Multiple risk factors in root survivorship: a 4-year study in Concord grape

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Summary

• Minirhizotron techniques were used to examine root lifespan in *Vitis labruscana* (Concord grape) for roots born in four different years that varied in rainfall.

• Experimental vines were given irrigation (irrigated or not) and canopy pruning treatments (minimal or balanced). Root survival was assessed from 1997 through 2000 and analysed using Cox proportional hazards regression. Model covariates included pruning, irrigation, vine yield, soil depth, root diameter, time of root birth, and numbers of neighboring roots.

• Soil depth, root diameter and time of birth consistently influenced root lifespan in all years ($P < 0.05$). Deeper and coarser roots had longer lifespans. Roots born near bloom were shorter-lived than roots born later in the season. Pruning and irrigation influenced root lifespan in some years but their effects seemed to vary with growing-season environmental conditions.

• These data underscore the value of long-term studies in distinguishing factors that consistently affect root lifespan from those that change annually with environmental conditions, and emphasize the diversity in life histories of fine roots within a species.

Key words: root lifespan, root survival, root turnover, root browning, root pigmentation, *Vitis labruscana* (Concord grape), minirhizotron.

Introduction

Fine roots, the major water and nutrient absorbing structures in plants, are ephemeral, requiring substantial plant resources for production and maintenance. Jackson *et al.* (1997) estimated that as much as 33% of global annual net primary productivity in terrestrial ecosystems is devoted to fine root production, and the growth and maintenance of fine roots may use up to 50% of daily photosynthate in crop plants (Lambers, 1987). Therefore, fine root lifespan has important implications for individual plant growth, crop productivity, plant interactions, and below-ground carbon (C) and nutrient cycling. Yet, we have little understanding of the factors that determine how long roots live. Root lifespan varies widely within and among species and across ecosystems, but our ability to predict root lifespan for particular species or systems is poor (Eissenstat & Yanai, 1997). Multiple 'risk' factors for root mortality must be examined simultaneously to identify those with the strongest influence on root lifespan. Moreover, while it is widely recognized that yearly variation in plant productivity may be substantial, rarely are multiple cohorts of roots examined from birth to death over several years. We examined potential links between root lifespan and 11 different environmental, morphological and physiological variables in the perennial *Vitis labruscana* (Concord grape) in a 4-yr field study using minirhizotron techniques.

We also have a very limited understanding of the factors affecting root 'aging' under field conditions. Fine roots, like other plant tissues, undergo conspicuous physical changes during maturation and senescence. Pigmentation is one such change, and may be associated with suberization or breakdown of cortical or epidermal cells in grape vines, depending on where pigmentation occurs along the root axis (Richards & Considine, 1981). Pigmentation is frequently observed in

minirhizotron studies, and has been associated with a marked decrease in root respiration and cell metabolic activity in Concord grape, suggesting that pigmentation in this species signals the end of root functions requiring active metabolism (Comas *et al.*, 2000). The influence of a variable on root lifespan may change depending on whether lifespan is defined as the time from root birth to the onset of pigmentation, or as the time from root birth to the onset of blackening, shriveling or disappearance (Comas *et al.*, 2000). Therefore, both pigmentation and obvious root death were examined in this study.

Factors both internal and external to the plant may affect root lifespan and pigmentation. One example of an internal factor is C demand in the shoot (i.e. the stem, leaves and fruit). If shoot demand for C increases (e.g. through canopy pruning, herbivory, or heavy fruit set), plants may respond by changing root lifespan, root production, or both (Atkinson, 1980; Eissenstat & Yanai, 1997). This factor is particularly relevant for crops, and some studies have examined the responses of root production to changes in shoot C status, such as defoliation in grape (reviewed in Richards, 1983), or yield variations in apple (Head, 1969; Heim *et al.*, 1979), peach (Williamson & Coston, 1989), citrus (Eissenstat & Duncan, 1992) and pistachio (Rosecrance *et al.*, 1996). However, relatively little is known about the interactions between shoot C sinks and root lifespan, particularly for woody plants in the field. In addition, only a few studies have examined multiple influences on root lifespan simultaneously (Wells & Eissenstat, 2001; Wells *et al.*, 2002). This approach is important because root responses to C supply may be mediated by soil resource availability, interactions with other roots and morphological variation within the fine root system (Eissenstat *et al.*, 2000). Our experimental vines were part of a long-term canopy pruning and irrigation study, which provided a unique opportunity to work with mature plants subjected to canopy manipulations for 6 yr before starting root observations, and routinely experiencing a strong, variable shoot C sink through fruit set. In addition, growing-season rainfall varied strongly over the 4 yr of our study, allowing us to observe root survival and pigmentation patterns under a wide range of environmental conditions.

Materials and Methods

Study site and species

The 0.2-ha study site was located at Cornell University's Vineyard Laboratory in Fredonia, NY, USA. Soils were a very deep (> 3 m), very well-drained, Chenango gravelly loam that was relatively uniform across the plot. Study plants were mature, 25-yr-old *Vitis labruscana* Bailey cv. Concord grapevines with permanent arms 1.8 m above the ground and spaced at 2.4 m between vines and 2.7 m between rows. Sets of five adjacent vines within a row (vine plots) were chosen for experimental treatments, with a buffer vine on each end that received the same treatment but was not measured. Vine plots were given one of two pruning treatments (balanced or minimally pruned), and one of two irrigation treatments (irrigated or not) in a 2×2 factorial design (four treatment combinations, four vine plots per combination). A blocking factor was also included to account for any unseen soil heterogeneity, with each treatment combination occurring once in each block.

Pruning treatments were initiated in 1991 and done each winter after leaf fall. Balanced pruning consisted of leaving 44 buds per kilogram of pruned stems from the previous season's shoot growth, while minimally pruned vines were unpruned except for a hedge undercut at 1 m high to keep shoots off the ground. Minimally pruned vines averaged about 350 shoots per vine and developed more canopy leaf area earlier in the growing season than balanced pruned vines, which averaged about 90 shoots per vine. Minimally pruned vines therefore intercepted a greater percentage of available sunlight early in the growing season, although total light interception was similar in the two treatments by the end of the growing season because the final canopy sizes were similar (Lakso *et al.*, 1997; Lakso, 1999a). In dry years, minimally pruned vines also depleted soil water more rapidly early in the season (Lakso *et al.*, 1999) and had, on average, about 35% higher annual yields (Lakso, 1999b). Balanced pruning is common in the Lake Erie region because this regime more consistently maintains fruit maturation across variations in vine vigor. Minimal pruning has been recently tested in the region because it is less labor-intensive, but has been associated with more variable yield (Lakso, 1999b).

Irrigation treatments also began in 1991. As a result of an average monthly rainfall of about 70 mm, irrigated vines received drip irrigation only when needed. Indicators used in deciding whether to irrigate included soil moisture volumetric soil water content (via neutron attenuation) below about 30%, declines in shoot growth rates, and vine mid-day stem water potentials below −0.9 MPa under sunny conditions. These thresholds were determined and adjusted over several years before the beginning of the root study. During the period of this study, 1997 and 2000 were rather wet and only two or three irrigations were given in late summer (Table 1). In 1998 and 1999, soils were very dry and weekly irrigations of 120–150 l per vine (16–22 mm) were provided with two 4-l per hour drippers.

In addition to neutron probe measurements, soil moisture was determined in 1999 and 2000 using time-domain reflectometry (TDR) to assess differences between irrigation treatments. The TDR probes (20 cm long) were installed vertically under 14 of the experimental vines (seven per irrigation treatment) within 0.5 m of the vine trunks, and measurements were done weekly during the growing season starting in July 1999. The vineyard was otherwise tended with cultural practices standard for Concord grape production. Dates of bloom,

Table 1 Weather conditions from April 1 to October 31 and dates of budbreak, bloom, veraison[a] and harvest of Concord grape (*Vitis labruscana*) at Cornell University's Vineyard Laboratory in Fredonia, NY, USA, 1997–2000

Year	Total precipitation (mm) for April 1–October 31	Mean daily maximum air temperature (°C) ± SE	Mean daily minimum air temperature (°C) ± SE	Budbreak date	Bloom date	Veraison date	Harvest date
1997	742	19.8 ± 0.45	10.2 ± 0.44	June 7	June 28	September 9	October 21
1998	551	22.3 ± 0.40	12.4 ± 0.41	April 27	June 5	August 17	September 15
1999	573	22.6 ± 0.43	12.1 ± 0.44	May 4	June 9	August 14	September 28
2000	692	21.1 ± 0.41	11.4 ± 0.41	May 3	June 12	August 22	October 11

Precip., precipitation; veraison refers to fruit colour change from green to purple.

veraison (fruit color change from green to purple, indicating the start of fruit ripening and rapid growth), and harvest for experimental vines were recorded each year. Annual fruit yields for each vine were measured at harvest in late September or early October from 1991 onwards.

Measurements of root lifespan and pigmentation

Roots were monitored through clear, butyrate (cellulose acetyl butyrate) minirhizotron tubes, 183 cm long and 5.7 cm external diameter, installed at 30° from vertical in the Fall of 1996. Four tubes were installed using hardened steel pipe, an angle guide and sledge hammers. The shale fragments were easily fragmented by the cutting edge of the steel pipe. Minirhizotrons were installed in each of the 16 plots of five vines, with tubes on the west sides of the vines, spaced equally between vines and approximately 0.5 m from their trunks (64 tubes total, 16 per pruning-irrigation treatment combination). After the minirhizotrons were inserted in the hole, dry sieved soil was added around the edges to permit good soil contact. Tube sections above the soil surface (*c.* 10 cm long) were wrapped with black electrical tape, and capped with black rubber stoppers to exclude light and moisture from the tube interior. White aluminum cans were also placed over the tube ends to minimize radiant heat exchange. Tubes were etched with a column of 127 numbered, 1.0 × 1.5 cm windows on the upper surface of the tube, oriented toward the interior of the vine row. Images of the windows were collected on Hi-8 video tape every 2 wk with a miniature video camera system (BTC-2; Bartz Technology, Santa Barbara, CA, USA) beginning in March 1997 and continuing during each growing season (March 1–October 31) to October 2000. Images were processed as previously described (Comas *et al.*, 2000; Wells & Eissenstat, 2001). A midwinter measurement was done each year, weather permitting.

Images from eight of the 16 tubes for each pruning-irrigation treatment combination were examined for this study (two tubes per plot of five vines, 32 tubes total). Dates that individual roots were born, became pigmented, turned black or shriveled, and disappeared were recorded as described by Comas *et al.* (2000). Roots born in 1997 and 1998 were followed through May 1999, roots born in 1999 were followed through May 2000, and roots born in 2000 were followed through October 2000. The total number of roots followed each year ranged from 971 in 1999 to 1726 in 1997. Root lifespan in days was calculated as the date the root was first observed as black, shriveled or disappeared minus the date the root was first observed on the tube (birth date). As root images were videotaped every 2 wk, observation dates were recorded as the date midway between video dates. Pigmentation time was calculated as the date the root was first observed as pigmented minus the root birth date, and was defined as the entire visible portion of the root changing from white to brown. 'Pigmentation' and 'lifespan' in this study corresponds to the 'functional lifespan' and 'total lifespan' categories, respectively, as described in Comas *et al.* (2000). The number of roots censored for lifespan (i.e. roots that did not become black or shriveled, or disappeared due to a tube shift during the study) ranged from 7.9% for the 1999 roots to 31.1% for the 2000 roots when monitoring ceased in October Roots censored for pigmentation ranged from 1.5% for the 1999 roots to 11.8% for the 1998 roots. Roots were assigned one of two diameter classes –1 (< 0.4 mm) or 2 (> 0.4 mm) from direct measurements of images on the computer screen. In grape, most of the finest lateral roots that bore no lateral roots were < 0.4 mm in diameter. Total numbers of roots appearing in each window during a given year were noted. The soil depth of each window was calculated from the installation angle and location of the window along the length of the tube (maximum window depth = 87 cm).

Data analysis

Variables with significant influences on individual root lifespan and pigmentation were identified with Cox proportional hazards regression using PROC PHREG in SAS (SAS Institute Inc., Cary, NC, USA). Proportional hazards regression is a modeling procedure that allows the effects of each covariate to be evaluated while controlling for effects of other covariates (Cox, 1972; Allison, 1995; Cantor, 1997). This is a powerful technique for working with root survival data in minirhizotron studies (Wells & Eissenstat, 2001; Wells *et al.*, 2002). Individual roots are evaluated for their 'hazard' of

Table 2 Variables tested in proportional hazards regression analyses of individual root survival and pigmentation of Concord grape (*Vitis labruscana*)

Variable	Coding and description
Canopy pruning	0 = balanced pruning, 1 = minimal pruning
Irrigation	0 = not irrigated, 1 = irrigated under dry conditions
Vine phenology	1 = roots produced between April 1 to bloom, 2 = bloom to 30 d postbloom, 3 = 30 d postbloom to veraison, 4 = veraison to harvest, 5 = harvest to March 31
Root diameter	1 (< 0.4 mm), 2 (> 0.4 mm)
Soil depth	Soil depth at which the root was born, measured in cm
Number of birth year neighbors	Total number of roots born in the same window in a given year
Number of previous year neighbors	Total number of roots born in the same window the previous year (no data for 1997-born roots)
Yield in birth year	Mean fruit yield (tonne ha^{-1}) in the year roots were born for each plot of five vines containing minirhizotron tubes
Yield in previous year	Mean fruit yield (tonne ha^{-1}) in the year before root birth for each plot of five vines containing minirhizotron tubes
Observer	Controls for individual bias in the data collector. Each year had two observers collecting data, coded as 1 and 2
Block	Controls for soil heterogeneity and other random environmental changes in the vineyard. Blocks are coded as 1–4

mortality, which can be considered as the instantaneous probability of mortality, although the hazard is not strictly a probability and can assume values greater than 1 (Allison, 1995). In a proportional hazards model, the hazard of an individual root at time t is defined as the product of a baseline hazard function that is unspecified and a linear function of k covariates which is exponentiated (Allison, 1995):

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

PROC PHREG uses the partial likelihood method (Cox, 1972) to estimate a β coefficient (or parameter estimate) for each covariate in the model and calculates a χ^2 statistic to test the null hypothesis that each β equals zero. The sign of a parameter estimate indicates whether the hazard decreases (negative sign) or increases (positive sign) as the covariate value increases (Wells & Eissenstat, 2001). PROC PHREG also generates a 'hazard ratio', which is e^{β}. For dichotomous categorical covariates (e.g. coded 0 and 1), the hazard ratio can be interpreted as the estimated hazard for roots with a value of 1 vs the estimated hazard for those with a value of 0. For quantitative covariates (e.g. soil depth), subtracting 1 from the hazard ratio and multiplying by 100 (i.e. $100(e^{\beta} - 1)$) gives the per cent change in the hazard of mortality associated with a 1-unit increase in the covariate, controlling for effects of other covariates (Allison, 1995).

Regression analyses were done using the forced-entry model building approach in PROC PHREG. Survival data for roots born in 1997, 1998, 1999 and 2000 were analysed separately, and roots for each year were given a common start date. Survival data can also be input into PROC PHREG so that roots enter the study at different dates (Alison, 1995), which is appropriate given that roots are actually born at different points during the year. Survivor function estimates generated in PROC PHREG are generally not as reliable when individuals enter the study at different times, although in this case preliminary analyses showed that the two methods gave similar results (data not shown). Covariates tested in the regression models included environmental variables, timing of root birth with respect to shoot phenology (equivalent to seasonal cohorts used in some studies), root diameter, number of neighboring roots, canopy pruning and irrigation treatments, and vine yield (Table 2). The interaction term between pruning and irrigation was also included in the analyses. The variable describing when roots were born relative to shoot phenology was divided into five developmental categories (Table 2). Therefore, dummy variables were created that contrasted roots produced during phenology categories 2–5 with roots produced during phenology category 1 (Allison, 1995).

Once an overall proportional hazards analysis had been done for each of the four years, a population of roots with the greatest risk of mortality was identified, based on these results. Follow-up proportional hazards analyses were done for this vulnerable population to further explore the influences of pruning, irrigation, yield, and neighboring roots on root lifespan. Follow-up analyses using soil moisture as a continuous variable were also done for a subpopulation of 1999 roots to specifically explore irrigation effects on root survival (see below).

In this study, large root numbers (n = 971–1726) provided high statistical power and large numbers of variables in each model (n = 11, Table 2) created the potential for statistically significant results that were not biologically meaningful. For each significant variable, survivor functions generated using the BASELINE command in PROC PHREG were examined to assess the true magnitude of the effect. Survivor functions describe the probability of individual root survival at time 't'. In addition, variables that were significant in at least three of

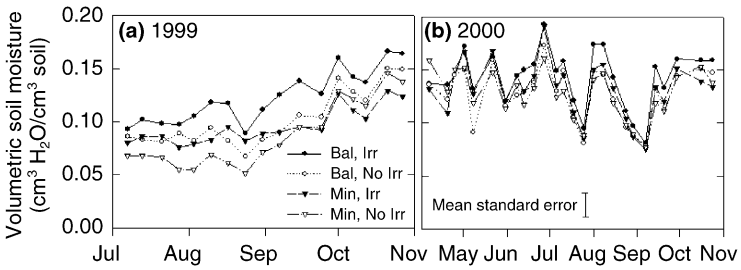

Fig. 1 Volumetric soil moisture in the top 20 cm as measured by time-domain reflectometry in four pruning–irrigation treatment combinations in 1999 and 2000. For each point $n = 7–8$. Note that measurements were taken over a longer period in 2000.

the four study years were considered to have stronger, more biologically relevant effects on root lifespan than those that were significant in only one or 2 yr.

Results

Environmental conditions

Growing season weather conditions at the experimental vineyard varied over the four yr of the study (1997–2000), providing the opportunity to examine treatment effects on root longevity under a wide range of environmental conditions. Generally, the growing seasons of 1997 and 2000 were cool and wet, while 1998 and 1999 were warm and dry (Table 1). The TDR measurements showed that soil moisture varied between the irrigated and unirrigated treatments during the relatively dry summer of 1999, and suggested that minimally pruned vines depleted soil moisture more than balanced pruned. There were no differences in soil moisture among treatments during the wet growing season of 2000 (Fig. 1).

Lifespan

Soil depth, root diameter, and the vine phenological stage at the time of root birth had significant, consistent effects on root lifespan (Table 3). Roots deep in the soil always had a lower risk of mortality (longer life span) than roots in shallow soil layers (Fig. 2). Each centimeter increase in soil depth reduced the risk of mortality by 0.3–0.7% according to the hazard ratios for each year (e.g. $100(e^{-0.00698} - 1) = 0.696\%$; Table 3), such that a root at 40 cm depth had a 9–21% lower risk of mortality compared with a root at 10 cm depth (e.g. $40–10 = 30$, $30 \times 0.3\% = 9\%$).

Roots born at least 30 d after bloom and before harvest (phenology categories 3 and 4, Table 2) had a significantly lower risk of mortality than roots born between April 1 and bloom (category 1) in at least three out of the 4 yr. The increased lifespan for roots born in midseason (category 3) in 2000 was marginally significant (Fig. 3, Table 3). Roots born just after bloom (category 2) and after harvest (category 5) tended to live longer than prebloom roots (category 1), but these effects were only significant in two of the 4 yr. Finally, roots greater than 0.4 mm in diameter had a significantly reduced risk of mortality (up to 37% reduced risk; e.g.

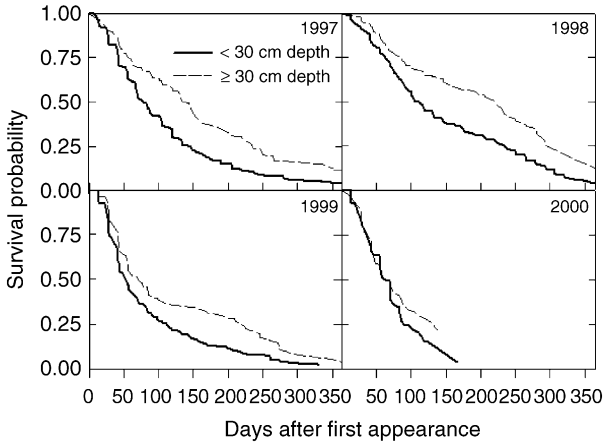

Fig. 2 Survivorship probabilities for roots of Concord grape (*Vitis labruscana*) in two depth categories, 1997–2000. Curves were generated using the BASELINE command in PROC PHREG of SAS, which produces baseline survivor functions for categories of the chosen covariate, evaluated at the means of all other covariates. Soil depth was analysed as a continuous variable (Table 2); the two depth categories of < 30 cm and ≥ 30 cm were chosen for purposes of illustration. The survivor functions for 2000 are truncated because roots were followed for a shorter period of time than in other years (see the Materials and Methods section).

100(0.628)) than finer roots in 1998, 1999, and 2000) (Fig. 4, Table 3). The year-to-year consistency in soil depth, phenology, and diameter effects suggest that these variables have strong, biologically important influences on grape root lifespan.

Canopy pruning had a statistically significant effect on root lifespan in three of the four years, although survivorship curves showed this effect to be relatively small (Fig. 5, Table 3). The direction of the pruning effect was linked to annual weather conditions. In 1997, a cool and wet year, roots lived longer on minimally pruned vines, which support more canopy leaf area than balance-pruned vines early in the growing season. In 1999, which was warm and dry, roots lived longer on balance-pruned vines (Fig. 5). The pruning effect showed a similar direction in 1998, also a warm, dry year, but was not statistically significant (Table 3).

The effects of irrigation were significant in only two of the four years, and were inconsistent (Table 3). Irrigation decreased root lifespan in 1997 (data not shown), but increased root lifespan and interacted significantly with pruning

Table 3 Results of proportional hazards regression analyses for individual root lifespan of Concord grape (*Vitis labruscana*) over 4 yr (1997–2000)

Variable and year	df	Parameter estimate	Standard Error	χ^2 Value	*P*-value	Hazard ratio
Pruning						
1997	1	–1.276	0.164	60.52	**< 0.001**	0.279
1998	1	0.235	0.187	1.58	0.209	1.265
1999	1	0.467	0.217	4.63	**0.031**	1.595
2000	1	–0.790	0.134	34.63	**< 0.001**	0.454
Irrigation						
1997	1	0.746	0.160	21.85	**< 0.001**	2.108
1998	1	0.298	0.159	3.51	0.061	1.347
1999	1	0.045	0.157	0.08	0.776	1.046
2000	1	–0.396	0.103	14.81	**< 0.001**	0.673
Pruning × Irrigation						
1997	1	–0.006	0.115	< 0.01	0.956	0.994
1998	1	0.084	0.252	0.11	0.739	1.088
1999	1	–0.309	0.216	2.05	0.152	0.734
2000	1	0.619	0.139	19.92	**< 0.001**	1.857
Soil depth						
1997	1	–0.00698	0.00169	17.12	**< 0.001**	0.993
1998	1	–0.00318	0.00127	6.30	**0.012**	0.997
1999	1	–0.00520	0.00141	13.55	**< 0.001**	0.995
2000	1	–0.00293	0.00124	5.61	**0.018**	0.997
Root diameter						
1997	1	–0.112	0.0647	2.99	0.084	0.894
1998	1	–0.465	0.0893	27.16	**< 0.001**	0.628
1999	1	–0.241	0.0839	8.26	**0.004**	0.786
2000	1	–0.289	0.0790	13.42	**< 0.001**	0.749
Vine phenology 2 vs 1						
1997	1	–0.443	0.0866	26.20	**< 0.001**	0.642
1998	1	–0.059	0.0910	0.41	0.520	0.943
1999	1	0.048	0.0965	0.25	0.616	1.050
2000	1	–0.167	0.0822	4.15	**0.042**	0.846
Vine phenology 3 vs 1						
1997	1	–0.578	0.102	31.98	**< 0.001**	0.561
1998	1	–0.474	0.078	37.23	**< 0.001**	0.622
1999	1	–0.464	0.101	20.97	**< 0.001**	0.629
2000	1	–0.163	0.087	3.53	0.0603	0.850
Vine phenology 4 vs 1						
1997	1	–0.696	0.187	13.80	**< 0.001**	0.499
1998	1	–0.949	0.128	55.07	**< 0.001**	0.387
1999	1	–0.780	0.191	16.68	**< 0.001**	0.458
2000	1	–1.350	0.244	30.62	**< 0.001**	0.259
Vine phenology 5 vs 1						
1997	1	–0.568	0.213	7.11	**0.008**	0.567
1998	1	–0.524	0.123	18.29	**< 0.001**	0.592
1999	1	–0.587	0.346	2.89	0.089	0.556
2000	1	–0.272	0.421	0.42	0.518	0.762
Birth year neighbors						
1997	1	< 0.0001	0.0059	< 0.01	0.997	1.000
1998	1	0.0071	0.0076	0.87	0.351	1.007
1999	1	–0.0156	0.0111	1.95	0.162	0.985
2000	1	–0.0069	0.0076	0.81	0.368	0.993
Previous year neighbors						
1998	1	0.0698	0.0198	12.46	**< 0.001**	1.072
1999	1	–0.0051	0.0122	0.17	0.680	0.995
2000	1	0.0195	0.0118	2.72	0.099	1.020
Birth year yield						
1997	1	0.156	0.022	48.21	**< 0.001**	1.168
1998	1	–0.034	0.022	2.26	0.133	0.967
1999	1	–0.025	0.024	1.05	0.307	0.976
2000	1	–0.073	0.019	15.27	**< 0.001**	0.930

Table 3 *Continued*.

Variable and year	df	Parameter estimate	Standard Error	χ^2 Value	*P*-value	Hazard ratio
Previous year yield						
1997	1	0.0218	0.0101	4.62	**0.032**	1.022
1998	1	0.0319	0.0218	2.14	0.144	1.032
1999	1	0.0028	0.0155	0.03	0.857	1.003
2000	1	0.1574	0.0280	31.63	**< 0.001**	1.170
Observer						
1997	1	0.0883	0.0665	1.76	0.184	1.092
1998	1	0.2387	0.1053	5.14	**0.023**	1.270
1999	1	0.0199	0.0752	0.07	0.792	1.020
2000	1	0.0727	0.0722	1.01	0.314	1.075
Block						
1997	1	–0.0567	0.0267	4.52	**0.034**	0.945
1998	1	0.236	0.0496	22.68	**< 0.001**	1.266
1999	1	0.0818	0.0440	3.46	0.063	1.085
2000	1	–0.0921	0.0289	10.18	**0.001**	0.912

Phe, vine phenology; Prev, previous. See Table 2 for explanations of variable coding and definitions of the phenology categories 1–5. Significant *P*-values are shown in bold ($P < 0.05$). For dichotomous categorical covariates (e.g. coded 0 and 1), the hazard ratio can be interpreted as the estimated hazard for roots with a value of 1 vs the estimated hazard for those with a value of 0. For quantitative covariates (e.g. soil depth), subtracting 1 from the hazard ratio and multiplying by 100 (i.e. $100(e^{\beta} - 1)$) gives the per cent change in the hazard of mortality associated with a 1-unit increase in the covariate, controlling for effects of other covariates (Allison, 1995).

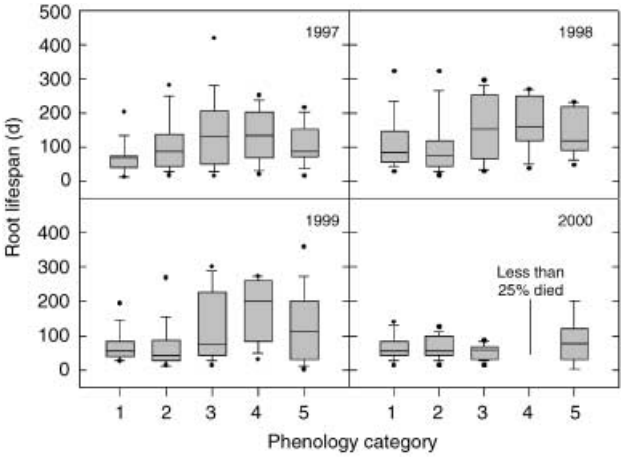

Fig. 3 Box plots of root lifespan of Concord grape (*Vitis labruscana*) for roots born at different stages of vine phenology (different seasonal cohorts), 1997–2000. See Table 2 for explanations of phenology categories. The horizontal line in each box is the median, the top and bottom box edges are the 75th and 25th percentiles, respectively, the top and bottom error bars are the 90th and 10th percentiles, respectively, and the black dots are outliers at the 95th and 5th percentiles. In 2000, there were only seven roots in category 5, so no outliers are shown.

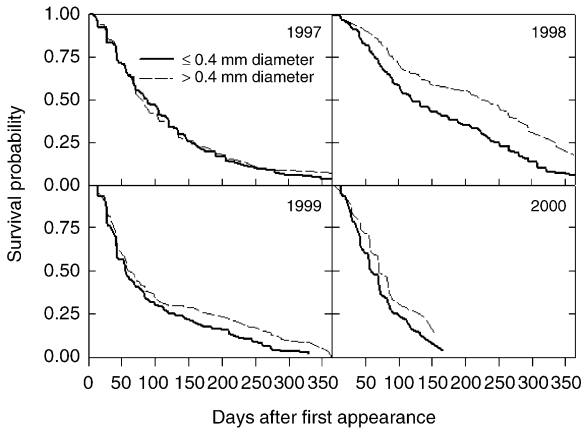

Fig. 4 Survivorship probabilities for roots of Concord grape (*Vitis labruscana*) in two diameter categories, 1997–2000. Curves were generated using the BASELINE command in PROC PHREG of SAS. Diameter was analyzed as a categorical variable (Table 2).

in 2000 (Fig. 6, Table 3). Surprisingly, irrigation did not significantly enhance root lifespan in 1998 and 1999, the driest years of the study (Table 1).

High vine yields in the previous year significantly increased the risk of root mortality in 1997 and 2000 (Table 3). Each additional metric ton per hectare increased the risk of root mortality by 2–17%. For these 2 yr, fruit yield in the year the root was born also significantly affected lifespan, but high yields decreased root lifespan in 1997 and increased it in 2000 (Table 3).

The number of neighboring roots in the same window the previous year had a significant effect on root lifespan in 1998 only. Neighbors increased the risk of mortality, with each additional neighbor increasing the risk by 7% (Table 3). The number of neighbors in the year a root was born never had a significant effect on lifespan in any of the four years (Table 3). There was significant variation in root lifespan owing to block effects in three of the four years, and observer bias was significant in 1998 (Table 3).

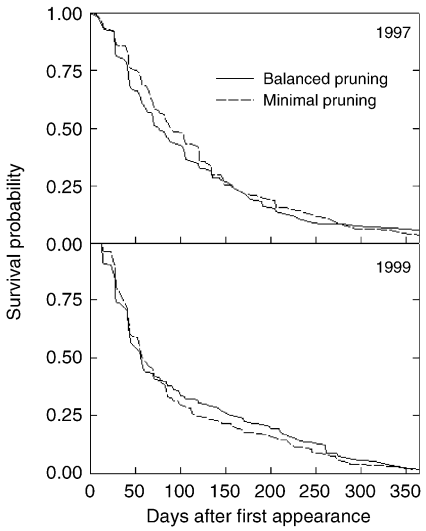

Fig. 5 Survivorship probabilities for roots of Concord grape (*Vitis labruscana*) in two pruning treatments for a wet year (1997) and a dry year (1999). Curves were generated using the BASELINE command in PROC PHREG of SAS.

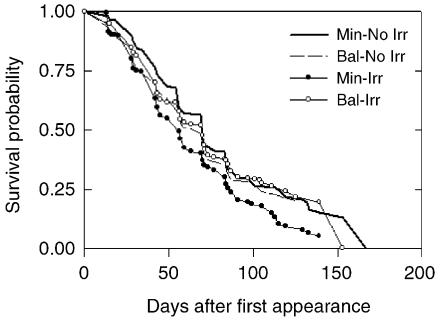

Fig. 6 Survivorship probabilities for roots of Concord grape (*Vitis labruscana*) in four pruning–irrigation treatment combinations showing the significant interaction between pruning and irrigation in 2000. Curves were generated using the BASELINE command in PROC PHREG of SAS.

Vulnerable roots

The overall analysis results for 1997–2000 showed that soil depth, root diameter, and timing of root birth had strong, consistent effects on root lifespan (Table 3, Figs 2–4). The roots with the highest risk of mortality were small-diameter roots born between April 1 and bloom in the surface soil layer. We hypothesized that other variables that had weak or inconsistent effects in the total population of roots (e.g. irrigation and yield) might have stronger influences on lifespan in this vulnerable root population. Therefore, separate proportional hazards analyses were done on data sets of those roots that were in phenology category 1, diameter class 1, and occurred at soil depths of less than 30 cm ('vulnerable roots'). Variables included in the models were pruning, irrigation, pruning × irrigation, birth year yield, previous year yield, birth year neighbors, previous year neighbors, observer, and block (Table 2). Generally, these variables had similar, or weaker effects (i.e. the variable was not significant at $P = 0.05$) on the vulnerable root population as compared with their effects in the total population of roots for all years (data not shown). Because this analysis and that in the following section were *post hoc* tests on a subset of the original data, *P*-values might be inflated.

Soil moisture effects on root lifespan

The lack of an irrigation effect on root survival in dry years was unexpected. One explanation for weak irrigation effects might be soil moisture heterogeneity in irrigated plots. Drip emitters were not always located directly over the minirhizotron tubes and the soil was well-drained, such that soil moisture distribution may have been patchy, obscuring influences of irrigation on root survival. To explore this possibility, a proportional hazards analysis was done for roots from 1999, a dry year, with a continuous soil moisture variable replacing the categorical irrigation variable in the analysis. The soil moisture value assigned to each tube was the mean of weekly TDR readings taken between July 1 and August 31, the driest period of 1999 for which data were available (Fig. 1). The population used in the analysis consisted of roots most likely to be influenced by soil moisture, as measured by TDR (i.e. roots from the tubes associated with TDR probes, located in the top 20 cm, and born between May 1 and July 31 in 1999; $n = 209$). All variables (Table 2) except irrigation were included in the proportional hazards model for the 1999 subpopulation.

There was no evidence that roots in moist soil lived longer than those in dry soil. Indeed, TDR soil moisture had a positive parameter value, indicating that higher soil moisture tended to increase the risk of root mortality, although this effect was not significant ($P = 0.0727$, data not shown). Survivor function curves suggested that soil moisture effects on root survival were complex, and relatively weak (data not shown). Therefore, this follow-up analysis supported the overall 1998 and 1999 results, indicating that irrigation had only modest effects, and tended to reduce root lifespan.

Pigmentation

Because pigmentation is part of the normal root maturation process and is correlated with cessation of metabolic activity in 'Concord' grape (Comas *et al.*, 2000), we predicted that variables with strong effects on root lifespan would also significantly influence the time to root pigmentation. This was the case for pruning, soil depth, and shoot phenology (Table 4). The magnitude and direction of these variables' effects on pigmentation were similar to their effects on lifespan, although generally variables were significant in fewer of the four years. The direction of the phenology effect on pigmentation differed from that on lifespan only in 2000,

Table 4 Results of proportional hazards regression analyses for time to pigmentation for individual roots of Concord grape (*Vitis labruscana*) over 4 yr (1997–2000)

Variable and year	df	Parameter estimate	Standard error	χ^2	*P*	Hazard ratio
Pruning						
1997	1	–1.357	0.158	73.38	**< 0.001**	0.257
1998	1	0.535	0.177	9.15	**0.003**	1.708
1999	1	0.360	0.214	2.81	0.094	1.433
2000	1	–0.128	0.121	1.10	0.293	0.880
Irrigation						
1997	1	1.151	0.155	55.01	**< 0.001**	3.162
1998	1	–0.035	0.146	0.06	0.808	0.965
1999	1	0.150	0.151	0.98	0.323	1.161
2000	1	0.027	0.094	0.09	0.769	1.028
Pruning × irrigation						
1997	1	–0.127	0.114	1.23	0.267	0.881
1998	1	0.132	0.222	0.35	0.553	1.141
1999	1	–0.440	0.212	4.30	**0.038**	0.644
2000	1	–0.024	0.121	0.04	0.843	0.976
Soil depth						
1997	1	–0.00244	0.00163	2.24	0.134	0.998
1998	1	–0.00578	0.00115	25.43	**< 0.001**	0.994
1999	1	–0.00602	0.00134	20.04	**< 0.001**	0.994
2000	1	–0.00534	0.00105	25.68	**< 0.001**	0.995
Root diameter						
1997	1	–0.002	0.063	< 0.01	0.970	0.998
1998	1	–0.137	0.074	3.38	0.066	0.872
1999	1	–0.053	0.080	0.45	0.504	0.948
2000	1	–0.092	0.068	1.83	0.176	0.912
Vine phenology 2 vs 1						
1997	1	–0.381	0.084	20.68	**< 0.001**	0.683
1998	1	–0.081	0.087	0.85	0.355	1.084
1999	1	–0.198	0.095	4.31	**0.038**	0.820
2000	1	0.159	0.078	4.19	**0.041**	1.173
Vine phenology 3 vs 1						
1997	1	–0.738	0.099	55.57	**< 0.001**	0.478
1998	1	–0.448	0.073	37.60	**< 0.001**	0.639
1999	1	–0.238	0.095	6.35	**0.012**	0.788
2000	1	0.149	0.079	3.57	0.059	1.161
Vine phenology 4 vs 1						
1997	1	–0.612	0.177	11.93	**< 0.001**	0.542
1998	1	–0.832	0.103	65.76	**< 0.001**	0.435
1999	1	–0.613	0.170	12.94	**< 0.001**	0.542
2000	1	–0.909	0.159	32.77	**< 0.001**	0.403
Vine phenology 5 vs 1						
1997	1	–0.577	0.207	7.75	**0.005**	0.562
1998	1	–0.758	0.107	50.51	**< 0.001**	0.468
1999	1	–0.971	0.345	7.91	**0.005**	0.379
2000	1	–0.269	0.419	0.41	0.521	0.764
Birth year neighbors						
1997	1	0.0186	0.0057	10.86	**0.001**	1.019
1998	1	0.0177	0.0066	7.24	**0.007**	1.018
1999	1	0.0292	0.0107	7.49	**0.006**	1.030
2000	1	0.0310	0.0069	20.41	**< 0.001**	1.031
Previous year neighbors						
1998	1	0.0288	0.0188	2.35	0.125	1.029
1999	1	0.0080	0.0121	0.44	0.508	1.008
2000	1	0.0230	0.0112	4.19	**0.041**	1.023
Birth year yield						
1997	1	0.2139	0.0217	97.39	**< 0.001**	1.238
1998	1	–0.0392	0.0197	3.94	**0.047**	0.962
1999	1	–0.0158	0.0234	0.43	0.511	0.984
2000	1	0.0170	0.0161	1.11	0.291	1.017

Table 4 *Continued*.

Variable and year	df	Parameter estimate	Standard error	χ^2	*P*	Hazard ratio
Previous year yield						
1997	1	–0.0227	0.0096	5.58	**0.018**	0.978
1998	1	–0.0149	0.0204	0.53	0.465	0.985
1999	1	< 0.0001	0.0154	< 0.01	0.997	1.000
2000	1	0.0276	0.0245	1.27	0.260	1.028
Observer						
1997	1	–0.072	0.065	1.20	0.273	0.931
1998	1	0.632	0.093	46.29	**< 0.001**	1.882
1999	1	–0.113	0.073	2.43	0.119	0.893
2000	1	0.133	0.063	4.47	**0.035**	1.143
Block						
1997	1	–0.046	0.026	2.97	0.085	0.955
1998	1	0.151	0.042	12.75	**< 0.001**	1.164
1999	1	0.119	0.044	7.35	**0.007**	1.126
2000	1	–0.060	0.025	5.81	**0.016**	0.942

Phe, vine phenology; Prev, previous. See Table 2 for explanations of variable coding and definitions of the phenology categories 1–5. Significant *P*-values are shown in bold ($P < 0.05$).

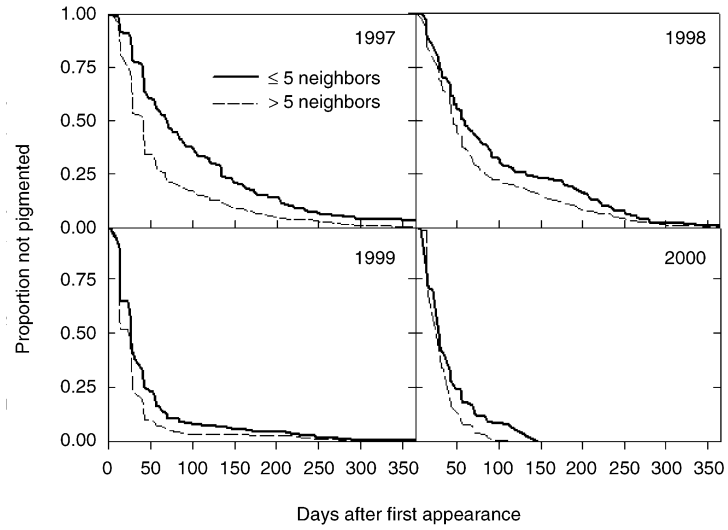

Fig. 7 Pigmentation probabilities for roots of Concord grape (*Vitis labruscana*) in two birth-year neighbor categories, 1997–2000. Neighbor number was analysed as a continuous variable (Table 2); the two neighbor categories of ≤ 5 and > 5 were chosen for purposes of illustration. Curves were generated using the BASELINE command in PROC PHREG of SAS.

where roots born between bloom and veraison (categories 2 and 3) pigmented more quickly than those born prebloom (category 1), which was opposite to the lifespan pattern. As with root lifespan, there was also significant variation in time to pigmentation associated with the block and observer variables (Tables 3 and 4). Effects of irrigation, pruning × irrigation, birth year yield, previous year yield, and previous year neighbors on pigmentation were significant in only one or 2 yr and inconsistent in direction, similar to the effects of these variables on root lifespan (Tables 3 and 4).

By contrast, diameter and number of neighbors in a year the root was born had very different effects on pigmentation than they had on root lifespan. Diameter had no significant influence on pigmentation (Table 4) whereas finer roots were significantly more likely to die than thicker roots in three of the four years (Table 3, Fig. 4). Roots with large numbers of neighbors in their year of birth pigmented significantly more quickly than roots with few neighbors in all four years, with the 'risk' of pigmentation increasing by 1.8–3.1% for each additional neighbor (Table 4, Fig. 7). By contrast, birth-year neighbors never significantly affected root lifespan (Table 3).

Discussion

Our goal was to identify factors with strong effects on root longevity. Recognizing that the effects of different variables may interact and change over time in a dynamic field environment, we took the approach of examining a large number of variables simultaneously over 4 yr in a system where most roots live less than 1 yr. Soil depth, root diameter,

and the timing of root birth at different vine phenological stages had strong, consistent year-to-year influences on root lifespan. The risk of root mortality decreased significantly with soil depth in all four years of the study (Table 3, Fig. 2). Increased root longevity in deeper soils has also been found in fruit trees (Kosola *et al.*, 1995; Wells *et al.*, 2002) and in a grassland community (Arnone *et al.*, 2000). By contrast, Hendrick & Pregitzer, 1992) observed greater root survivorship in more shallow soils for sugar maple (*Acer saccharum*). The mechanisms underlying increased root lifespan at depth are not known, but deep roots probably experience fewer extremes in soil temperature and moisture, and possibly reduced pathogen and herbivory stress (Eissenstat & Yanai, 1997).

We observed a positive correlation between root diameter and lifespan in three of the four years (Table 3, Fig. 4), which has also been reported in apple (*Malus domestica*, Wells & Eissenstat, 2001) and peach (*Prunus persica*, Wells *et al.*, 2002). Differences in lifespan among roots of different diameter may be related to different functional roles within the fine root system. Large-diameter roots serve as transport conduits and initiate new laterals as well as absorbing soil resources, and may be preferentially preserved by the plant (Wells & Eissenstat, 2001). Our work adds to a small but growing body of data suggesting that diameter may be an important predictor of root lifespan within and perhaps across species (Eissenstat *et al.*, 2000).

The timing of root birth with respect to shoot phenology had a strong influence on root lifespan in all four years, with roots born before bloom (in early spring) having the shortest lifespans (Table 3, Fig. 3). A similar pattern was seen for pine roots (*Pinus ponderosa*): in two of three years, roots born between February and June tended to have shorter lifespans than roots born between August and December (Johnson *et al.*, 2000). The mechanism associated with these patterns is unclear because shoot phenological changes are confounded with seasonal environmental changes. For example, soil temperature has been suggested to affect root lifespan, with warmer temperatures linked to higher root mortality in a field study of sugar maple (Hendrick & Pregitzer, 1993) and in a pot study using ryegrass (*Lolium perenne*, Forbes *et al.*, 1997, reviewed in Pregitzer *et al.*, 2000). Alternatively, root lifespan may to be correlated with seasonal changes in root carbohydrates. Bates *et al.* (2002) working with 'Concord' grape in the same vineyard, showed that the lowest seasonal starch levels occurred in fine roots in a period 15 d before to 30 d after bloom, corresponding with our phenology categories 1 and 2. By 75 d after bloom, starch levels were three to six times higher. Therefore, roots born near bloom may have relatively less C reserves for long-term root maintenance than those born later in the season.

Variables related to C sinks in the shoot (canopy pruning and yield) occasionally had significant influences on root lifespan (Table 3). However, the direction and strength of these effects varied from year to year, suggesting that linkages between root lifespan and shoot C demand are complex. Our data also suggest interactions between shoot C, environmental conditions, and root lifespan. For example, roots lived significantly longer on minimally pruned vines and vines with low previous year yields in cool, wet years (1997, 2000, Table 3), but during warm, dry years (1998, 1999), roots tended to live longer on balance-pruned vines, and yield did not have a significant influence on root lifespan. The significant interaction between pruning and irrigation treatments in 2000 also underscores the potential for shoot C influences on roots to be modified by other variables (Fig. 6).

There has been very little work on the effects of shoot C demand on root lifespan in woody plants, but the few published studies suggest that defoliation and strong sinks in the shoot increase root mortality (reviewed by Eissenstat & Yanai, 1997). Heavy croploads led to higher root mortality in *Citrus* (Smith, 1976; Graham *et al.*, 1985), and experimental pruning and defoliation increased root mortality in *Citrus* (Eissenstat & Duncan, 1992), apple (*Malus* sp., Head, 1969), and blackcurrant (Atkinson, 1972). We found some reductions in root lifespan with heavy pruning and high yields in our study, but the effects were not consistent. Pruning and high previous year yields reduced root lifespan in 1997 and 2000, but not in 1998 or 1999. Root longevity was reduced by high current year yields for 1997 roots, but had the opposite effect in 2000 (Table 3). Some differences between studies may be related to the timing of pruning during the growing or dormant seasons (Faust, 1989). Alternatively, grape roots may be less sensitive than other species to changes in pruning or yield. For example, Hunter *et al.* (1995) did not see a strong effect of defoliation on root growth or carbohydrate content in wine grapes, although McLean *et al.* (1992) found increased root growth when fruit clusters were removed from drought-stressed vines, and smaller root masses have been observed in vines with heavy crops (Clingeleffer & Krake, 1992). The control of C allocation to roots is still not well understood and the shoot may not be the site of primary control in grape or other species (Farrar & Jones, 2000). In addition, to fully understand the influences of shoot manipulations on grape roots, both root lifespan and root production must be examined.

The effect of irrigation on grape root lifespan was weak and inconsistent, and the literature is equivocal with regard to soil moisture and irrigation influences on root lifespan. Herbaceous plants with fine, succulent roots appear to be very sensitive to dry conditions and experience substantial root mortality during drought (Huang *et al.*, 1997; Kirkham *et al.*, 1998). Pregitzer *et al.* (1993) found that root lifespan was higher in irrigated patches in a hardwood forest community, and Marshall (1986) found that root mortality increased for Douglas fir seedlings exposed to drought, especially if coupled with shading. However, *Citrus* species retain their roots in dry soil (Kosola & Eissenstat, 1994; Bryla *et al.*, 1997; Espeleta & Eissenstat, 1998), and root turnover rates were not affected

when rainfall was experimentally reduced in a mixed hardwood forest (Joslin *et al.*, 2000). In addition, a survey of global patterns of root turnover found no significant relationship between mean annual precipitation and fine root turnover for several different ecosystems (Gill & Jackson, 2000). Thus, the effects of irrigation on root lifespan appear to be species- and system-specific, making generalizations difficult.

Most of the variables that significantly affected root lifespan (estimated by the blackening or shriveling of the root) had similar effects on the time to pigmentation (Table 4). This is logical if we assume that variables that influence root lifespan do so by accelerating or decelerating the root aging process. Pigmentation signals a sharp decrease in metabolic activity in grape, suggesting that pigmented roots are dying (Comas *et al.*, 2000). However, pigmentation in many woody species is associated with root aging without necessarily being associated with root death (reviewed by Wells & Eissenstat, 2001). In this study, root diameter and number of neighbors differed in their effects on pigmentation compared to root lifespan (Tables 3 and 4, Fig. 7), emphasizing that pigmentation and mortality are not always tightly coupled. Root diameter strongly affected root lifespan, but had no significant effect on pigmentation, while increasing numbers of neighbors in the year a root was born increased the risk of pigmentation in all four years, but never affected root lifespan (Tables 3 and 4). The mechanisms behind these patterns are unknown, but the neighbor effect on pigmentation suggests there may be trade-offs between root proliferation and the length of time roots remain metabolically active. Also, using a black and/or shriveled appearance to indicate root death may include some decomposition of the root in the total lifespan estimate (Comas *et al.*, 2000). Root diameter may affect root decomposition more strongly than root metabolism, providing a stronger linkage between total lifespan and root diameter than between root diameter and pigmentation. These questions deserve investigation.

Our data may have practical implications. Irrigation and minimal pruning are relatively new cultural practices for Concord grape in cool humid climates, and may benefit growers by reducing vine stress and pruning labor (Lakso *et al.*, 1999; Lakso, 1999a,b). It is important to understand how these new practices influence the Concord root system, which has received little study. Overall, irrigation effects on root lifespan were modest. Pruning had stronger effects, but these influences varied from year to year. Our data do not strongly recommend one cultural practice over another with regard to root lifespan, although root production in this system may be significantly improved by irrigation in dry years (unpubl. data).

As has been emphasized by many other researchers, long-term data collection is crucial for ecological and agricultural studies. Our work underscores this point, demonstrating that experimental treatments can be significant in one year, and then change direction or lose significance in the following year. By taking a long-term approach, we showed that soil depth, root diameter, and the timing of root birth had consistent, predictable influences on root lifespan. By contrast, shoot C effects were more complex and appeared to be modified by environmental conditions. Irrigation and neighboring roots had relatively modest effects on root lifespan, although increases in neighbor roots significantly increased the risk of pigmentation. These data emphasize the diversity that exists in the life histories of fine roots, and future studies should focus on the mechanisms that underlie these variable patterns of mortality and pigmentation.

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