 M Na_2HPO_4-KH_2PO_4 buffer with 50 μL Triton X-100 added) and incubated in the dark for 48 h at 30°C. Root segments were then examined under a stereomicroscope for the presence of pink coloration caused by the enzymatic reduction of tetrazolium to formazan, an insoluble red precipitate.

Statistical analysis

Data from the video images were used to construct survivorship curves for three cohorts of autumn roots each year. Roots from cohort 1 were further divided into the following diameter classes: 0.1–0.2 mm, 0.2–0.3 mm, 0.3–0.5 mm, and 0.5–1.1 mm. Roots whose diameters were equal to one of the cutoff values were placed in the larger diameter category. Differences in survivorship among diameter classes were assessed by making pairwise comparisons between all diameter class combinations using the Wilcoxon test (Pyke and Thompson 1986, Fox 1993) as performed using SAS PROC LIFETEST (SAS Institute 1997).

A stratified Cox proportional hazards regression (SAS PROC PHREG) was performed to assess the effects of three covariates on root life span: diameter, pigmentation, and number of neighbors. Pigmentation, which changed from white to brown in a number of roots, was treated as a time-dependent covariate. The four inner and four outer minirhizotrons associated with an individual tree constituted a plot. Roots were assigned to plots based on the minirhizotron tube on which they appeared, and survival data were stratified by plot in order to control for potential effects of tube location on root life span.

We include here a brief review of the proportional hazards model as it applies to root life span data in order to facilitate subsequent interpretation of our results. For further details on the design and interpretation of proportional hazards models see Allison (1995) and Cantor (1997).

The term ''hazard'' refers to the instantaneous risk of mortality at time *t*, conditioned on survival to *t*. The hazard function, $h(t)$, is written mathematically as

$$h(t) = \lim_{\Delta t \to 0} \frac{\Pr\{t \le T < t + \Delta t \,|\, T \ge t\}}{\Delta t} \qquad (1)$$

where T is the failure time (or in our case, the date of death) for the individual in question. 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 which remains unspecified and a linear function of k covariates which is exponentiated (Allison 1995):

$$h_i(t) = h_0(t)\exp(\beta_1 x_{i1} + \ldots + \beta_k x_{ik}). \qquad (2)$$

PROC PHREG employs the method of partial likelihood (Cox 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. When interpreting the results of a PHREG analysis, it is important to remember that a negative parameter estimate indicates that increasing values of the covariate are associated with a decreasing risk of mortality. Likewise, a positive parameter estimate indicates that increasing values of a covariate are associated with an increasing risk of mortality. Also reported for each covariate is a risk ratio, defined as e^{β}. The risk ratio has several interpretations, depending on the nature of the covariate. For a covariate such as ''pigmentation'' which was assigned only two values (0 and 1), the risk ratio can be interpreted as the ratio of the hazard of a brown root (1) to that of a white root (0), controlling for all other covariates. For a quantitative covariate such as diameter, subtracting one from the risk ratio and multiplying it by 100 gives the estimated percent change in the hazard associated with a one unit change in the covariate, controlling for other covariates (Allison 1995).

RESULTS

The fine roots in our videos ranged from 0.09 to 1.1 mm in diameter, with a mean (±1 SD) diameter of 0.32 ± 0.26 mm in 1994–1995 and 0.32 ± 0.20 mm in 1995–1996 (Fig. 1). The majority of roots (69.3% in 1994–1995, 64.5% in 1995–1996) fell between 0.1 and 0.3 mm in diameter. Brown pigmentation developed in 10% of the roots in 1994–1995 and 44% of the roots in 1995–1996. Larger diameter roots were more likely to be pigmented. In 1994–1995 only 2% of the roots

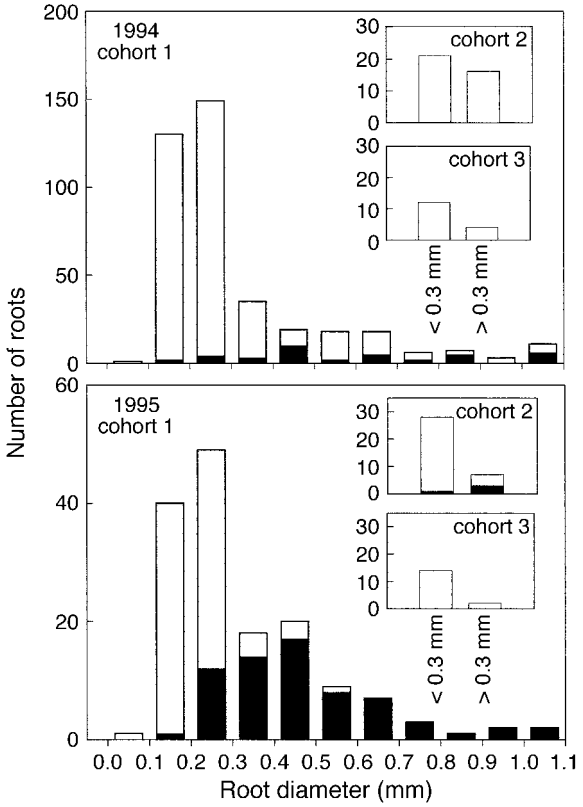

FIG. 1. Frequency distributions of fine root diameters for the fall standing crop of roots (cohort 1) in 1994–1995 and 1995–1996. Dark portions of bars represent pigmented roots. Diameter was measured on the first date that a root appeared. Insets: frequency distributions of fine root diameters for cohorts 2 and 3.

<0.3 mm in diameter were pigmented, compared with 35% of the roots >0.3 mm in diameter. A similar trend was observed in 1995–1996. There were more roots of diameter >0.3 mm and more pigmented roots in all diameter classes in 1995–1996. In neither year did we observe fine roots that increased in diameter with time, although in some cases roots decreased in diameter due to sloughing of epidermal and cortical tissue.

Fine root survivorship over winter was low: 13–16% in 1994–1995 and 0–20% in 1995–1996 (Table 1). Median survival times for fall root cohorts ranged from 36 to 48 d in 1994–1995 and from 55 to 114 d in 1995–1996. Median survival times for cohort 2 in both years represent the most accurate estimates of fine root median life span because cohort 2 was the largest group of roots whose production date was known with certainty.

The time course of root mortality differed between the two years of our study (Fig. 2). In 1994–1995, the bulk of fine root mortality took place in the fall. Sixty percent of the fine roots present on 26 October had died by 28 December, and an additional 14% died over the period between 28 December and 5 May. In 1995–1996, appreciable root mortality occurred during both fall and winter: 30% of the fall standing crop died between 30 October and 15 January, and another 40% died between 1 January and 3 May. In both years, there was very little root mortality in the spring; roots that survived over winter were maintained after the onset of higher temperatures.

Root diameter was significantly related to the risk of root mortality in 1994–1995 and 1995–1996 (Table 2). In both years, there was a large group of roots <0.3 mm in diameter whose overwinter survivorship was very low (3–4% in 1994–1995 and 5–12% in 1995–1996; Table 3) and whose median survival times were <2 mo (34–36 d in 1994–1995 and 45–57 d in 1995–1996; Fig. 3). Roots of diameter 0.3–0.5 mm were also unlikely to survive over winter (30% in 1994–1995 and 1995–1996; Table 3), but had considerably longer

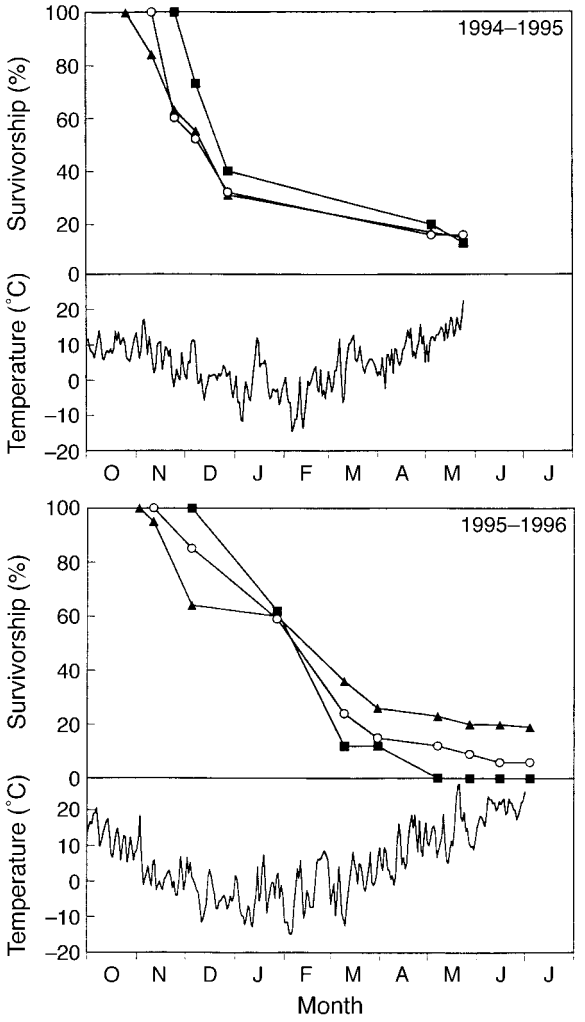

FIG. 2. Percentage survival of three fall root cohorts over winter in 1994–1995 and 1995–1996 (▲, cohort 1; ○, cohort 2; ■, cohort 3). Observation periods were between 26 October 1994 and 25 May 1995 and between 30 October 1995 and 6 July 1996.

TABLE 2. Results of proportional hazards regression for root survivorship data collected in 1994–1995 and 1995–1996.

a) 1994–1995

Variable	df	Parameter estimate	SE	Wald chi-square	*P*	Risk ratio
Diameter	1	−2.262529	0.41692	29.44979	0.0001	0.104
Neighbors	1	0.068305	0.01427	22.90875	0.0001	1.071
Browning	1	−0.491416	0.25443	3.73041	0.0534	0.612

b) 1995–1996

Variable	df	Coefficient estimate	SE	Wald chi-square	*P*	Risk ratio
Diameter	1	−2.003237	0.92399	4.70035	0.0302	0.135
Neighbors	1	−0.044616	0.05163	0.74679	0.3875	0.956
Browning	1	−0.535815	0.30427	3.10101	0.0782	0.585

Note: Analyses were performed using data from cohort 1 only (see *Methods*) in order to ensure adequate numbers of roots in all categories.

median survival times (97 d in 1994–1995 and 143 d in 1995–1996). Finally, roots between 0.5 and 1.1 mm in diameter often survived over winter (55% and 60%; Table 3) and had median survival times of 211 d or more.

Smaller diameter roots were likely to have large numbers of neighboring roots, while larger diameter roots were more likely to be found alone or with a single neighbor (Fig. 4). Controlling for the effects of diameter, number of neighbors had a significant ($P < 0.0001$) positive effect on the risk of root mortality in 1994–1995: each additional neighbor was associated with a 7.1% increase in the hazard, controlling for other covariates (Table 2). A significant relationship between number of neighbors and root life span was not observed in 1995.

Pigmentation had a marginally significant effect on the risk of root mortality ($P < 0.053$, 1994–1995; $P < 0.078$, 1995–1996); the hazard of mortality for brown roots was estimated to be 61.2% (1994–1995) and 58.5% (1995–1996) that of white roots, controlling for other covariates (Table 2).

In both years, the surviving fine root population present in the spring had a greater percentage of large diameter roots and pigmented roots relative to the initial fall population (Fig. 5; compare to Fig. 1). Significant production of new white roots did not occur until ~1 mo after bud break and flowering in May of both years (C. Wells, *personal observation*). The production of flowers and the initiation of the next year's fruit crop occurred when the fine root system consisted predominantly of roots that had survived from the previous fall. Tetrazolium staining of pigmented fine roots harvested from our orchard in March of 1996 indicated that the majority (90% of pigmented fine roots sampled) were viable. Video observations in the late spring and early summer indicated that older, pigmented roots gave rise to new, white laterals during the spring root flush.

DISCUSSION

Diameter classes within the fine root system

Although roots <1 mm in diameter are generally treated as a single homogeneous population, we have shown considerable variation in life history among

TABLE 3. Mean root diameter (±1 SD) and survivorship data for four diameter classes of fall apple roots in 1994–1995 and 1995–1996.

Diameter class (mm)	*n*	Diameter (mm)	Mortality (d)†			Final survivorship (%)‡
			25%	50%	75%	
1994–1995						
0.1–0.2	132	0.16 ± 0.03	19	34	56	3
0.2–0.3	153	0.24 ± 0.03	19	36	58	4
0.3–0.5	53	0.38 ± 0.06	46	97	>211	30
0.5–1.1	66	0.66 ± 0.36	56	>211	>211	55
1995–1996						
0.1–0.2	41	0.15 ± 0.03	35	57	117	5
0.2–0.3	49	0.24 ± 0.03	32	45	146	12
0.3–0.5	38	0.40 ± 0.07	110	143	>242	30
0.5–1.1	24	0.71 ± 0.25	151	240	>242	60

Note: All roots were drawn from cohort 1 of each year (see *Methods*).

† Number of days until 25%, 50%, and 75% mortality were calculated by linear interpolation between observed data points of survivorship curves.

‡ Percentage of roots in each class that survived to May of the following year.

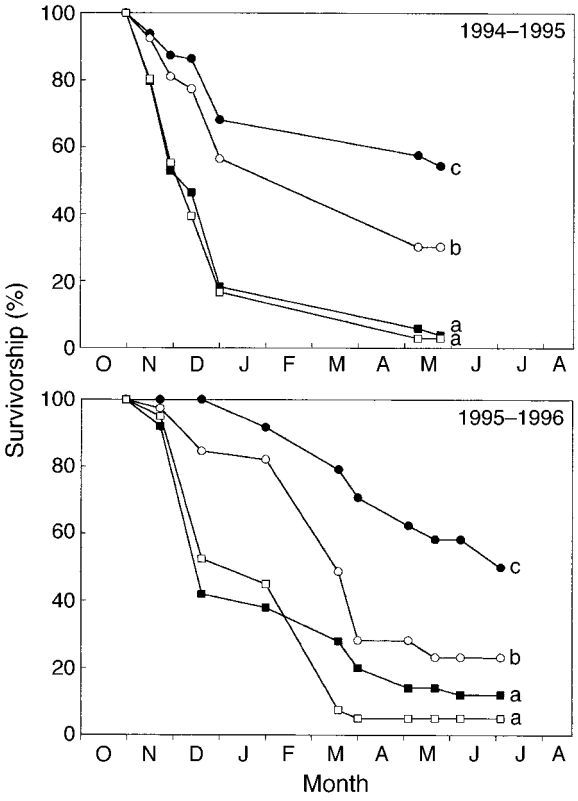

FIG. 3. Percentage survival of cohort 1 roots from four diameter classes in 1994–1995 and 1995–1996 (□, 0.1–0.2 mm; ■, 0.2–0.3 mm; ○, 0.3–0.5 mm; ●, 0.5–1.1 mm). Survivorship curves followed by different letters are significantly different ($P < 0.0001$; Wilcoxon test).

roots whose diameters differ by only tenths of a millimeter. Not all fine roots in our study turned over rapidly: those >0.5 mm in diameter were likely to survive over winter and function for more than one growing season. Roots <0.3 mm in diameter, on the other hand, were unlikely to survive over winter and had median survival times of under two months. These smaller diameter fine roots made up the majority of the fine root population and tended to be found in densely proliferated patches. They were also less likely than larger diameter roots to develop pigmentation. In addition to the differences in life span reported here, differences in tissue nitrogen content (McClaugherty et al. 1984, Camire et al. 1991, Pregitzer et al. 1997), root respiration rate (Pregitzer et al. 1998), and decomposition rate (Berg 1984, McClaugherty et al. 1984, Camire et al. 1991) have also been linked to fine root diameter in a number of tree species. Taken together, these results suggest that the fine root system may be more complex than is often appreciated.

The importance of fine root demographic data for modeling terrestrial carbon, nitrogen, and hydrological cycles is increasingly recognized (Hendricks et al. 1993, Jackson et al. 1997). Belowground production represents a significant fraction of annual net primary productivity and can substantially exceed aboveground production in some ecosystems (Caldwell and Camp 1974, Grier et al. 1981, Santantonio and Grace 1987). Belowground root mortality returns considerable amounts of carbon and nitrogen to the soil (Cox et al. 1978, Hendricks et al. 1993), exceeding the amounts returned by leaf litterfall in some systems (Vogt et al. 1986). The widespread use of minirhizotrons has simplified the collection of root production and mortality data and added to our knowledge of belowground processes. Nonetheless, while the fine root system is clearly composed of roots with different diameters, orders, ages, and degrees of pigmentation; it is generally treated as a single entity. The results presented here suggest that the practice of combining all roots <1 mm in diameter into one class may obscure ecologically relevant information about the timing and consequences of root turnover.

For example, nutrient inputs from roots to soil may be influenced not only by absolute root mortality rates but also by which roots die during a given interval. In our study, 35% of the roots present in the fall standing crop on 3 October 1995 had died by 28 November. However, these deaths were not evenly distributed among diameter classes. Of the 54 roots that died during that interval, 91% were 0.1–0.3 mm in diameter, 9% were 0.3–0.5 mm in diameter, and none were >0.5 mm in diameter. Assuming that diameter classes of roots also differ in their tissue nutrient concentrations (Pregitzer et al. 1998) and resistance to decomposition (Pregitzer et al. 1997), then the proportion of roots in each diameter class that die over a given interval could strongly affect nutrient input to the soil.

Faster rates of decomposition in smaller diameter roots coupled with the difficulty of recovering them from the soil may also help to explain the contradictory results obtained by various methods of root analysis. Pregitzer et al. (1997) point out that fine root decomposition appears to take place very rapidly when measured with minirhizotrons but has been estimated to take several years when measured using buried bag techniques. They suggest that buried bag studies may not account for the large number of very small diameter roots which are difficult to remove intact from the soil but which are readily visible on minirhizotrons. Therefore, most of the roots which are measured in bag studies, while defined as fine (<1 or 2 mm), may in fact be semipermanent components of the root system whose higher levels of structural tissue make them more resistant to decomposition.

The recognition that all fine roots are not alike has implications for the way we think about root life span. Fine roots are often considered to be analogous to leaves; both are ephemeral tissues whose primary function is the acquisition of resources. However, unlike leaves, all but the most distal links of the fine root system have additional functions such as the transport

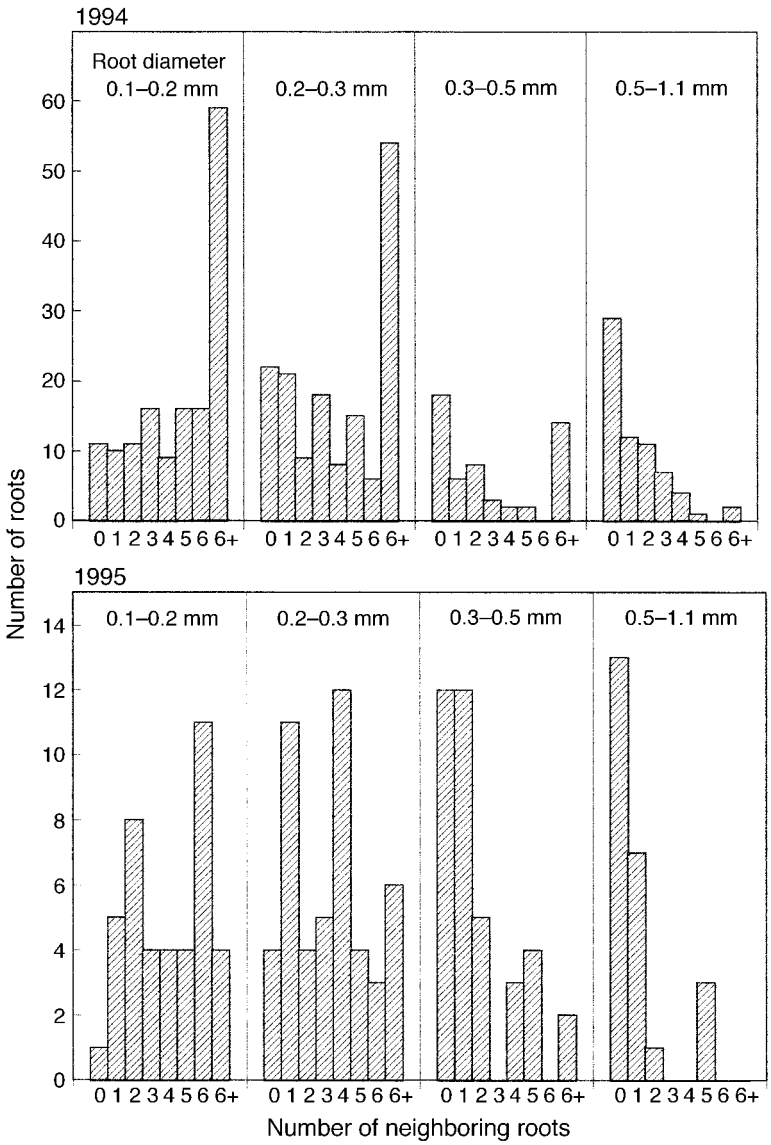

FIG. 4. Frequency distribution of roots with different numbers of neighbors in the fall of 1994 and 1995. Roots are divided into four diameter classes. Data were taken from cohort 1 in both years. The number of roots in each diameter class that shared a minirhizotron window with 1–6 or more (6+) neighboring roots is shown.

of materials to and from lateral roots and the production of new laterals. In some ways, lower order roots (i.e., roots with at least one hierarchical level of dependent laterals) may be more analogous to stems than leaves. Lower order roots are generally of greater diameter (Fitter 1991, Pregitzer et al. 1997; C. Wells, *unpublished data*). While the limited viewing area of the minirhizotrons did not permit us to categorize our roots by order, it is likely that the larger diameter, longer lived roots in our study were of lower order and supported additional lateral roots. The multiple functions of lower order roots imply that we may not be able predict their life span solely from models based on resource acquisition efficiency at the single root level (Yanai et al. 1995).

Proportional hazards model for root life span data

In previous work, we have used differences in median life span estimates as rough indicators of differences in survivorship among populations (e.g., Kosola et al. 1995). Simple survival analysis tools such as the log-rank and Wilcoxon tests have also been employed to test for differences in survivorship curves among treatment groups (Pregitzer et al. 1993). Recently, Ruess et al. (1998) demonstrated the utility of the MARK parameter estimation program in generating

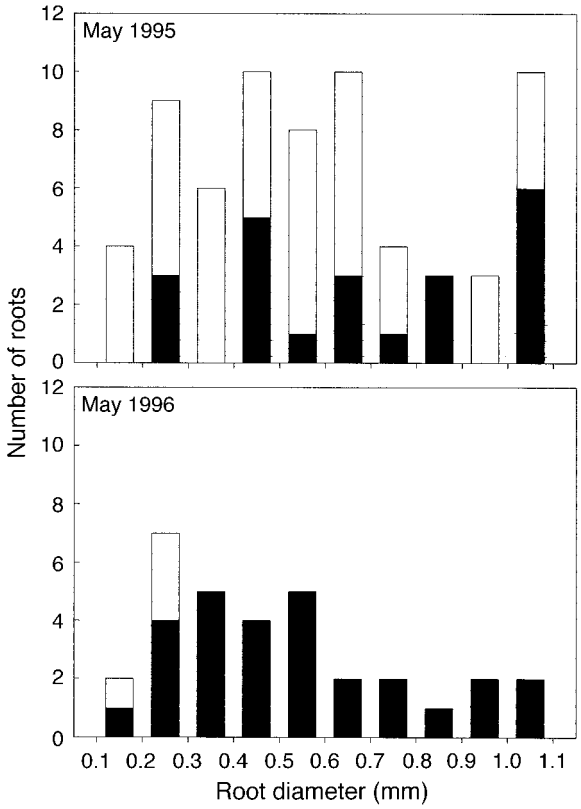

FIG. 5. Frequency distributions of fine root diameters for cohort 1 roots that survived over winter and were present at bud break the following May. Dark portions of bars represent pigmented roots.

survival and decomposition estimates for fine roots in an Alaskan taiga forest.

We used a Cox proportional hazards regression approach (SAS PROC PHREG) to assess the effects of diameter, color, and number of neighbors on fine root life span. This method allowed us to quantify the effects of individual covariates on fine root mortality while controlling for the effects of other covariates. In addition, it did not require us to specify an underlying probability distribution of survival times and was capable of handling both time-dependent covariates and stratifying variables.

The proportional hazards model can been interpreted biologically to mean that all individuals in the population experience periods of high hazard at the same time, but that the likelihood of an individual succumbing during a period of high hazard is dependent upon its set of covariates. This contrasts with accelerated failure time models in which individuals with ''bad'' sets of covariates enter periods of high risk sooner (Fox 1993). This model was particularly appropriate for our data, since roots at our study site were likely to experience periods of high hazard at approximately the same time due to shifts in temperature, moisture, herbivore abundance, etc. We did not expect covariates such as diameter or color to change the timing of high risk periods, but we did expect them to influence the chance that a root failed during a period of high risk.

The influence of neighboring roots

Number of neighboring roots had a significant, negative effect on root life span in 1994–1995: each additional neighbor was associated with a 7.1% increase in the hazard of mortality, controlling for other covariates. This is the first reported instance of density-dependent mortality in roots, although the factors responsible for it are not clear and a similar relationship was not found in 1995–1996 (Table 2). In 1994–1995, there were a larger number of densely proliferated patches, and some roots had as many as 17 neighbors. It may be that the effects of neighboring roots do not become apparent until roots reach very high densities. The larger number of roots produced in 1994–1995, as well as the greater number of densely proliferated patches, may have resulted from compensatory root regrowth in response to wounding during the minirhizotron installation process (Joslin and Wolfe 1999). Soil disturbance may have also resulted in the formation of transient high nutrient patches which encouraged root proliferation. Density-dependent root mortality observed in 1994–1995 may therefore not be representative of root turnover under undisturbed conditions.

A number of factors could potentially contribute to higher mortality for more crowded roots. Highly proliferated roots may be connected to the same lower-order root, and the death of this parent root may result in the simultaneous death of all its dependent laterals. Root crowding may also result in overlapping depletion zones for more immobile nutrients such as phosphorus, causing roots to become less efficient and more likely to be shed (Nye and Tinker 1977, Eissenstat and Yanai 1997). High density roots may be more apparent to soil herbivores and pathogens. Finally, high-density root patches may result from the rapid proliferation of cheaply built roots in response to wounding or localized resource patches, and these roots may be intrinsically more vulnerable to biotic and abiotic stresses. All of these possibilities deserve further study.

The significance of root pigmentation

Brown pigmentation tended to decrease the risk of root mortality. Browning has been observed previously in apple and numerous other woody species (Rogers 1929, Atkinson 1985, Hendrick and Pregitzer 1992, Reid et al. 1993, Wang et al. 1995). It has been variously attributed to root death (see Wang et al. 1995), root cortical cell death or senescence (Rogers 1968, McKenzie and Peterson 1995, Spaeth and Cortes 1995), root suberization (Head 1966), and accumulation of condensed tannins (McKenzie and Peterson 1995). In numerous studies, very dark brown coloration has been

used as a criterion for determining the date of root death, although this method has been shown to be particularly inaccurate for tree species (Wang et al. 1995). Brown coloration in and of itself is not indicative of root death, as has clearly been shown by numerous authors (Nightingale 1935, Richards and Considine 1981, McKenzie and Peterson 1995), and its use as an indicator of death undoubtedly results in an underestimation of root life span. Brown roots clearly retain the ability to take up water and nutrients in many species (Crider 1933, Chapman and Parker 1942, Kramer 1946, Atkinson and Wilson 1979), although they are generally considered inferior to white roots in this regard (Head 1966).

Vital staining of brown roots that had overwintered in our orchards revealed that 90% of the roots were viable. These results suggest that browning is not indicative of root death in apple, but is simply associated with root aging and the transition of the root to a different physiological state. Browning, if caused by phenolic accumulation, may confer benefits to the root such as reduced palatability to soil organisms.

The apple fine root system over winter

In general, fine root survivorship over winter was low: 13–16% in 1994 and 0–20% in 1995. These observations are consistent with the pattern of fine root mortality reported for both apple and other tree species, in which fine root length or root counts peak during the growing season and decline during the fall and winter (McClaugherty et al. 1984, Atkinson 1985, Joslin and Henderson 1987, Hendrick and Pregitzer 1992, Hansson et al. 1995, Katterer et al. 1995, Ruess et al. 1998). The median survival times calculated here for apple fine roots fall within the range of 28–84 d reported by Atkinson (1985) for apple roots produced in October and November, and are considerably longer than the median life spans of 14–21 d that he observed for roots produced earlier in the year. These numbers put apple at the low-to-middle range of the tree species whose root life spans have been measured in the field (reviewed by Eissenstat and Yanai 1997).

The time course of fine root mortality differed between the two years of our study, as reflected both in the shapes of the survivorship curves (Fig. 2) and in the fine root median survival times (Table 3). In 1994, most fine root mortality was confined to the 2-mo interval between 26 October and 28 December, whereas in 1995 root mortality occurred at a lower, more constant rate throughout fall and winter. Several factors may have contributed to this year-to-year variation. Winter temperatures were considerably colder in 1995–1996 and the onset of cool temperatures also occurred earlier in the year. Lower temperatures are associated with both reduced root respiration (Marshall and Waring 1985, Palta and Nobel 1989, Eissenstat and Yanai 1997) and reduced activity of soil organisms (Head 1973, Brown and Gange 1990), two factors which have been suggested to play a role in root turnover. In addition, the roots we observed in 1995–1996 had fewer neighbors and were more likely to be brown; proportional hazards regression indicated that both of these factors were associated with a decreased risk of mortality (Table 2). As previously mentioned, root system characteristics observed in 1994–1995 may have been influenced by the disturbance of minirhizotron installation. The time course of root mortality observed in 1995–1996 may therefore better represent root dynamics in undisturbed soil.

The production of leaves and flowers in May of both years took place while the fine root system consisted almost entirely of overwintered roots from the previous fall. The extent to which uptake by these over-wintered roots contributed nitrogen and other nutrients for new leaf and flower production is unknown, although previous studies indicate that spring growth is supported primarily by nitrogen remobilized from bark proteins (Tromp and Ovaa 1973, Millard and Thomson 1989). Carbon isotope studies have indicated that the root system can serve as a source of carbohydrate for spring aboveground production in apple (Kandiah 1978); however, the specific role of over-wintered fine roots in spring carbohydrate partitioning has not been examined.

Defining the fine root: implications for future studies

The considerable variation in life span reported here for roots <1 mm in diameter leads us to question the appropriateness of defining the term ''fine root'' on an arbitrary basis of diameter alone. A more functional definition of the term might take into account the time scale of root turnover, assigning the category of ''fine root'' only to those size classes of root whose median life spans are relatively short. Clearly, the size classes exhibiting rapid turnover will differ among species, and ''fineness'' will need to be defined in a species-specific context. While not technically feasible in all experimental situations, such a definition would more accurately separate ephemeral from permanent roots and would reflect real differences in physiology and life history within the root population.

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