Soil insects alter fine root demography in peach (*Prunus persica*)

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ABSTRACT

Minirhizotrons were used to assess the effects of soil insect suppression on the demography of peach fine roots (<1 mm diameter) over two growing seasons. The experiment was conducted at the USDA–ARS Appalachian Fruit Research Station in Kearneysville, WV, USA using six 15-year-old peach trees. Clear butyrate minirhizotrons were installed beneath each tree in April 1996. Soil drench treatments were applied around individual minirhizotron tubes at monthly intervals and consisted of 1 L of water or 250 μL of a broad-spectrum insecticide in 1 L of water. Roots were videotaped at 2- to 4-week intervals during the 1996 and 1997 growing seasons. Insecticide application was associated with a significant increase in fine root longevity: the median lifespans of insecticide-treated roots were 46–125 d longer than those of control roots. In addition, the development of brown pigmentation was significantly delayed in insecticide-treated roots. Insecticide application did not appear to increase soil fertility, as accumulation of NO_3^-, NH_4^+, and PO_4^{2-} on mixed bed ion-exchange resin was similar in treated and untreated soil. These results suggest that interactions with below-ground insects can significantly influence root longevity and may alter the rate at which roots undergo developmental changes in anatomy and physiology.

Key-words: Minirhizotron; proportional hazards; root herbivory; root pigmentation; root turnover.

INTRODUCTION

The effects of above-ground insect herbivory on plant performance in both natural and agricultural systems are well documented (Crawley 1983; Gange 1990; Trumble 1993). However, our understanding of below-ground herbivory is much more limited (Anderson 1987; Brown & Gange 1991) and generally focuses on the effects of specific crop pests such as the corn rootworm (*Diabrotica* spp.; Levine & Oloumi-Sadeghi 1991) or the grape phylloxera (*Daktulosphaira vitifoli*; Ordish 1987). Below-ground herbivory has been responsible for dramatic reductions in above-

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ground production (reviewed in Brown & Gange 1990) and can significantly affect host plant physiology by changing carbon allocation patterns (McNaughton 1983; Schmid, Miao & Bazzazz 1990), creating or exacerbating water stress (Ridsell-Smith 1977; Gange & Brown 1989), and altering tissue nitrogen levels (Goldson *et al*. 1985; Steinger & Muller-Scharer 1992). In addition, a number of studies indicate that substantial below-ground herbivory can occur in systems where no single dominant root pest has been identified and in which above-ground symptoms of root feeding are not readily apparent (Henderson & Clements 1974; Henderson & Clements 1977; Brown & Gange 1989; Wilson, Gunn & Cherrett 1995).

Frequently, the effects of below-ground herbivory do not manifest themselves above ground until a significant portion of the root system has been removed. The 'damage threshold' for root loss, defined as the percentage of the root system that can be removed without measurable reduction in above-ground production (Brown & Gange 1991), has been estimated at 50–60% for grasses (Davidson 1979) and can be as high as 67% in maize under well-watered conditions (Dunn & Frommelt 1998). Maron (1998) found little effect of ghost moth root herbivory on bush lupin growth and fecundity over the course of 2 years despite considerable damage to the root system, although delayed effects on fecundity appeared in the third year. Such high tolerances for root loss suggest that moderate levels of below-ground herbivory may be common and may represent an important cause of root mortality in the field.

In recent years, the contributions of fine roots to whole plant carbon budgets (Lambers 1987) and to ecosystem level carbon and nitrogen cycles (Jackson, Mooney & Schulze 1997) have gained wider appreciation. Non-destructive root observation tubes (minirhizotrons) have permitted detailed measurements of root longevity in a number of systems (Hendrick & Pregitzer 1992; 1993; Reid, Sorenson & Petrie 1993; Hansson, Aifen & Andren 1995; Majdi & Kangas 1997; Fitter *et al*. 1998; Ruess, Hendrick & Bryant 1998; Wells & Eissenstat 2001) and have revealed differences in longevity among roots of various sizes and positions within the root system (Gill *et al*. 2001, Wells & Eissenstat 2001; Wells, Glenn & Eissenstat 2001). Nonetheless, we have little insight into the physiological and ecological processes that determine the longevity of individual fine roots. To what extent root lifespan is controlled by the plant to optimize the efficiency of resource capture (Eissen-

stat & Yanai 1997) and to what extent it is controlled by external biotic and abiotic factors remains unclear.

A number of authors working with rhizotrons and minirhizotrons (Rogers 1929; Head 1968; Fogel 1990; Wilson *et al.* 1995) have noted insects feeding on and around roots, although the consequences of these interactions for root life history have rarely been examined in detail. Rogers (1929) noted that insects appeared to feed on dead or decaying cortical tissue which had been sloughed from apple roots, and Head (1968) observed that the activities of insects on the surface of white roots were responsible for significant losses of root–soil contact in apple. Wilson *et al.* (1995) measured a decrease in root turnover and a 30% increase in above-ground dry matter production following soil insecticide treatment of a British grassland. Although insect numbers declined in this system following pesticide application, none of the specific insects affected were considered to be pests.

In the present experiment, we used minirhizotron observations to assess the effects of soil insect suppression on the production and longevity of peach (*Prunus persica*) fine roots during two growing seasons. Soil insect populations were reduced through the application of chlorpyrifos, a broad-spectrum organophosphate insecticide that has been widely used in studies of below-ground herbivory (McGonigle & Fitter 1988; Brown & Gange 1989; Maron 1998). We used a Cox proportional hazards regression approach to compare the risk of mortality for treated and untreated roots and to determine whether all fine roots responded equally to the pesticide treatments. Previous root demographic work in peach has shown that fine roots of different orders, diameters, and depths differ in their risk of mortality (Wells *et al.* 2001), and we therefore hypothesized that these different classes of roots would also differ in their susceptibility to herbivory. Destructive harvest data were used to determine whether differences in fine root longevity in response to pesticide application were associated with changes in the root length density of the bulk soil.

MATERIALS AND METHODS

Study site and minirhizotron installation

The experiment was conducted at the United States Department of Agriculture–Agricultural Research Service Appalachian Fruit Research Station in Kearneysville, West Virginia using six 15-year-old Loring peach trees on Halford rootstock growing in a Hagerstown silt loam soil (fine, mixed Mesic Typic Hapludalf). The trees at this site exhibited no above-ground symptoms of below-ground herbivory, and the soil did not contain significant numbers of insects considered to be pests in peach (D. M. Glenn, personal communication). Minirhizotron observations and inspection of soil samples revealed the soil arthropod population to consist primarily of members of the orders Acarina and Collembola, the class Diploda, and Lepidopteran and Coleopteran larvae.

In April 1996, six clear butyrate observation tubes (minirhizotrons) were installed beneath each tree at an angle of 30° from the vertical, pointing towards the tree base. Tubes were placed approximately 0·7 m from the trunk and at least 0·8 m from one another. They were 70 cm in length (50 cm viewable), 6 cm in outer diameter, and were inscribed with a single vertical transect of 30 1.8×1.2 cm windows on the upper surface. 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 aluminium cans. Two minirhizotrons from each tree (12 minirhizotrons total) were randomly chosen for use in the present experiment; the remaining tubes were used in additional experiments not reported here.

Insecticide application

Soil drench treatments were applied around individual minirhizotron tubes at monthly intervals from August to October in 1996, from April to October in 1997, and from April to June in 1998. One tube per tree received 1 L of water, and the other received 250 μL of Lorsban 4E insecticide (chlorpyrifos, 44·9% by volume; DowElanco Corporation, Midland, MI, USA) in 1 L of water. Assignment of drench treatments to tubes was random. The drenches were poured slowly around the circumference of each tube where it emerged from the ground, allowing the liquid to move down the soil–tube interface. Care was taken to treat only the soil immediately adjacent to the minirhizotron tube. Initial trials in which a miniaturized camera (Bartz Technology, Santa Barbara, CA, USA) was used to videotape the progress of dyed water along the soil–tube interface confirmed that this method successfully treated all roots adjacent to the minirhizotron windows.

Chlorpyrifos is a non-systemic, broad-spectrum organophosphate insecticide which acts as a cholinesterase inhibitor in the insect nervous system. It is labelled for use against numerous arthropod pests, including Coleopteran and Lepidopteron larvae, mites, aphids, scale insects, ants, thrips, and wireworms. Chlorpyrifos is primarily a contact poison, although death may also result from ingestion or exposure to vapours (Thomson 1982). Following application, chlorpyrifos adsorbs quickly to soil particles and remains relatively immobile in the soil (USEPA 1989; Racke 1992); its half-life ranges from 60 to 120 d, although this figure can be modified by soil and climatic factors (Hartley & Kidd 1983; Howard 1989).

Although the chlorpyrifos molecule does contain an atom of phosphorus, it is unlikely that the application of Lorsban 4E directly increased soil fertility in our experiment due to the low amounts of active ingredient applied (approximately 97·5 mg chlorpyrifos containing 8·7 mg P per drench). Chlorpyrifos has been widely used in studies of below-ground herbivory, and a number of authors have reported no effect of the pesticide on plant growth and nutrient status in the absence of herbivores (McGonigle &

Fitter 1988; Brown & Gange 1989; Maron 1998). Greenhouse trials with saplings grown in sterilized soil confirmed that peach exhibited no growth response to chlorpyrifos in the absence of herbivores (data not shown). Evidence suggests that the effect of chlorpyrifos on non-arthropod fauna is also minimal (Clements & Bale 1988; Pozo *et al.* 1995).

Ion exchange resin bags

It is unlikely that soil fertility was directly affected by the pesticide applications, but it may have been enhanced by the death and decomposition of target organisms. We tested this possibility using an mixed-bed ion exchange resin (IER) bag method (Binkley & Matson 1983), which provided an integrative measure of soil nitrate, ammonium and phosphate availability over the weeks following pesticide application. Buried IER bags intercept gravitational and diffusional water flow and accumulate nutrient ions in amounts which reflect their activities in the soil solution (Yang, Skogley & Schaff 1991; Wyland & Jackson 1993). Accumulation is sensitive to the water regime (Binkley 1984), temperature (Yang *et al.* 1991), and short-duration nutrient pulses (Gibson 1986) and is thought to provide a reasonably accurate reflection of nutrient availability to the root system through time.

Ion exchange resin bags were made by placing 15 g wet weight (7·45 g dry weight) of mixed bed cation + anion exchange resin (Dowex MR-3, Sigma # I9005, Sigma-Aldrich Co., St Loris, MO, USA) into nylon stocking tubes which were sewn shut with cotton-polyester thread for finished bag dimensions of 8·0 cm × 5·0cm × 0·5 cm. Twelve bags were buried beneath each of the six experimental trees (72 bags total) at a depth of 8 cm and a distance of 0·8 m from the trunk in May 1998. Following burial, each bag received one of two soil drench treatments: 500 mL of water or 500 mL of insecticide as described above.

Six bags from each tree were removed after 1 month, whereas the other six received a second soil drench treatment at 1 month and were removed after 2 months. The exposure times of the resin bags were chosen to reflect the time frame within which we saw responses to our pesticide treatments in the minirhizotron videos. Resin recovered from the bags was air-dried and extracted in 200 mL of 1 M KCL. Nitrate and phosphate concentrations in the extract were analysed by ion chromatography (DX-500 system with conductivity detector and chemical suppression, Dionex Corp., Sunnyvale, CA, USA). Ammonium concentrations were determined colorimetrically using the automated phenate method (American Public Health Association 1998) on a Technicon AutoAnalyzer II system (Bran + Luebbe, Buffalo Grove, IL, USA). Availability indices (μg nutrient g^{-1} dry resin) for each nutrient were calculated as described in Binkley & Matson (1983).

Root videotaping and final harvest

Roots that grew against the surface of the minirhizotron tubes were videotaped at two-to four-week intervals during the 1996 and 1997 growing seasons using a miniaturized camera system and portable VCR (Bartz Technology). Videotaping was suspended during the winter, as prior observations indicated that there was little root activity during this time. Frames from the resulting videotapes were digitized and archived on a Macintosh 7500 computer (Wells & Eissenstat 2001). Data recorded for each root included the date of appearance, date of browning, date of death, depth, diameter, and order. Death was defined as either (a) disappearance or (b) a blackened and shrivelled appearance. Roots that turned brown but appeared intact were not counted as dead. Root diameter was measured to the nearest tenth of a millimetre on the first date that the root appeared using RooTracker software (Dave Tremmel; Duke University Phytotron, Durham, SC, USA). Root order was assigned according to a topological classification scheme in which roots with no dependent laterals (external links) were considered to be order 1, roots with a single set of dependent laterals were considered to be order 2, and so on.

After the last video sampling date in June 1998, a 1·25 cm thick annulus of soil was removed from around each minirhizotron tube (approximately 2000 cm^3 of soil per tube). Roots from each sample were washed free of soil and scanned on a flat bed colour scanner; root length per sample was measured using WinRhizo software (Regent Systems, Quebec, Canada) and used to calculate the root length density (cm root cm^{-3} soil) of the soil surrounding each minirhizotron tube.

Statistical analyses

The effects of insecticide treatment on nutrient availability indices and root length density were each assessed using analysis of variance with tree as a random effect and treatment as a fixed effect. Average root browning times were compared using the Mann–Whitney *U*-test (SPSS 8·0; SPSS, Inc. Chicago, IL, USA).

The effects of insecticide treatment, root order, root diameter, depth (0–20 cm or 20–40 cm) and browning on the risk of root mortality in each year were estimated using a Cox proportional hazards regression (Cox 1972; Allison 1995; Wells & Eissenstat 2001) performed with SAS PROC PHREG (SAS Institute, Cary, NC, USA). Roots from all cohorts produced after the initiation of treatments in a given year were combined for this analysis to ensure adequate numbers of roots in all categories. The data were stratified by cohort in order to control for the effects of production date on the risk of mortality. Terms representing the interactions between each covariate and insecticide treatment were also tested. Non-significant interaction terms ($P > 0.05$) were subsequently removed to arrive at the simplest model that described the data.

RESULTS

The use of a broad-spectrum insecticide to suppress soil insect populations around individual minirhizotron tubes
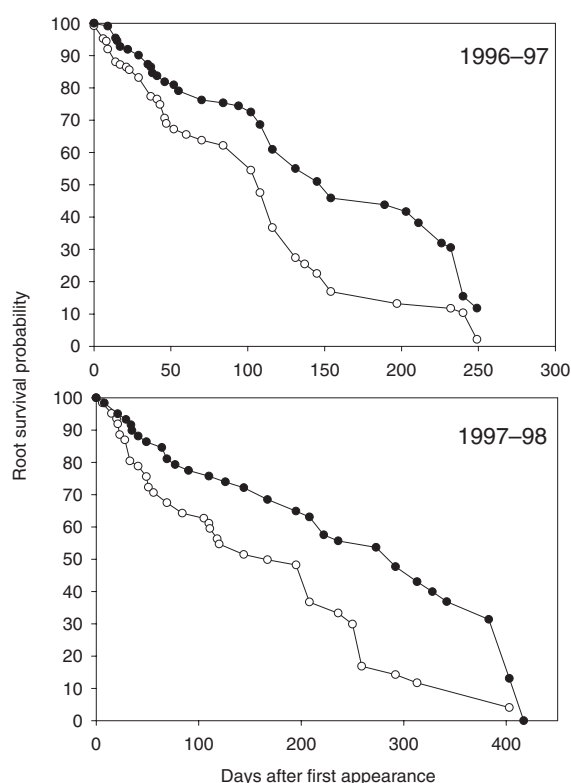
Figure 1. Survival probabilities for insecticide-treated (●) and control (○) roots in 1996–97 and 1997–98. All roots produced after the initiation of treatments in a given year were pooled and given a common start date. Survival probabilities were generated using the baseline statement of PROC PHREG in SAS. Median lifespan is defined as the first time-point at which the survival probability is less than or equal to 50%.

was associated with a significant decrease in the risk of fine root mortality. Application of insecticide extended fine root median lifespan by 46 d in 1996 and 125 d in 1997 (Fig. 1). The risk of mortality for insecticide-treated roots was 59% that of control roots in 1996 [exp (−0·5285) = 0·59], and 47% that of control roots in 1997 (Table 1; see footnote for explanation of relative risk calculations).

Higher root order was associated with a significant decrease in the risk of mortality in 1996 ($\beta = -0.5691$, $P < 0.0004$), but not in 1997 ($\beta = -0.0652$, $P < 0.8923$) when the number of higher order roots in the sample was quite small ($n = 9$). Larger root diameter was also associated with a decrease in the risk of mortality in 1997 ($\beta = -3.0596$, $P < 0.0545$), and a similar trend was noted in 1996 ($\beta = -1.3712$, $P < 0.1500$). There was insufficient evidence to conclude that root depth influenced the risk of mortality in either year. Root browning had no effect on root mortality in 1996 and decreased the risk of root mortality in 1997 ($\beta = -0.8095$, $P < 0.0142$; Table 1). Terms representing the interaction of insecticide treatment with order, diameter, depth and browning were not significant at the $P < 0.05$ level in either year and were dropped from the model (data not shown).

Insecticide treatment did not influence the overall percentage of roots which became brown (41% treated versus 40% control in 1996; 30 versus 34% in 1997). However, insecticide application was associated with a significant delay in the development of brown pigmentation in both 1996 and 1997 (Mann–Whitney *U*-test; $P < 0.02$ in 1996; $P < 0.015$ in 1997; Fig. 2). Among roots that became brown, the mean time required for browning was 22 (1996) and 66 (1997) days longer for insecticide-treated roots than control roots.

Table 1. Results of proportional hazards regression performed on pooled root lifespan data collected in 1996–97 and 1997–98. The parameter estimate and chi-square statistic for each model covariate is shown
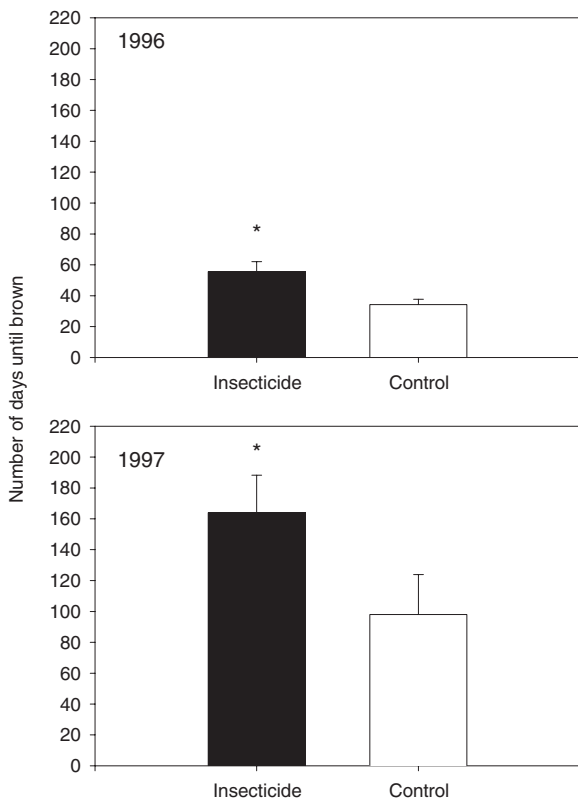
Variable	d.f.	Parameter estimate[a]	Standard error	Wald chi-square	P > chi-square
1996–97[b]					
Treatment	1	−0·5285	0·1660	10·1340	0·0015
Depth	1	−0·3022	0·1726	3·0679	0·0799
Diameter	1	−1·3712	0·9525	2·0726	0·1500
Order	1	−0·6775	0·2393	8·0127	0·0046
Browning	1	−0·0256	0·1790	0·0268	0·8701
1997–98[c]					
Treatment	1	−0·7499	0·2881	6·7763	0·0092
Depth	1	−0·4653	0·3106	2·2434	0·1342
Diameter	1	−3·0596	1·5914	3·6962	0·0545
Order	1	−0·0652	0·4813	0·0183	0·8923
Browning	1	−0·8095	0·3300	6·0172	0·0142

[a]Parameter estimates (values of β) can be interpreted as follows: for quantitative covariates such as diameter, a one unit change in the covariate is associated with a $100[\exp(\beta) - 1]$ percentage change in the hazard of mortality, controlling for other covariates. For dummy variables such as treatment, the ratio of the hazard of an individual coded '1' to that of an individual coded '0' is $\exp(\beta)$, controlling for other covariates.
[b]$n = 223$; mean diameter ± SD = 0·29 ± 0·10 mm; number of higher order roots = 29.
[c]$n = 117$; mean diameter ± SD = 0·31 ± 0·12 mm; number of higher order roots = 9.

Figure 2. Mean number of days required for the development of brown pigmentation in roots that became brown. Bars represent 1 SE. Asterisk denotes significant difference at $P < 0.05$ level (Mann–Whitney U-test).

Figure 4. Cumulative root production (a) and standing root crop (b) on insecticide-treated (●) and control (○) minirhizotron tubes for each sampling date from June 1996 to June 1998. Data are expressed both as the total number of roots observed on two minirhizotrons per tree (left-hand axis) and as the number of roots observed per cm^2 of minirhizotron surface area (right-hand axis). Error bars represent 1 SE. Dark bars in (a) represent the time periods during which insecticide treatments were applied.

There was no evidence to suggest that pesticide treatment increased the availability of NO_3^-, NH_4^+ or PO_4^{2-} in the months following the initiation of treatments. No differences were apparent in the nutrient availability indices of any of the ions at one month (data not shown) or at two months (Fig. 3) after pesticide application ($P > 0.25$ for all ions and dates).

We did not detect differences in cumulative fine root production or standing root numbers on treated and control tubes over the 2 years of the experiment (Fig. 4). Although cumulative production and standing root numbers were always higher on treated tubes, this difference
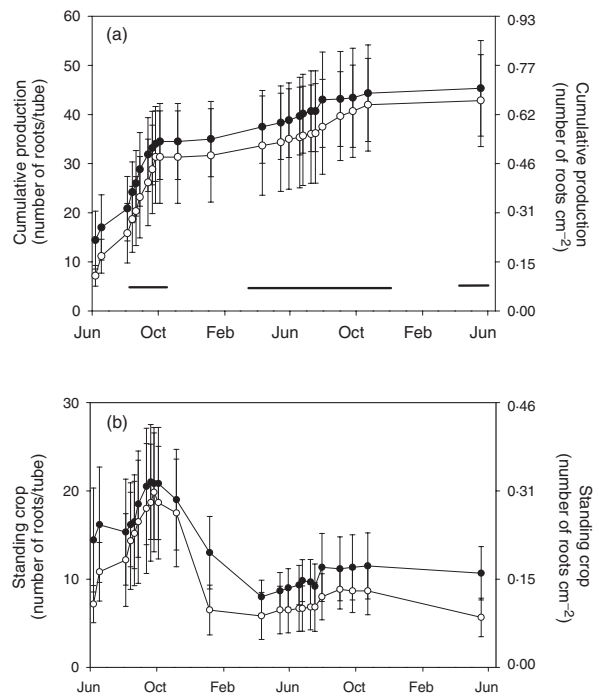
was not significant (differences evaluated with Student's t-test on individual dates) and may have reflected differences in initial fine root length on treated and control tubes prior to the application of treatments. The coefficient of variation for standing root numbers on a per tube basis ranged from 57 to 106%, depending on the date. The number of minirhizotrons used in the experiment may not have been sufficient to detect treatment differences in standing root numbers given this level of variation. We could also detect no difference in root length density in the soil surrounding treated and untreated tubes ($F_{1,5} = 0.076$, $P < 0.794$; Fig. 5). However there was a significant relationship between the
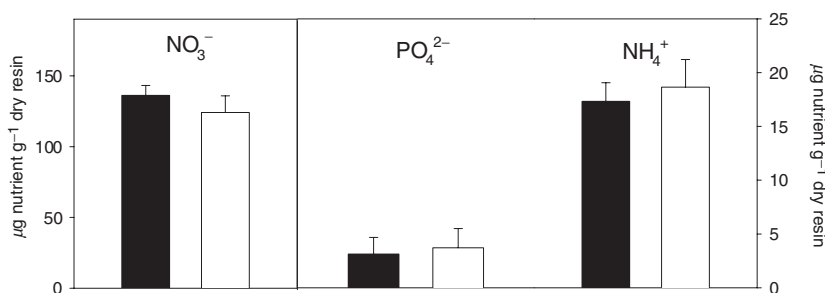

Figure 3. Nutrient availability indices (μg nutrient g^{-1} dry resin) for NO_3^-, NH_4^+ and PO_4^{2-} on ion exchange resin bags from insecticide-treated soil (dark bars) and control soil (white bars) after 2 months of insecticide application.
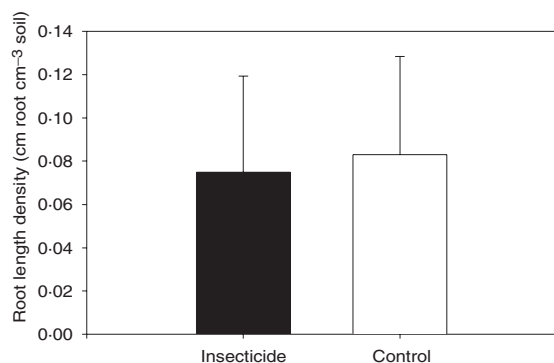
Figure 5. Root length density (cm root cm^{-3} soil) in a 1·25 cm ring of soil surrounding treated and control minirhizotron tubes.

number of roots visible on the minirhizotron surface and the root length density in the surrounding soil ($r^2 = 0\cdot3818$, $P < 0\cdot0191$; data not shown).

DISCUSSION

Insecticide treatment and root longevity

Application of a broad-spectrum insecticide around individual minirhizotron tubes was associated with increased longevity of peach fine roots (< 1 mm diameter) over the course of two growing seasons. The median lifespan of insecticide-treated roots was 46–125 d longer than that of control roots, depending on the year. The effect of insecticide application on root longevity did not appear to be due to increased soil fertility, as accumulation of NO_3^-, NH_4^+, and PO_4^{2-} on mixed bed ion-exchange resin was similar in treated and untreated soil. This study represents the first time that the effect of soil faunal suppression on fine root longevity has been directly quantified, and the results suggest that soil insects may strongly influence fine root mortality.

Although direct removal of root tissue through feeding is the most obvious manner in which soil insects may affect root physiology and lifespan, it is not the only way in which they may do so. Numerous species – including aphids, scale insects, phylloxerids and cicada nymphs (Brown & Gange 1990) – feed from the phloem or xylem sap of intact roots. Much of the damage associated with the grape phylloxera results not from the feeding activities of the insects themselves, but from the introduction of a fungal pathogen into feeding wounds (Granett *et al.* 1998). Collembola reduce plant growth through feeding on mycorrhizal hyphae, although the effect of hyphal consumption on the roots themselves is not known (Warnock, Fitter & Usher 1982; McGonigle & Fitter 1988). The activity of insects moving along the surface of the roots, perhaps grazing on rhizosphere micro-organisms (Head 1968), reduces root–soil contact and may decrease nutrient uptake by individual roots. In addition, not all interactions with below-ground herbivores are necessarily negative: moderate levels of root

feeding can stimulate root growth (Simberloff, Brown & Lowry 1978; Riedell 1989), increase lateral root branching (Kahler *et al.* 1985) and enhance rhizosphere bacterial populations (Denton *et al.* 1999). Although we use the term 'below-ground herbivory' for the sake of brevity, we recognize that interactions with soil insects may take on many forms in addition to the direct removal of root tissue. Application of a broad-spectrum insecticide undoubtedly altered a number of below-ground plant–insect interactions, and the observed changes in root longevity represent a response to the sum of these alterations. Nonetheless, this study provides strong evidence that peach fine root mortality is exacerbated by insects or other arthropods that are naturally present in the soil of an apparently healthy orchard.

It has previously been shown that the risk of root mortality in peach is related to a number of single-root level characteristics including root order, diameter, depth and age. Median survival times of 200 d or more are common for larger diameter fine roots (>0·5 mm diameter) with dependent laterals, whereas smaller diameter fine roots (<0·25 mm diameter) with no laterals have median survival times of approximately 70 d (Wells *et al.* 2001). We expected that these different classes of fine roots would also differ in their susceptibility to herbivory, especially in light of the differences in tissue nitrogen content (and, presumably, nutritive value) with diameter and order that have been reported for other tree species (McClaugherty, Aber & Melillo 1984; Camire, Cote & Brulotte 1991; Pregitzer *et al.* 1997). However, fine roots of different orders, diameters and depths responded similarly to soil insecticide treatment, implying that they experienced similar levels of below-ground herbivory under control conditions. All roots considered in the study were less than 1 mm in diameter and non-woody; it may be that soil insects do not discriminate among roots within this size class in peach.

Insecticide treatment and root pigmentation

In addition to its effects on root longevity, soil insect suppression also delayed the development of pigmentation, implying that browning may be enhanced by the presence of soil insects under natural conditions. This result is consistent with those of previous authors who noted that interactions with the soil biota altered both the pattern (Rogers 1929) and rate (Dunn 1979) of root browning. Root browning is a natural developmental process that occurs weeks to months after initial root production and appears to involve condensed tannin accumulation and cortical senescence (McKenzie & Peterson 1995). Although the physiological significance of browning is poorly understood, a number of studies have indicated that brown roots have a reduced nutrient uptake capacity relative to younger white roots (Chapman & Parker 1942; Head 1966; Harrison-Murray & Clarkson 1973; but see Atkinson 1983). If browning is associated with reduced nutrient uptake capacity, then organisms which enhance the rate of root browning could also influence whole plant nutrition and growth without directly removing root tissue.

Brown pigmentation was associated with a lower risk of root mortality in 1997, and a similar effect of browning on mortality has also been shown in apple (Wells & Eissenstat 2001). Although the rate of browning was enhanced in the presence of soil insects and brown pigmentation was associated with increased root longevity in 1997, there was no evidence to suggest that browning itself protected roots against herbivory. Treatment by browning interaction terms were not significant in either year of the study.

Insecticide treatment and root production

Despite marked differences in root longevity with insect suppression, we detected no changes in cumulative root production in response to our treatments. Numerous authors have reported compensatory re-growth of roots following below-ground herbivory (McNaughton 1983; Schmid *et al.* 1990; Steinger & Muller-Scharer 1992, Dunn & Frommelt 1998), whereas Wilson *et al.* (1995) reported a decrease in root production when soil insects were suppressed. Most of these studies dealt with below-ground herbivory at the level of the whole plant or ecosystem, and it is not surprising that the loss of a significant portion of the root system at this scale shifted whole-plant carbon allocation to support root re-growth. In the present experiment, only a small portion of each tree's root system was protected from herbivory, and it is unlikely that whole-tree carbon allocation patterns were in any way affected by the treatments. Equivalent fine root production on treated and untreated minirhizotrons was not unexpected given the highly localized nature of the insecticide applications and the fact that all trees received the same set of treatments (one treated tube, one untreated tube).

Although we observed changes in root longevity with insecticide treatment, we did not detect significant differences in minirhizotron root numbers or bulk soil root length density on insecticide-treated tubes. We had expected higher standing root numbers on treated tubes as a result of reduced root mortality rate. Although the average number of roots visible was always higher on treated tubes, especially over winter, extremely high spatial variability in root numbers limited our ability to draw strong conclusions in this regard.

CONCLUSIONS

There is clearly a qualitative difference between the protection of individual roots from herbivory and the protection of an entire root system. The localized treatments used in the present experiment indicated that soil insects could influence lifespan, but provided no information on the complex feedback between below-ground and above-ground physiology that undoubtedly characterizes root–insect interactions at the whole-tree level. A complete understanding of the effect of below-ground herbivory on root dynamics in peach awaits further investigation at the whole plant level and the identification of specific plant–insect

interactions at the single root level. Nonetheless, the results presented here indicate that interactions with below-ground insects can strongly influence root longevity and can alter the rate of at which roots undergo developmental changes in anatomy and physiology. The potential for soil fauna to influence above-ground production as well as below-ground carbon and nutrient cycling through interactions with the root system is an area of root biology that deserves further investigation.

REFERENCES

Allison P.D. (1995) *Survival Analysis using the SAS System: a Practical Guide.* SAS Institute, Cary, NC.

American Public Health Association. (1998) *Standard Methods for the Examination of Water and Wastewater* 20th edn. American Public Health Association, New York.

Anderson D.C. (1987) Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *Quarterly Review of Biology* **62**, 261–286.

Atkinson D. (1983) The growth, activity and distribution of the fruit tree root system. *Plant and Soil* **71**, 23–35.

Binkley D. (1984) Ion exchange resin bags: Factors affecting estimates of nitrogen availability [Adsorption of ammonium and nitrate, *Pinus taeda*]. *Soil Science Society of America Journal* **48**, 1181–1184.

Binkley D. & Matson P. (1983) Ion exchange resin bag method for assessing forest soil nitrogen availability. *Soil Science Society of America Journal* **47**, 1050–1052.

Brown V.K. & Gange A.C. (1989) Differential effects of above- and below-ground insect herbivory during early plant succession. *Oikos* **54**, 67–76.

Brown V.K. & Gange A.C. (1990) Insect herbivory below-ground. *Advances in Ecological Research* **20**, 1–58.

Brown V.K. & Gange A.C. (1991) Effects of root herbivory on vegetation dynamics. In *Plant Root Growth: an Ecological Per-*

spective (eds D. Atkinson, D. Robinson & I.J. Alexander), pp. 453–479. Blackwell Scientific, Oxford, UK.

Camire C., Cote B. & Brulotte S. (1991) Decomposition of roots of black alder and hybrid poplar in short-rotation plantings: nitrogen and lignin control. *Plant and Soil* **138**, 123–132.

Chapman H.D. & Parker E.R. (1942) Weekly absorption of nitrate by young bearing orange trees growing out of doors in solution cultures. *Plant Physiology* **17**, 366–376.

Clements R.O. & Bale J.S. (1988) The short term effects on birds and mammals of the use of chlorpyrifos to control leatherjackets in grassland. *Annals of Applied Biology* **112**, 41–47.

Crawley M.J. (1983) *Herbivory, the Dynamics of Animal–Plant Interactions.* Blackwell Scientific, Oxford, UK.

Cox D. (1972) Regression models and life tables. *Journal of the Royal Statistical Society* **34**, 187–220.

Davidson R.L. (1979) Effects of root feeding on foliage yield. In *Proceedings of the 2nd Australian Conference on Grassland Invertebate Ecology* (eds T.K. Crosby & R.F. Pottinger), pp. 117–120. Government Printer, Wellington, New Zealand.

Denton C.S., Bardgett R.D., Cook R. & Hobbs P.J. (1999) Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland. *Soil Biology and Biochemistry* **31**, 155–165.

Dunn R. (1979) Effect of *Xiphinema americanum* and *Rhizoctonia solani* on browning of apple roots. *Fungicide and Nematicide Tests* **35**, 214.

Dunn J.P. & Frommelt K. (1998) Effects of below-ground herbivory by *Diabrotica virgifera virgifera* (Coleoptera) on biomass allocation and carbohydrate storage of maize. *Applied Soil Ecology* **7**, 213–218.

Eissenstat D.M. & Yanai R.D. (1997) The ecology of root lifespan. *Advances in Ecological Research* **27**, 1–60.

Fitter A.H., Graves J.D., Self G.K., Brown T.K., Bogie D.S. & Taylor K. (1998) Root production, turnover and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. *Oecologia* **114**, 20–30.

Fogel R. (1990) Root turnover and production in forest trees. *Hortscience* **25**, 270–273.

Gange A.C. (1990) Effects of insect herbivory on herbaceous plants. In *Pests, Pathogens and Plant Communities* (eds J.J. Burdon & S.R. Leathers), pp. 49–62. Blackwell Scientific, Oxford, UK.

Gange A.C. & Brown V.K. (1989) Effects of root herbivory by an insect on a foliar-feeding species, mediated by changes in the host plant. *Oecologia* **81**, 38–42.

Gibson D.J. (1986) Spatial and temporal heterogeneity in soil nutrient supply measured using *in situ* ion-exchange resin bags. *Plant and Soil* **96**, 445–450.

Gill R.A., Burke I.C., Laurenroth W.K. & Milchunas G. (2001) Longevity and turnover of roots in the shortgrass steppe: influence of diameter and depth. *Plant Ecology* (in press)

Gill R.A., Burke I.C., Laurenroth W.K., & Milchnnas G. (1999) *Bouteloua gracilis* root dymanles through a soil profile. Oral Presentation, Session 34. Annual Meeting, Ecological Society of America, Spokare, WA, 8–13 August 1999.

Goldson, S.L., Dyson, C.B., Proffitt, R.J., Frampton, E.R. & Logan, J.A. (1985) The effect of *Sitona discoides* Gyllenhal (Coleoptera: Curculionidae) on lucerne yields in New Zealand. *Bulletin of Entomological Research* **75**, 429–442.

Granett J., Omer A.D., Pessereau P. & Walker M.A. (1998) Fungal infections of grapevine roots in phylloxera-infested vineyards. *Vitis* **37**, 39–42.

Hansson A., Aifen Z. & Andren O. (1995) Fine-root production and mortality in degraded vegetation in Horqin sandy rangeland in Inner Mongolia, China. *Arid Soil Research and Rehabilitation* **9**, 1–13.

Harrison-Murray R.S. & Clarkson D.T. (1973) Relationship between structural development and the absorption of ions by the root system of *Cucurbita pepo. Planta* **114**, 1–16.

Hartley D. & Kidd H. (1983) *The Agrochemicals Handbook.* Royal Society of Chemistry, Nottingham, UK.

Head G.C. (1966) Estimating the seasonal changes in the quantity of white unsuberized root on fruit trees. *Journal of Horticultural Science* **41**, 197–206.

Head G.C. (1968) Studies of growing roots by time lapse cinematography. *Ninth International Congress of Soil Science Transactions* **1**, 751.

Henderson I.F. & Clements R.O. (1974) The effect of pesticides on the yield and botanical composition of a newly sown ryegrass ley and of an old mixed pasture. *Journal of the British Grassland Society* **29**, 185–190.

Henderson I.F. & Clements R.O. (1977) Grass grown in different parts of England in relation to invertebrate numbers and pesticide treatment. *Journal of the British Grassland Society* **32**, 89–98.

Hendrick R.L. & Pregitzer K.S. (1992) The demography of fine roots in a northern hardwood forest. *Ecology* **73**, 1094–1104.

Hendrick R.L. & Pregitzer K.S. (1993) Patterns of fine root mortality in two sugar maple forests. *Nature* **361**, 59–61.

Howard P.H. (1989) *Handbook of Environmental Fate and Exposure Data for Organic Chemicals, Vol III: Pesticides.* Lewis Publishers, Chelsea, MI.

Jackson R.B., Mooney H.A. & Schulze E.D. (1997) A global budget for fine root biomass, surface area, and mineral nutrients. *Proceedings of the National Academy of Sciences of the United State of America* **94**, 7362–7366.

Kahler A.L., Olness A.E., Sutter G.R., Dybing C.D. & Devine O.J. (1985) Root damage by western corn rootworm and nutrient content in maize. *Agronomy Journal* **77**, 769–773.

Lambers H. (1987) Growth, respiration, exudation and symbiotic association: the fate of carbon translocated to roots. In *Root Development and Function* (eds P.J. Gregory, J.V. Lake & D.A. Rose), pp. 123–145. Cambridge University Press, London.

Levine E. & Oloumi-Sadeghi G. (1991) Management of Diabroticite rootworms in corn. *Annual Review of Entomology* **36**, 229–255.

Majdi H. & Kangas P. (1997) Demography of fine roots in response to nutrient applications in a Norway spruce stand in southwestern Sweden. *Ecoscience* **4**, 199–205.

Maron J.L. (1998) Insect herbivory above- and belowground: individual and joint effects on plant fitness. *Ecology* **79**, 1281–1293.

McClaugherty C.A., Aber J.D. & Melillo J.M. (1984) Decomposition dynamics of fine roots in forested ecosystems. *Oikos* **42**, 378–386.

McGonigle T.P. & Fitter A.F. (1988) Ecological consequences of arthropod grazing on VA mycorrhizal fungi. *Proceedings of the Royal Society of Edinburgh* **94B**, 25–32.

McKenzie B.E. & Peterson C.A. (1995) Root browning in *Pinus banksiana* Lamb. & *Eucalyptus pilularis* Sm. 1. Anatomy and permeability of the white and tannin zones. *Botanica Acta* **108**, 127–137.

McNaughton S.J. (1983) Compensatory plant growth as a response to herbivory. *Oikos* **40**, 329–336.

Ordish G. (1987) *The Great Wine Blight.* Sidgwick and Jackson, London.

Pozo C., Martinez-Toledo M.V., Salmeron V., Rodelas B. & Gonzalez-Lopez J. (1995) Effect of chlorpyrifos on soil microbial activity. *Environmental Toxicology and Chemistry* **14**, 187–192.

Pregitzer K.S., Kubiske M.E., Yu C.K. & Hendrick R.L. (1997)

Relationships among root branch order, carbon and nitrogen in four temperate species. *Oecologia* **111**, 302–308.

Racke K.D. (1992) The environmental fate of chlorpyrifos. *Review of Environmental Contamination and Toxicology* **131**, 1–154.

Reid J.B., Sorenson I. & Petrie R.A. (1993) Root demography in kiwifruit (*Actinidia deliciosa*). *Plant, Cell and Environment* **16**, 949–0957.

Ridsell-Smith T.J. (1977) Effects of root feeding by scarabeid larvae on the growth of perennial ryegrass plants. *Journal of Applied Ecology* **14**, 73–80.

Riedell W.E. (1989) Western corn rootworm damage in maize: Greenhouse technique and plant response. *Crop Science* **29**, 412–415.

Rogers W.S. (1929) Root studies VIII. Apple root growth in relation to rootstock, soil, seasonal and climatic factors. *Journal of Pomology and Horticultural Science* **17**, 99–130.

Ruess R.W., Hendrick R.L. & Bryant J.P. (1998) Regulation of fine root dynamics by mammalian browsers in early successional Alaskan taiga forests. *Ecology* **79**, 2706–2720.

Schmid B., Miao S.L. & Bazzazz F.A. (1990) Effects of simulated root herbivory and fertilizer application on growth and biomass accumulation in the clonal perennial *Solidago canadensis*. *Oecologia* **84**, 9–15.

Simberloff D., Brown B.J. & Lowrie S. (1978) Isopod and insect root borers may benefit Florida mangroves. *Science* **201**, 630–632.

Steinger T. & Muller-Scharer H. (1992) Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nitrogen availability. *Oecologia* **91**, 141–149.

Thomson W.T. (1982) *Insecticides, Acaricides and Ovicides*. Agricultural Chemicals, Book I. Thomson Publications, Fresno, CA.

Trumble J.T. (1993) Plant compensation for arthropod herbivory. *Annual Review of Entomology* **38**, 93–119.

USEPA (US Environmental Protection Agency) (1989) *Registration Standard (Second Round Review) for the Reregistration of Pesticide Products Containing Chlorpyrifos*. Office of Pesticide Programs, USEPA, Washington, DC.

Warnock A.J., Fitter A.H. & Usher M.B. (1982) The influence of a springtail *Folsomia candida* (Insecta, Collembola) on the mycorrhizal association of the leek *Allium porrum* and the vesicular-arbuscular mycorrhizal endophyte *Glomus fasiculatus*. *New Phytologist* **90**, 285–292.

Wells C.E. & Eissenstat D.M. (2001) Marked differences in survivorship among apple roots of different diameters. *Ecology* **82**, 882–892.

Wells C.E., Glenn D.M. & Eissenstat D.M. (2001) Changes in the risk of fine root mortality with age: a case study in peach (*Prunus persica*). *American Journal of Botany* (in press).

Wilson K., Gunn A.G. & Cherrett J.M. (1995) The application of a rhizotron to study the subterranean effects of pesticides *Pedobiologia* **39**, 132–143

Wyland L.J. & Jackson L.E. (1993) Evaluating nitrate recovery by ion-exchange resin bags. *Soil Science Society of America Journal* **57**, 1208–1211.

Yang J.E., Skogley E.O. & Schaff B.E. (1991) Nutrient flux to mixed-bed ion exchange resin: temperature effects. *Soil Science Society of America Journal* **55**, 762–767.