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Consequences of insect herbivory on grape fine root systems with different growth rates

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ABSTRACT

Herbivory tolerance has been linked to plant growth rate where plants with fast growth rates are hypothesized to be more tolerant of herbivory than slower-growing plants. Evidence supporting this theory has been taken primarily from observations of aboveground organs but rarely from roots. Grapevines differing in overall rates of new root production, were studied in Napa Valley, California over two growing seasons in an established vineyard infested with the sucking insect, grape phylloxera (*Daktulosphaira vitifoliae* Fitch). The experimental vineyard allowed for the comparison of two root systems that differed in rates of new root tip production (a 'fast grower', *Vitis berlandieri* × *Vitis rupestris* cv. 1103P, and a slower-growing stock, *Vitis riparia* × *Vitis rupestris* cv. 101–14 Mgt). Each root system was grafted with a genetically identical shoot system (*Vitis vinifera* cv. Merlot). Using minirhizotrons, we did not observe any evidence of spatial or temporal avoidance of insect populations by root growth. Insect infestations were abundant throughout the soil profile, and seasonal peaks in phylloxera populations generally closely followed peaks in new root production. Our data supported the hypothesis that insect infestation was proportional to the number of growing tips, as indicated by similar per cent infestation in spite of a threefold difference in root tip production. In addition, infested roots of the fast-growing rootstock exhibited somewhat shorter median lifespans (60 d) than the slower-growing rootstock (85 d). Lifespans of uninfested roots were similar for the two rootstocks (200 d). As a consequence of greater root mortality of younger roots, infested root populations in the fast-growing rootstock had an older age structure. While there does not seem to be a trade-off between potential growth rate and relative rate of root infestation in these cultivars, our study indicates that a fast-growing root system may more readily shed infested roots that are presumably less effective in water and nutrient uptake. Thus, differences in root tip production may be linked to differences in the way plants cope with roots that are infested by sucking insects.

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INTRODUCTION

Belowground herbivory may be a major cause of root turnover in both agricultural and natural ecosystems (Brown & Gange 1989; Eissenstat *et al.* 2000). Root feeding by insects can cause large changes in plant productivity and plant community structure (Ingham & Detling 1990; Dunn & Frommelt 1998; Hunter 2001). Surprisingly, few direct quantitative observations of the effects of root herbivory on root turnover or productivity exist from field observations. Indirect evidence comes from the use of non-selective insecticides, where an increase in median lifespan of 79 d (from 46 to 125 d) was observed for peach tree fine roots (Wells, Glenn & Eissenstat 2002), a reduced rate of new root production has been observed in pasture systems (Dawson *et al.* 2003), and an increase in herb species richness and reduced seedling mortality has been noted in old fields (Brown & Gange 1989). Direct evidence from artificial systems indicates that maize roots grown in containers show a 13.8% reduction in root biomass when plants were infested with 50 western corn rootworm larvae, and 49.5% when infested with 100 larvae (Dunn & Frommelt 1998).

Defence against herbivory has been linked to plant growth rate (Coley 1988), in the sense that Coley and co-workers (Coley, Bryant & Chapin 1985) proposed that slow-growing plants allocate more resources for defence against herbivory than do plants with faster growth rates. Fast-growing plants, on the other hand, would be able to suffer higher levels of damage from herbivores and still maintain an amount of growth commensurate with reproductive success. Controversy still exists concerning the relationship between plant growth rate and susceptibility to insect damage, and most of this research has focused on plant responses to foliar damage. These various investigations have shown that differences in plant growth rate can have a positive correlation (Cebrian & Duarte 1994; Strauss & Agrawal 1999), a negative correlation (Hoffland *et al.* 1996; Mutikainen *et al.* 2002) or no correlation (Almeida-Cortez, Shipley & Arnason 1999; Messina *et al.* 2002) with aboveground herbivory.

As an alternative to a response based solely on evaluation of foliar biomass or photosynthetic area loss to herbivory, loss may also occur in proportion to the number of actively growing tips. Insect herbivores have been hypothesized to preferentially utilize these vigorously growing plant tissues (Price 1991). In this scenario, rapidly growing aboveground plant organs have a higher probability of herbivore attack in comparison with slower-growing plant organs. This supports the hypothesis that more vigorously growing plants would better tolerate herbivory damage (Craig, Itami & Price 1989; Kimberling, Scott & Price 1990; Woods *et al.* 1996; Fritz, Crabb & Hochwender 2000) and would result in a higher absolute consumption of growing tips of faster-growing plants by herbivores, but a proportionally similar amount to that of slower-growing plants (Woods *et al.* 1996).

Plants may also avoid herbivory. Gall-forming insects, for example, need to be present when the plant organ is at its susceptible stage for successful gall formation (Weis, Walton & Crego 1988). With respect to herbivore avoidance and plant vigor, plants with higher potential growth rates may possess greater phenological or morphological plasticity (Van Schaik, Terborgh & Wright 1993). In a woody perennial like grape, such plasticity may lead to root or shoot flushes during periods of low herbivore population densities (Murali & Sukumar 1993; Mopper & Simberloff 1995). In addition to temporal avoidance, perennial plant roots may be able to spatially avoid insect herbivory by growing into soil locations not tolerated by the insects.

We are unaware of any reports that have directly observed root herbivory and its consequences on root population dynamics. Our main objective in this study was to contrast the influence of root herbivory by grape phylloxera (*Daktulosphaira vitifoliae* Fitch) on root survivorship and root population dynamics for two genetically distinct rootstocks with different rates of root tip production, but with genetically identical shoot systems. The sucking insect grape phylloxera provided us with a novel system because unlike many root-feeding insects that do not produce visible changes in root morphology, phylloxera infestations cause roots to increase in diameter and to curl or bend at 90°–180° angles (Riley 1874; Forneck, Walker & Merkt 1996) and form galls. These morphological changes allowed us to make non-destructive observations of insect infestations and at regular time intervals under field conditions. We did this by using minirhizotron technologies that have presumably minimal impact on plant–herbivore interactions. In addition, gall-forming insects such as phylloxera are dependent on the growth of new meristems for survival (Abrahamson & Weis 1987), thus allowing us to directly determine the proportion of actively growing tips in a root system and numbers of roots that were infested. By directly observing roots and their surrounding environment, we were better able to determine whether new root production and phylloxera populations were situated in the same locations at the same time.

We evaluated our data in a manner that allowed us to test whether it was more consistent with the potential growth rate-defence hypothesis (Coley *et al.* 1985) in as much as we hypothesized that vines with root systems with higher root tip production would exhibit fewer defence responses compared with root systems with lower root tip production. Support for this hypothesis would be reflected in both higher absolute numbers of roots infested with phylloxera, and a higher percentage of total roots infested. Alternatively, if root infestation were simply proportional to the number of growing tips (Woods *et al.* 1996), then per cent infestation would be similar in fast- and slow-growing root systems. Secondly, we were able to test the hypothesis that the faster-growing root system would respond faster to infestation, leading to more rapid shedding of infested roots. Thirdly, we were able to examine the hypothesis that the faster-growing root system might exhibit greater avoidance of the insects by temporally or spatially producing roots at times or locations of limited insect activity. Lastly, we hypothesized that root herbivores could alter the root system age structure by selectively feeding on the youngest roots of the finest branching orders. In this case, selective herbivory would cause portions of root systems with herbivore grazing to exhibit older root age structure than portions uninfested with insects, and this might occur to a greater extent in the fast-growing cultivar. We acknowledge that the above responses are not mutually exclusive and that a perennial plant in response to belowground herbivory may employ several such strategies.

MATERIALS AND METHODS

Site and study system

The experiments were conducted in an established research vineyard in Oakville, CA (latitude 38.44° N, longitude 122.40° W) planted with *Vitis vinifera* cv. Merlot. Soils were Bale (variant) gravely clay loams (fine-loamy, mixed, superactive, thermic Cumulic Ultic Haploxeroll). The climate was characterized by relatively warm, dry growing season conditions (May–August average daily maximum temperature of 30.7 °C; average total precipitation of 80 cm) as determined from an on-site weather station (CIMIS 2006). Vines were planted in 1995 using a spacing of 2.4 meters between rows and 2.2 meters between vines, and were trained to a bilateral cordon with vertical shoot positioning (VSP). The entire experimental vineyard covered about 1 ha and was laid out in a completely randomized block design with three irrigation treatments in each of six blocks. Within each irrigation treatment are subplots of 18 vines (two buffer rows with a central treatment row of six vines) of each of two rootstocks (1103P, *Vitis berlandieri* × *Vitis rupestris*, and 101–14 Mgt, *Vitis riparia* × *Vitis rupestris*). These rootstocks were chosen for our experiments because of their clear differences in growth rate with 1103P known for being 'highly vigorous' (Wolpert *et al.* 2002) and would be identified as the 'fast-growing' rootstock. Rootstock 101–14 Mgt is reported to be of 'moderate vigor' (Wolpert *et al.* 2002) and would be referred to as the 'slower-growing' rootstock. All vines were only rooted by the rootstock and exhibited no scion rooting. Rootstock 1103P conferred much more vegetative growth on scions than the rootstock 101–14 Mgt for this experiment where it produced nearly two times the shoot biomass and greater yields than scions on 101–14 Mgt. As we will show, the total number of new root tips produced is also much higher in 1103P.

The parents of the *Vitis* hybrid rootstocks are all native to North America, as is the insect herbivore, phylloxera (*D. vitifoliae* Fitch). The native distribution of *Vitis berlandieri* is mainly Texas and northern Mexico; *Vitis rupestris* is mainly in the central USA as far north as Kentucky, southern Missouri and southern Kansas. *Vitis riparia*, however, is not only widespread in the Gulf States as far east as Florida, but also along the west coast appearing as far north as British Columbia (Winkler *et al.* 1974). Grape phylloxera is common throughout most of this range.

Vines reported in this study were irrigated at 40% of the maximum estimated vineyard evapotranspiration (ET_c), which is a common practice in the Napa region. ET_c was determined from a reference evapotranspiration (ET_c) multiplied by a dimensionless crop coefficient K_c that takes into account crop leaf area, reflectance, canopy resistance and evapotranspiration from exposed soil (Hunsaker *et al.* 2003). The vineyard was irrigated using a micro-irrigation system with one drip emitter located about 50 cm from the trunk of each vine.

In April 2002, clear cellulose acetate butyrate (CAB) root observation tubes (minirhizotrons) were installed at an angle of 30° from the vertical. While CAB may affect the survivorship of roots of some woody species (Withington *et al.* 2003), it seems improbable that this plastic would differentially affect two closely related cultivars or the relative sensitivity of roots to phylloxera. One minirhizotron tube was placed through the drip zone, and the other was placed 60 cm from the trunk on the opposite side of the vine in an area that was not irrigated. Tubes were 1.5 m in length, 6 cm in outside diameter and had a viewing area of 0.0192 m^2. The bottoms of the tubes were sealed with polyvinyl chloride (PVC) plugs, and the top of the tubes were wrapped with black electrical tape and sealed with rubber stoppers to prevent light penetration. The tops of the tubes were covered with a white aluminum can to prevent radiant heating when not in use. Beginning in June of 2002, a specially designed digital imaging camera (BTC-2; Bartz Technology, Santa Barbara, CA, USA) was used to observe roots every 2 weeks during the growing season and typically every month after leaf fall and before bud break. A sub-population of roots was sampled at a shorter interval of every 3 h for 5 d to examine short-term changes in root diameter and nodule development. The images were captured directly to a computer using software designed by Bartz Technology (ICAP v.4.1, Bartz Technology). All images were analysed using Win Rhizo Tron MF software (Regents Inc. Quebec, Canada). In addition to monitoring the date of root birth, lifespan, root diameter, root order, number of first-order lateral roots (roots with no laterals) and number of neighboring roots present at birth and death events of an individual root (index of competition), the date of insect infestation was recorded. Root births were estimated by calculating the date midway between the observation date when a root was first observed and the previous observation date. Similarly, root death was estimated as being midway between the date the root was first observed dead and the previous observation date. Root death was identified by a black and shriveled appearance (Comas, Eissenstat & Lakso 2000) or if the root had disappeared from the window and did not reappear. Roots that transected more than one minirhizotron observation window vertically within the same minirhizotron observation tube were only counted once. Phylloxera-infested roots were identified as those exhibiting a typical bulbous swelling and with an associated phylloxera population immediately adjacent to the root.

Environmental data collected, including daily maximum air temperature and precipitation, were downloaded daily from a weather station located on site (CIMIS 2006). Volumetric soil water content was estimated approximately every 2 weeks using time domain reflectrometry. The minirhizotrons were used as an access tube for soil moisture determination at three depths, 0–30, 30–60 and 60–90 cm, intervals that corresponded with highest root densities, using a Trime soil moisture probe (Mesa Systems Co., Medfield, MA, USA).

Total root production was normalized using a log transformation and analysed using analysis of variance (version 11.0; SPSS Inc., Chicago, IL, USA). Block and year were not significant and thus were removed from the model. Root lifespan data were analysed with the Cox proportional hazards regression (PROC PHREG; SAS Institute Inc., Cary, NC, USA). This type of analysis allows the influence of all other covariates to be held constant while the 'hazard' of an individual covariate is determined (Cox 1972). The 'hazard' of a covariate refers to the risk of mortality of a root at any point in time (Allison 1995).

Statistical Analysis System's PROC PHREG (SAS Institute Inc.) uses the partial likelihood method (Cox 1972) to estimate a parameter coefficient of β for each tested covariate, and calculates a chi-square statistic to test the null hypothesis that each β equals zero. A parameter estimate can have either a negative or positive sign depending on the effect it has on the covariate. In this case, a negative sign indicated a decreased hazard of mortality with an increase in the covariate (Wells & Eissenstat 2001). Covariates tested included root diameter, number of daughter roots and the number of neighbor roots present in the window. Effects of year of observation and depth of roots on root population size were analysed using the general linear model (GLM) procedure (version 11.0, SPSS Inc.). *Z* tests were used to determine differences and significance values for cumulative grape root mortality between the two rootstocks. Root age–class differences were examined by comparing age distributions of roots in observation windows containing infested roots with adjacent windows containing no infested roots. Age class differences were analysed using PROC FREQ (SAS Institute Inc.).

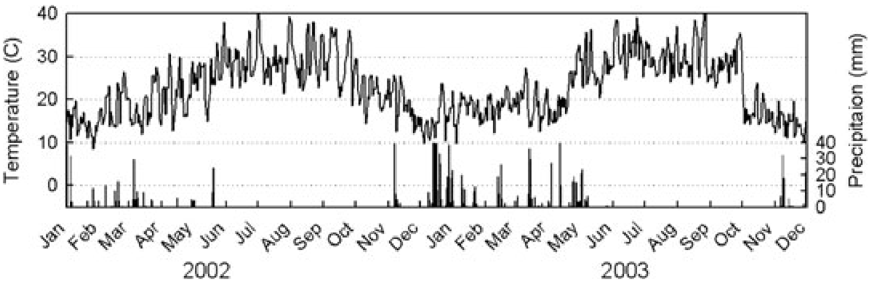

Figure 1. Daily maximum temperatures (line) and precipitation (bars) for Oakville, CA in 2002 and 2003. Total rainfall in 2002 was 94.5 cm, and in 2003, it was 91.1 cm.

RESULTS

The years 2002 and 2003 were typical for weather patterns of Napa Valley, Napa, CA with cool wet winters and warm summers with no rainfall (Fig. 1). Soil moisture generally increased with the soil depth, with 2003 being moister at all three depths as a result of greater precipitation amounts in late spring (Table 1 and Fig. 1). Per cent soil moisture only decreased by about 5% of volumetric content over the summer season at all three depths measured, which probably reflect the high clay content of soils at the site (18–36% clay) and their high resistance to root growth as it dries.

The fast-growing root system (1103P) produced three- to fourfold more roots than the slower-growing root system (101–14 Mgt) ($P = 0.03$, Fig. 2), and differences in root production between the two root systems were similar in 2002 and 2003. Consistent with the hypothesis that herbivory is proportional to the number of growing tips, we observed a similar percentage of roots infested with phylloxera in the two root systems ($P = 0.202$). Thus, the absolute number of roots infested was higher in the fast-growing root system ($P = 0.004$), but the relative number was similar to that in the slower-growing root system in both years of the study.

Populations of roots infested with phylloxera reached peaks in mid summer and generally overlapped with periods of peak root production (Fig. 3). Peaks in insect activity also corresponded with peaks in seasonal temperature and an extended period of time without rainfall but with irrigation (Fig. 1). Thus, there was no evidence for temporal avoidance in root production of either root system in relation to timing of peak insect infestations. There was also little evidence that roots at particular locations in the soil escaped insect infestations. Rather, it appeared that fine root production was related to soil temperature increase and development of a photosynthetic canopy during spring. Per cent of infested roots was distributed differently with depth for the two rootstocks (rootstock × depth interaction: $P = 0.0251$, Fig. 3). The majority of infested roots were located in the 60–90 cm depth (56%) for the fast-growing rootstock, while the slower-growing root system had equal distribution of infested roots at 30–60 cm (39%) and 60–90 cm (41%). For both root systems, the surface roots (0–30 cm) had the lowest percentages of infestation with 12.5% of roots for the fast-growing rootstock and 19.5% for the slower-growing rootstock. We did not observe that root diameter ($P = 0.62$) or number of neighboring roots ($P = 0.21$) had an effect on a root's susceptibility to grape phylloxera infestation in either year.

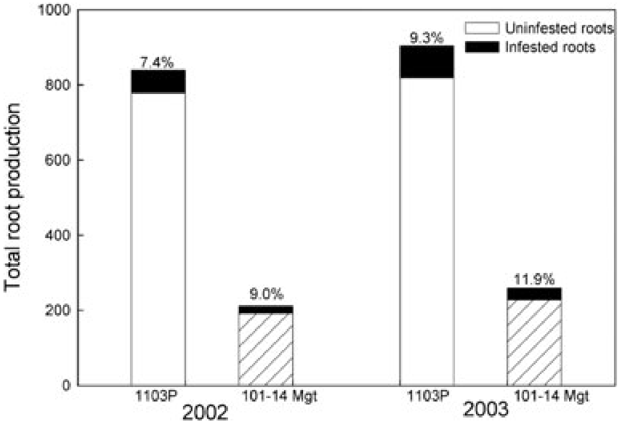

Figure 2. The total number of roots produced annually for both irrigated and non-irrigated sides of the vine (number of roots m^{-2} of viewing surface) for the years 2002 and 2003. Shown by the white bars are uninfested roots of a fast-growing rootstock (1103P), and by the hatched bars uninfested roots of a slower-growing rootstock (101–14 Mgt). The solid black bars show the number of infested roots. Differences in total root production between the two rootstocks for the 2 years were significant ($P = 0.03$). Percentages above the bars indicate the per cent frequency of infested roots (cultivar effect: $P = 0.202$).

Table 1. Soil moisture (per cent) averaged over both sides of the vine during July 2002 and July 2003 at three depths (±1 SE)

	2002		2003	
Depth (cm)	1103P	101–14 Mgt	1103P	101–14 Mgt
0–30	25.2 (±2.0)	22.8 (±2.3)	26.4 (±1.7)	24.3 (±1.4)
30–60	26.5 (±1.9)	23.8 (±1.6)	28.7 (±1.6)	26.5 (±1.7)
60–90	29.4 (±1.8)	30.2 (±2.2)	30.9 (±1.2)	31.4 (±1.7)

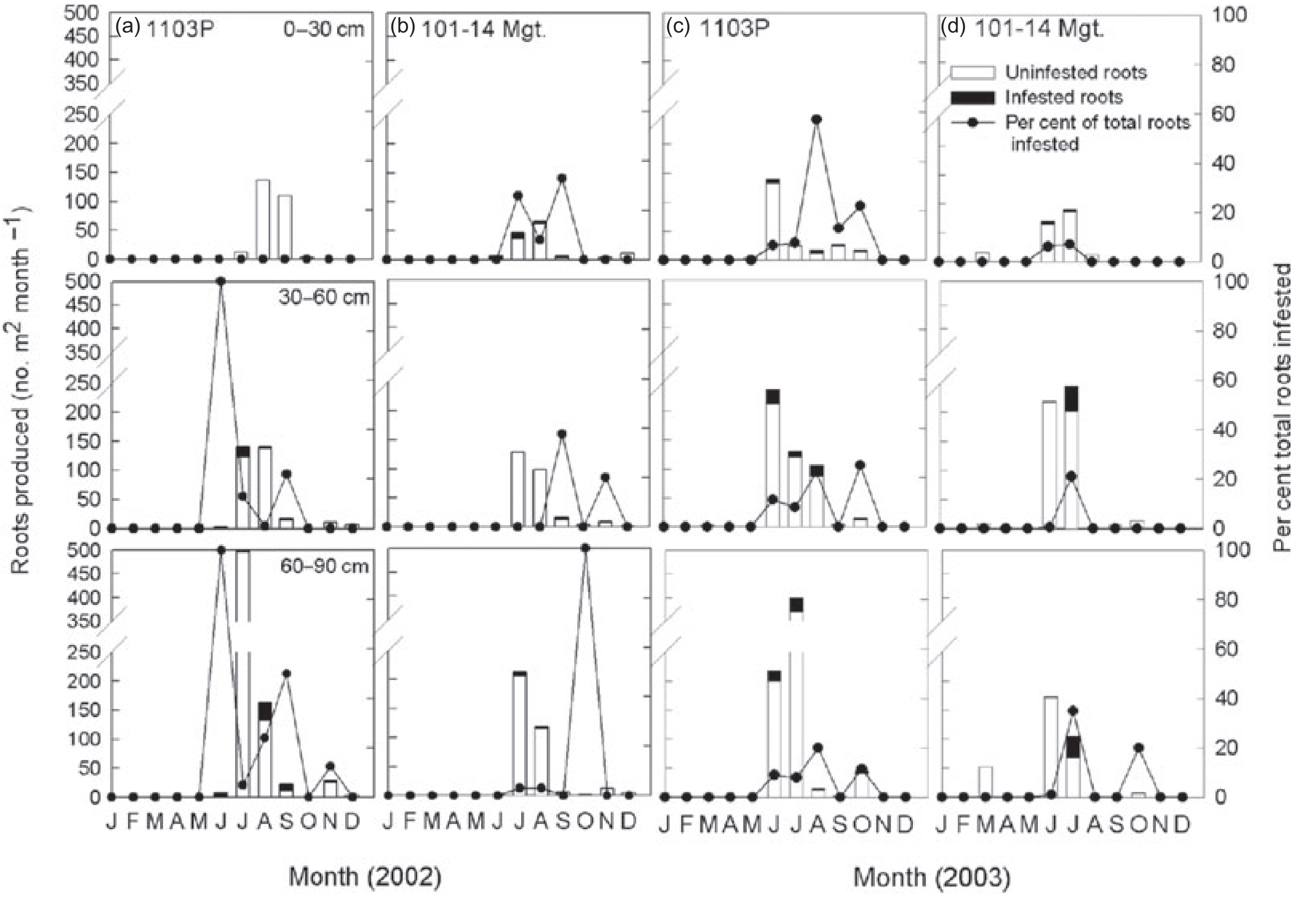

Figure 3. Monthly patterns of root production and per cent infestation on the irrigated side of the vine for a fast-growing root system, 1103P (a & c) and a slower-growing root system, 101–14 Mgt (b & d) for 2002 (a & b) and 2003 (c & d) and at 0–30, 30–60 and 60–90-cm-depth intervals (upper, middle and bottom row of plots, respectively). Shown by the histogram bars is the monthly new root production by depth interval of uninfested roots (unshaded) and infested roots (shaded) (mean of six plots with 1 tube per plot). Total monthly root production is indicated by the sum of the two bars. Data are expressed per square meter of viewing surface of minirhizotron (total viewing surface per plot over a 30 cm vertical depth = 63 cm^2). The line graph represents the monthly percentage of the total new roots that were infested over the year.

Percentages of infested roots that perished were highest immediately following tube installation, but declined to more constant values shortly afterward (Fig. 4). By October of the first year of the study, approximately two times the percentage of total roots that were apparently infested with phylloxera died at each sampling date for the more vigorous root system (1103P), compared to that of the less vigorous root system (101–14 Mgt). Statistically significant differences were detectable between rootstocks for nearly all sampling dates ($P < 0.05$).

Cumulative root mortality observed from the beginning of the experiment by the month reported ranged from 18.8 to 6.0% for the fast-growing root system and from 25.0 to 3.6% for the slower-growing root system. A twofold increase in root mortality attributable to phylloxera infestation initially occurred for roots on the irrigated side of the vine. Too few roots were born on the non-irrigated sides (dry soils) that were visible through the minirhizotron windows to permit a direct statistical comparison between the irrigated and non-irrigated roots. Nonetheless, the observed mortality patterns for non-irrigated versus irrigated roots were nearly identical to those shown in Fig. 4.

Root survivorship of uninfested roots was similar for the root systems of both rootstocks ($P = 0.991$), but infested roots died more quickly in the fast-growing rootstock, 1103P ($P = 0.022$, Fig. 5). According to hazard ratios, roots deeper in the soil had a 14% lower risk of mortality with every 30-cm-depth increment, and larger diameter roots had 37% lower risks of mortality for every 1 mm increase in diameter ($P = 0.001$). More roots present at the time of birth decreased risks of individual root mortality by 3% with each additional neighbor ($P < 0.001$), while the opposite was true for the number of roots present at time of death, with an increased risk of mortality of about 5% with each additional neighbor ($P < 0.001$).

Roots that became visibly infested with phylloxera usually did so within 2 weeks following their birth for both root systems (7.5 ± 1.5 SE days). Once infested, root survivorship of phylloxera-infested roots was lower than that of uninfested roots (contrast Fig. 5b with 5c, $P < 0.001$).

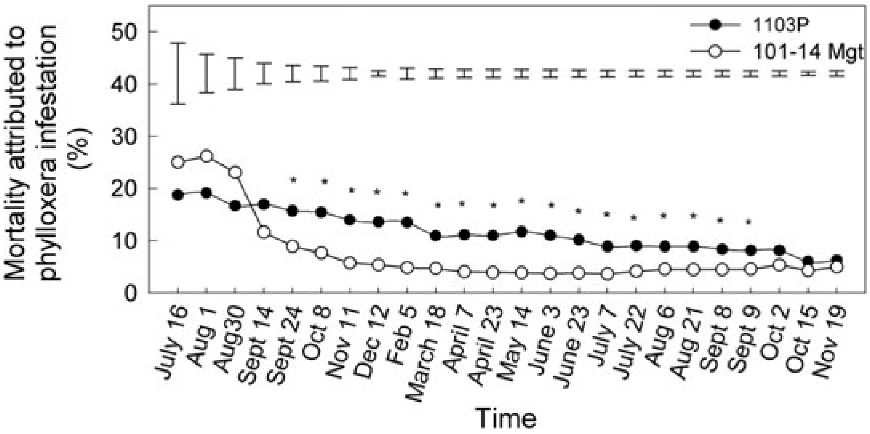

Figure 4. Shifts in cumulative grape root mortality that was attributable to phylloxera infestation for a rootstock with fast growth (1103P) and a slower-growing rootstock (101–14 Mgt). Data represent the percentage of total cumulative root mortality from the beginning of the experiment (June 2002) that was attributed to phylloxera infestation on the date indicated, relative to total cumulative mortality observed up to that time. Observations were combined for minirhizotron observation tubes located on both the irrigated and the non-irrigated sides of the vine. Vertical bars represent pooled SEs. Significant differences observed between rootstocks are indicated by asterisks ($P < 0.05$).

Regardless of the population size of the infestation, if a fine lateral root became infested with phylloxera, and a nodosity was formed, the median lifespan was about 60 and 85 d for the fast- and slow-growing rootstock roots, respectively. Uninfested roots, however, were not significantly different between the two rootstocks ($P = 0.991$) and had a lifespan of greater than 200 d (Fig. 5).

Infested and uninfested roots in June 2003 displayed a bimodal age structure with the majority of roots falling into either a 'young' age class (<100 d) or an 'old' age class (>300 d, Fig. 6). For the entire root population, similar age structures were observed for both rootstocks (Fig. 6a); however, for the fraction of the population infested with phylloxera, the fast-growing root system exhibited an older root age structure than that of the slower-growing root system (Fig. 6c, $P < 0.001$).

Among roots less than 100 d old, no significant age class differences were observed between rootstocks. Consequently, the data were combined for the two rootstocks to evaluate the effects of phylloxera infestation on root age structure of this 'younger' root population (Fig. 7). The age structures of infested and uninfested roots were similar in the 100 d age class during June and July; however, significant differences in root age structure developed in August ($P < 0.001$) and September ($P < 0.002$). Populations of uninfested roots alive in August and September had an older age class distribution than infested roots, although the majority of roots were still younger than 60 d.

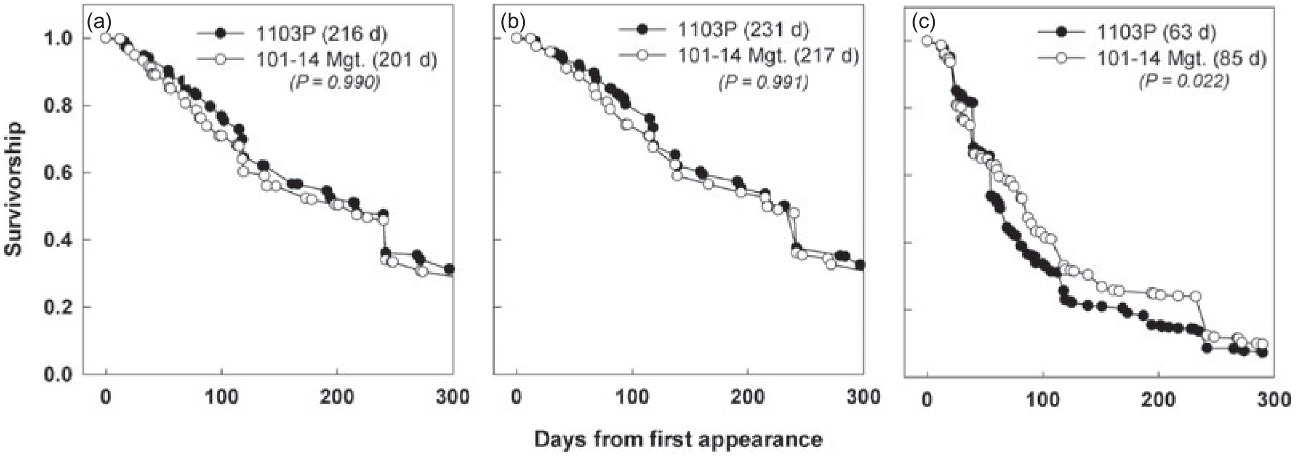

Figure 5. Root survivorship in a vineyard in Oakville, California for (a) total, (b) uninfested and (c) phylloxera-infested roots of a rootstock with fast (1103P) and slow (101–14 Mgt) growth rates. Median lifespans are indicated in parentheses. Data are for all roots observed through minirhizotron windows located in both irrigated and non-irrigated soils in 2002 and 2003. The rootstock of slower growth rate, which produced fewer roots, had significantly higher root survivorship ($P = 0.022$). Differences in survivorship between uninfested (b) and infested (c) roots were significant for both root systems ($P < 0.001$).

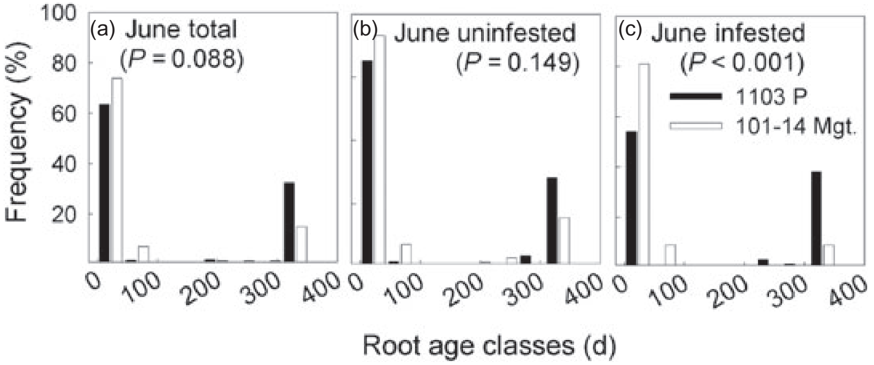

Figure 6. Root age structure of (a) total, (b) uninfested and (c) infested fine roots over the growing season in 2003 for the rootstock of high (1103P, shaded) and low (101–14 Mgt, unshaded) rates of root production. Age structure is indicated by the relative frequency of roots in a given age class. Age classes are in 50 d intervals.

DISCUSSION

Trade-offs between plant growth rate and herbivory defence have been proposed for foliar herbivory (Coley 1988; Cebrian & Duarte 1994), but this is the first study to our knowledge to report on the relationship between growth rate of root systems and root herbivory tolerance. In grape, we found that root systems with a threefold difference in root tip production (Fig. 2) exhibited similar proportional rates of infestation by phylloxera, an indication of similar defences against the insect. However, roots of the faster-growing root system that became infested with phylloxera had shorter lifespans than those of the slower-growing root system (Fig. 5), suggesting that differences exist in how woody perennial plants and resistant rootstocks of different growth potentials may tolerate a root-feeding insect.

According to the plant vigor hypothesis established from aboveground studies, insect infestation level may be related to the number of young growing tissues (Woods *et al.* 1996). The two root systems we examined had similar per cent infestation of the finest roots. The root system of the cultivar that produced many more root tips (1103P, Fig. 2) had more infested roots than that of the cultivar with the slower-growing root system (101–14 Mgt, Fig. 3) but the proportion infested was similar. Therefore, the number of meristematic tips, and perhaps the insect's ability to find them, appears to be the driving factor in herbivory damage by the galling insect in this system.

Root lifespan of infested roots of the faster-growing cultivar, however, was shorter than infested roots of the slower-growing rootstock (Fig. 5). This led to higher total root mortality attributed to phylloxera in the faster-growing root system after the initial root establishment phase (Fig. 4). Thus, fine roots of the grape rootstock of higher apparent growth rate seemed less tolerant of infection by the sucking insect phylloxera.

Differences in root longevity between the rootstocks may also have important implications for the insect. The greater longevity of infested roots in the slower-growing rootstock would allow for longer lifespans of the phylloxera, possibly leading to greater egg production and subsequently higher insect population densities.

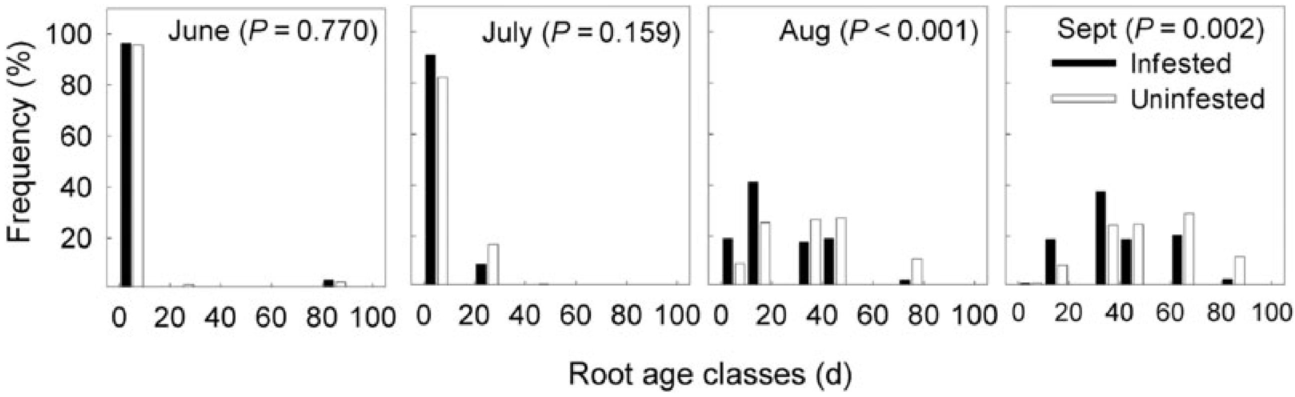

Figure 7. Root age structure of roots less than 100 d old from June through September of 2003 for both rootstocks combined. Age classes are in 10 d intervals (e.g. the large June age class represents infested and uninfested roots that are 0–10 d old). The 80–90-day-old age class in June represents a very small fraction of roots born in the early spring of 2003. The probability of a significant difference in age structure between infested and uninfested roots is indicated in parentheses.

The high rates of root mortality at the beginning of the study (Fig. 4) may have been a consequence of the large new root flush following soil disturbance or disturbance of the root system caused by minirhizotron installation (Smart *et al.* 2005). It is possible that insect populations increased as a consequence of increased carbohydrate availability during these root flushes or the high concentration of very young, susceptible new root meristems. Similar root flushes can be caused by nematode attack in natural systems or root pruning in agricultural systems (Murray & Clements 1998). Nonetheless, during 2003, the percentage of roots succumbing to insect infestation appeared to be stabilizing in both root systems with fine root mortality attributable to the insects at only about 5–10% (Fig. 4). As a consequence of shorter root lifespans, insect-related root mortality in the faster-growing rootstock was twice that of the slower-growing rootstock in spite of the similar percentage of infestation.

Laboratory studies have indicated that phylloxera feeding on nodosities of *Vitis vinifera* roots is rarely tolerated and results in root death (Kellow, Sedgley & Van Heeswijck 2004). Our field study indicates that the rootstock that produced fewer roots (101–14 Mgt) had a lower risk of root mortality caused by phylloxera infestation as compared with the rootstock that produced more roots (1103P). In both cases, infested roots sustained relatively long median lifespans (63–85 d, Fig. 5) similar to those (90 d) found under optimal laboratory study conditions (Forneck *et al.* 1996).

Previous research has suggested several causes for decreased lifespan of phylloxera-infested roots. One theory suggests that the phylloxera insect may serve as a vector for introduction of fungal or bacterial pathogens (Granett *et al.* 1998; Omer & Granett 2000). Fungal opportunists may enter the root upon damage by grape phylloxera probing and be the ultimate cause of root death. Root swelling may also be linked to the main cause for the increased risk of mortality in roots (Kellow *et al.* 2004). However, we observed little change in root diameter of insect-infested roots after initial swelling for either root system.

Unlike aboveground plant structures, roots reside in an often extremely heterogeneous environment in regard to oxygen, moisture, temperature and physical impedance, all of which may influence root–insect interactions. Nonetheless, we did not see any evidence of temporal or spatial avoidance of insect herbivory by the root systems. Root growth was primarily concentrated at depths of 30–90 cm, which was also the location where phylloxera populations were most abundant and where temperature and moisture conditions should be more stable. Both root systems had readily visible insect populations at times of highest root abundance (spring) regardless of soil depth. Because the insect does not apparently cause high rates of root mortality in the germplasm where the vine and insect co-evolved, there may have been little selection pressure for growth mechanisms that would allow for avoidance of the insect.

Root system function is tightly coupled to root age (Volder *et al.* 2005). In woody perennial plants where root systems undergo seasonal flushes of new root cohorts, the age structure of the finest roots is dynamic (Wells & Eissenstat 2003). High levels of nutrient uptake potential and metabolic activity are associated with very young roots (Comas *et al.* 2000; Volder *et al.* 2005). Thus, selective mortality of the youngest roots that alters a root system's age structure towards an older age distribution may diminish the ability of roots to acquire water and nutrients. The higher mortality rate of infested roots for the cultivar that produced more new roots led to a somewhat older infested root population than that of the slower-growing root system (Fig. 6c). Because infested roots represented only a small fraction of the total root population, the age structure of the total root population (infested and non-infested roots) was similar for the two root systems (Fig. 6a). Nonetheless, insects infested much larger percentages of newly formed roots for both root systems (Fig. 7). This resulted in proportionally fewer roots reaching older age classes in root populations infested with insects as compared with uninfested roots. Infestation in August, for example, caused relatively fewer roots to reach ages older than 40 d, and resulted in a greater percentage of roots in the 10 and 20-day-old categories (Fig. 7). Because roots older than 40 d are not likely to show high metabolic activity and nutrient uptake capacity (Comas *et al.* 2000; Volder *et al.* 2005), the effect of root age structure on nutrient and water acquisition of the overall plant may therefore depend on the seasonal availability of nutrients and extent of plant water demand. Thus, a shift to an older average age structure of roots over 40 d old may not necessarily result in large decreases in overall performance of the root system based on these seasonal considerations.

SUMMARY

In summary, we found that grape phylloxera infested rootstocks with different growth rates (new root production) in proportion to the number of growing tips. The rootstock with inherently more new root production was also observed to be less tolerant of insect feeding at the local level as indicated by more rapid mortality of infested roots. We observed no evidence of temporal or spatial avoidance of the insect in either root system cultivar.

The overall impact of phylloxera on these root systems and aboveground production is still in question. While we observed reductions in root survivorship of infested compared to uninfested root populations, the cumulative loss of only 4–6% of the total roots attributable to insect infestation (Fig. 4) suggests only a small decrease in root biomass. Moreover, it appeared unlikely that a reduction of 'middle-age' classes of roots (40–60 d old) in infested root populations substantially affected the plant's ability to take up water and nutrients (Comas *et al.* 2000; Volder *et al.* 2005). Not known is the amount of carbon loss caused by direct feeding of the insects or in gall formation and maintenance, which may be lower in the faster-growing rootstock cultivar if it sheds phylloxera-infested roots more rapidly.

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