



Ring nematodes (*Mesocriconema xenoplax*) alter root colonization and function of arbuscular mycorrhizal fungi in grape roots in a low P soil

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ABSTRACT

A reduction of arbuscules in roots of grapevines (*Vitis vinifera*) observed when ring nematodes were added to field microplots led to the hypothesis that nematode feeding suppresses arbuscules by competing for root carbohydrates. Support for this hypothesis was tested by growing 'Pinot noir' grapevines in a factorial experiment with three levels of initial nematode densities (0, 0.1, 1.0 nematodes g^{-1} soil), two levels of light (full sun, 50% sun), and two levels of AMF (nonAMF, +AMF). Effects on plant growth were primarily driven by a light and AMF treatment interaction, such that low light increased stem dry matter accumulation at the expense of roots in +AMF vines only. Nematodes had only a minor influence on plant growth (leaf mass was reduced at the highest nematode density), but nematodes did not affect overall plant dry matter accumulation. Since nonAMF vines were severely limited by P and their growth was so poor, the impact of nematode and light treatments was further analyzed in +AMF plants only. Nematode populations, AMF colonization, and root carbohydrates were differentially affected by initial nematode density or light levels. Root biomass, and reducing sugar and starch concentrations in fine roots were reduced by low light, but the final nematode populations and arbuscule frequencies in roots were unaffected by light. Nematodes reduced arbuscules and starch concentrations in fine roots, but did not affect total colonization by AMF (hyphae, vesicles or arbuscules). Nematodes reduced plant P and K uptake at the highest density, and low light reduced Mg uptake. These findings are consistent with the hypothesis that ring nematodes suppress arbuscules in roots via competition for root carbohydrates. However, the lack of a treatment interaction between light and nematodes in our study suggests that ring nematode-AMF interactions in grape roots are controlled by more than competition for photosynthate.

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1. Introduction

The ring nematode *Mesocriconema xenoplax* (Raski) Loof & Degrisse is commonly found in vineyards worldwide (Klinger, 1975; Pinochet and Cisneros, 1986; Walker, 1995) and is the most abundant plant parasitic nematode in some grape-growing regions (Güntzel et al., 1987; Pinkerton et al., 1999). Ring nematodes feed from a single root cortical cell for many days; the nematode stylet does not penetrate the host plasmamembrane or cause obvious damage to the feeding cell (Hussey et al., 1992). Reduction in vine vigor is sometimes, but not always associated with high *M. xenoplax* populations in soil (Klinger, 1975; Ramsdell et al., 1996; Pinkerton et al., 1999). McKenry (1992) estimated that yield reductions are significant whenever *M. xenoplax* populations approach 0.5 individuals g^{-1} soil. In Oregon, populations up to 2 nematodes g^{-1} soil were not associated with reduction in vigor or yield of mature vines in commercial vineyards (Pinkerton et al., 1999).

However, populations of 6–8 ring nematodes g^{-1} soil reduced vigor and yield of young vines grown in infested microplots (Pinkerton et al., 2004). The reduction of growth and productivity of these young vines was associated with lower root density and altered root colonization by beneficial arbuscular mycorrhizal fungi (AMF).

Roots of grapevines in Oregon vineyards are intensely colonized by AMF, particularly in the red hill soils (Ultisols) that are the major vineyard soils in western Oregon (Schreiner and Linderman, 2005). This intense colonization is due to the high dependence of grapevines on AMF to obtain P from these low fertility soils. For example, self-rooted 'Pinot noir' grapevines were stunted when grown in a red hill soil (Jory series) without AMF, but vine biomass increased ~threefold when AMF were added to the soil which was associated with ~eightfold improvement in P uptake (Schreiner, 2007).

Ring nematodes introduced in field microplots were previously shown to reduce arbuscules in fine roots of grapevines without altering overall root colonization by AMF (Pinkerton et al., 2004). Since ring nematodes feed on root cortical cells for long periods of time and presumably enhance the sink strength of the feeding cell (to allow for an increased nutrient flow; Hussey et al., 1992), and

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since arbuscule-containing cells in the root cortex also have an increased metabolism (Smith and Read, 1997) and demand for reduced carbon (Blee and Anderson, 1998), we hypothesized that ring nematodes specifically suppress arbuscules in roots by reducing carbohydrates available to the fungus that support arbuscule development. Indeed the overall effect of ring nematodes on above-ground, grapevine growth and crop yield may be mediated through competition for carbohydrates (Pinkerton et al., 2004). The predisposition of *Prunus* spp. to bacterial canker disease caused by *Pseudomonas syringae* when ring nematodes are present in soil (Lownsbery et al., 1977; Nyczepir, 1990) has been attributed to a progressive weakening of trees that is related to lower carbohydrate concentrations in roots and scions of nematode infested trees (Nyczepir et al., 1987; Olien et al., 1995; Ferris et al., 2004).

The purpose of this study was to test the hypothesis that ring nematodes specifically reduce arbuscules in grapevine roots by consuming carbohydrates that are likely to be required for arbuscular development. Our focus here on carbohydrates was due in part to the lack of necrosis or other visible symptoms having occurred in the fine roots of grapevines exposed to ring nematodes in field microplots (Pinkerton et al., 2004). The hypothesis was tested by growing plants under different light levels (full sun and 50% sun) intended to manipulate the supply of photosynthate to roots. We reasoned that ring nematodes would have a greater impact on arbuscule development in plants grown at 50% sun than in plants grown at full sunlight. We also tested if the presence of AMF in roots would have an impact on ring nematode development by comparing nonAMF vines to vines inoculated with mycorrhizal fungi, which was not possible in the previous field microplot study (all plants were mycorrhizal). Prior research on interactions between AMF and plant parasitic nematodes has generally focused on endoparasitic nematodes (i.e. root knot nematode) that cause obvious damage, induce gross morphological changes in roots, and often trigger plant defense reactions (Ingham, 1988; Pinochet et al., 1996; Borowicz, 2001). Interactions between AMF and ectoparasitic, ring nematodes that cause minimal disruption to root cortical cells (Hussey et al., 1992), may be fundamentally different from interactions with more aggressive root-feeding nematodes.

2. Materials and methods

2.1. Experimental design, biological materials, and soil

The experiment was conducted in a greenhouse located in Corvallis, OR, USA (44.568°N, 123.289°W). A $3 \times 2 \times 2$ factorial experiment with three levels of nematodes (0, 0.1, and 1.0 nematodes g^{-1} soil initial density), two light levels (full sun and 50% sun), and two AMF levels (nonAMF and +AMF) was conducted using self-rooted 'Pinot noir' grapevines grown in 41 pots. Each treatment combination was replicated 8 times for a total of 96 experimental units (potted plants). The soil used was a Jory series, silty-clay loam (fine, mixed, active, mesic Xeric Palehumult) collected from the Oregon State University, Woodhall Research Vineyard (OSU-WRV) located near Alpine, OR, USA. The experimental soil was mixed 1:1 (vol./vol.) with coarse sand (pre-stress sand mix, Knife River Inc., Corvallis, OR), and dolomite lime (50% $CaCO_3$, 40% $MgCO_3$) was added at a rate of 35 $g\ kg^{-1}$ dry soil to raise soil pH to ~ 6.0 . The resulting soil mix was fumigated with methyl bromide at a rate equivalent to 448 $kg\ ha^{-1}$ (Trident Inc., Vancouver, WA) to kill resident AMF and soil borne pests. Soil was stored for 3 months at room temperature after fumigation before use. Available soil nutrients ($mg\ kg^{-1}$) and pH were: NO_3-N , 5.3; P (Bray 1), 14; K, 121; Ca, 1158; Mg, 285; SO_4-S , 77; Fe, 62; Mn, 70; B, 0.20; Zn, 1.3; Cu, 1.0; pH, 5.8.

The equivalent of 5 kg dry soil mix was placed into each pot, and half of the pots received a mixed inoculum of three AMF (+AMF) or

not (nonAMF). AMF inoculum consisted of *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders INVAM #OR219, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe INVAM #OR218, and *Glomus* sp. INVAM #OR215, which had been previously isolated from Jory soil. Each fungus was isolated and propagated by hand-picking spores from trap cultures and re-culturing with *Sorghum bicolor* L. in a low P, sandy loam soil. The +AMF pots received 10 g of whole soil inoculum (containing spores, hyphae and colonized root fragments) from each fungal species. All pots received a microbial extract from the live experimental soil and the AMF inoculum soils to provide similar microflora in different treatments. This extract was prepared by wet-sieving live Jory soil and AMF inoculum soils (25 g each) through a 38 μm sieve 3 times.

Pre-rooted, three-node cuttings of 'Pinot noir' (Pommard clone, FPS 91) were transplanted into pots and thinned to a single shoot per vine. Ring nematodes were introduced into the pots 21 days after planting (DAP), by pipetting 5 ml aliquots of a nematode-water suspension into eight small holes located between the plant stem and side of the pots to reach a final density of either 0.1 or 1.0 nematodes g^{-1} soil (500 or 5000 individuals pot^{-1}). The ring nematode population used in this study was isolated from the OSU-WRV and maintained in greenhouse cultures on 'Pinot noir' grapevines in steam-pasteurized, sandy loam soil. Ring nematodes were extracted from these soil cultures by wet-sieving/sucrose centrifugation (Ayoub, 1977) and suspensions were adjusted to 12.5 or 125 nematodes ml^{-1} prior to infestation.

2.2. Growth conditions

All plants were grown under full sun conditions for 50 d before applying different light levels intended to manipulate root carbohydrates. At this time, half of the pots in each treatment were moved under shade cloth, which reduced incident photosynthetically active radiation (PAR) by $\sim 50\%$. Typical midday light levels (PAR) in the canopy of the full sun plants averaged $\sim 1100\ \mu mol\ m^{-2}\ s^{-1}$ and this was reduced to $\sim 500\ \mu mol\ m^{-2}\ s^{-1}$ in the 50% sun treatment.

Plants were grown during the spring and summer of 2003 (30 April–8 October) for a total of 161 DAP. Plants were watered as needed by monitoring soil surface wetness. Each pot was watered to field capacity whenever the soil on the surface became dry. Pots were checked 3 times daily. A one-half strength Hoagland's solution (with P) was applied to all pots once every 2 weeks to maintain a moderate level of soil fertility and to supply nonAMF vines with a source of P (Hoagland and Arnon, 1950). Daytime soil temperatures in the shaded and full sun pots were not different when measured on several occasions throughout the growing season. The full sun plants were watered more often than the shaded plants and hence experienced greater fluctuations in soil moisture content. However, no plants showed signs of water stress (wilted tips or leaves) at any time during the experiment.

2.3. Sampling and assays

Periodic measurements of shoot length were made throughout the growing cycle using a flexible tape measure. Root length density, root carbohydrates, AMF colonization of roots, and nematode populations in soil were determined on 78, 121, and 161 DAP. Root and soil samples were obtained using a 2 cm diameter core taken halfway between the plant (in center of pot) and the side of the pot. Soil core samples were taken from only half of the experimental units ($n = 4$) on each sample date so that all samples could be processed within a single day. Soil cores were gently washed over a 1 mm sieve with tap water catching all rinse water used in a large bucket. The water remaining in the bucket (containing nematodes) was mixed and decanted over a 20 μm

sieve. Nematodes retained on the sieve were transferred to centrifuge tubes using cold tap water, stored at 4 °C overnight, and extracted from the remaining soil mineral particles by wet-sieving/sucrose centrifugation (Ayoub, 1977). Nematodes were counted in a grid dish under a stereoscope at 40 \times .

Fine roots (primary roots with an intact cortex) retained on the 1 mm sieve were thoroughly rinsed clean (no adhering soil), blotted dry, and weighed. A subsample of ~0.3 g fresh weight was stored in formaldehyde:acetic acid:alcohol (FAA; 1%:10%:50% by volume) to assess root length and colonization by AMF after clearing in KOH and staining with trypan blue as described by Schreiner (2003). Root length of this subsample was determined using the grid line intercept method (Newman, 1966). The proportion of root length colonized by any AMF structures (total colonization = hyphae, vesicles or arbuscules) and by arbuscules alone was determined on root fragments that were mounted and squashed between microscope slides (see McGonigle et al., 1990).

A second fine root subsample (~0.1 g fresh weight) was frozen in liquid N, and stored at -80 °C for carbohydrate determinations. Reducing sugars, sucrose and starch were measured after extraction/precipitation in 80% ethanol and conversion to reducing sugars (sucrose and starch) using the copper-bicinchoninate microplate assay described by Fox and Robyt (1991). Briefly, roots were ground in a mortar pestle in 80% ethanol and centrifuged at 5000 \times g (washing the pellet 2 times with fresh 80% ethanol) retaining the supernatant (reducing sugars and sucrose) and pellet (starch) for further analysis. The supernatant was dried under nitrogen gas at 50 °C and the residue containing soluble sugars was resuspended in distilled water. Reducing sugars were then determined in appropriately diluted aliquots of the resuspended supernatant with and without pre-treatment with excess invertase (to convert sucrose to reducing sugars, Sigma Chemical Company, St. Louis, MO, USA). Sucrose concentrations were derived from subtracting the sugar concentration in the +invertase samples from the untreated samples. Starch in the pellet was solubilized by boiling the sample in Na-acetate buffer at pH 4.2 and was converted to glucose by reacting with excess amylase and amyloglucosidase at 50 °C (Sigma Chemical Company). All carbohydrate fractions were determined in duplicate and expressed as glucose equivalents mg^{-1} root fresh mass.

Plants were destructively harvested 161 DAP and separated into leaves (blades and petioles), stems (shoots with leaves removed), wood (original cutting), and roots. Roots were washed free from all soil. All plant parts were washed with distilled water, oven dried at 70 °C for 8 d, and weighed. All dried plant material was ground to pass through a 40 mesh (425 μm) screen for later determination of nutrient concentrations. N was determined by combustion analysis (CNS-2000 Macro Analyzer, Leco Inc., St. Louis, MO, USA) and P, K, Ca, Mg, S, Fe, Mn, B, Zn, and Cu were determined by ICP-OES (Optima 3000 DV, Perkin-Elmer Inc., Wellesley, MA, USA) after microwave-assisted, acid digestion of tissue samples (Jones and Case, 1990).

2.4. Statistical analysis

Data were analyzed using ANOVA. A three factor ANOVA employing AMF, light and nematode treatments was used to analyze the final dry matter accumulation data. However, since the nonAMF plants grew so little compared to +AMF vines, and since nematodes did not significantly reproduce in nonAMF soils, we focused our further analysis on +AMF plants only using sample date, light, and nematode treatments as factors. When necessary, log-transformed values were used to satisfy assumptions of variance (Cochran's test). Shoot and root length data across all sample dates (six sample dates for shoots, three sample dates for roots) could not be transformed to satisfy assumptions of variance,

so these data were analyzed separately at each sample date. Means were compared using Tukey's HSD test at 95% confidence. Data presented in tables and figures represent the back-transformed means (when a transformation was used) and the standard errors of the arithmetic mean. Correlations between arbuscular colonization and other root variables were conducted using data that were expressed per quantity of root (arbuscules per unit root length, nematodes per unit root length and carbohydrates per unit root fresh mass). The impact of nematodes and light on plant nutrient uptake was evaluated using a two factor ANOVA for the total plant content (roots + wood + stem + leaves) of each mineral analyzed at the final harvest. All analysis was carried out using Statistica software (version 8.0, Statsoft Inc., Tulsa, OK).

3. Results

3.1. Plant growth and dry matter

Shoot growth of 'Pinot noir' was strongly influenced by AMF and light treatments (Fig. 1A). NonAMF vines grew only slightly after transplanting, reaching a final height of only about 20 cm in all respective nematode and light treatments. NonAMF vines did not respond to the different levels of light or to nematodes because growth was so minimal (the pooled average for all the nonAMF treatments is shown in Fig. 1). NonAMF vines began to show symptoms of P deficiency by about 60 DAP (red spots on leaves beginning at the leaf margin between major veins), and these symptoms became more obvious over time.

Plants inoculated with AMF began to grow about 50 DAP and reached a final height between 60 and 160 cm, depending on light

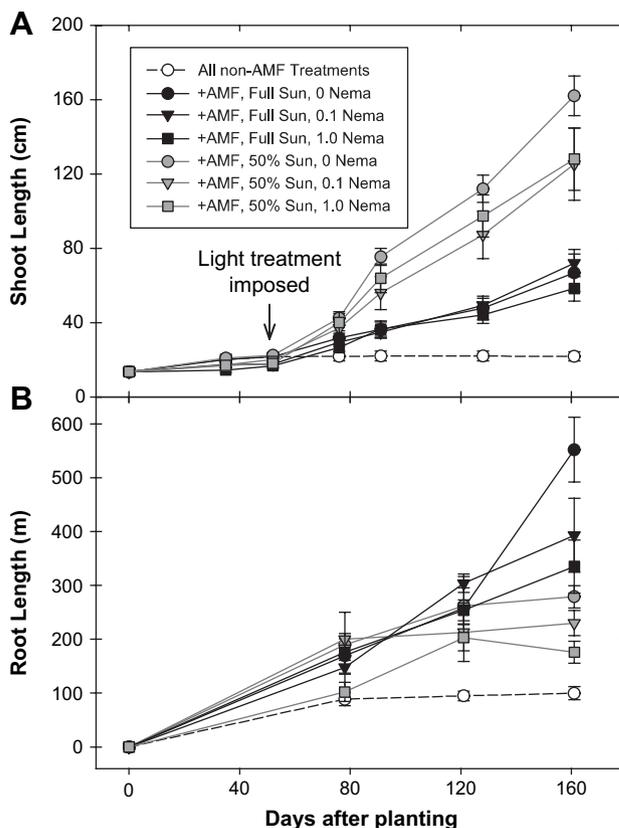


Fig. 1. Effect of light and ring nematodes on shoot (A) and root (B) growth of mycorrhizal (+AMF) 'Pinot noir' grapevines. The mean of all nonAMF treatment combinations are shown for comparison purposes. Data represent back-transformed means (\pm standard errors of arithmetic mean). Root data are from core samples from one-half of the experimental units.

treatment. +AMF vines grown at 50% of full sun quickly responded to the lower light level by increasing shoot length compared to plants in full sun. Vines grown at 50% sunlight were significantly taller than those grown at full sun from 76 DAP onward. The increased shoot growth of +AMF vines at 50% sun occurred at the expense of roots, since root growth was reduced compared to the full sun vines (Fig. 1B). The effect of light on roots was not significant until our last sample date at 161 DAP. By this time, root length of vines grown in 50% sun was roughly half that of the vines grown in full sun (main effect). Nematodes did not affect shoot length of vines at any sample date, but nematodes at the highest density reduced root length of vines compared to noninfested plants at 161 DAP (main effect).

The final dry mass of various plant parts followed a pattern similar to shoot and root length (Table 1). AMF and light treatments had a large impact on dry matter accumulation. The +AMF vines accumulated approximately 5 times more leaf mass, 4 times more stem mass, and 4 times more root mass than nonAMF vines. The plants grown under 50% sunlight allocated more biomass to stems and less biomass to roots, but this only occurred in +AMF plants (resulting in a significant interaction between AMF and light treatments). The dry mass of stems in +AMF plants grown at 50% sun was increased by 2.3 g over that of the +AMF plants grown at full sun, while root dry mass decreased by 4.8 g. Leaf mass was not affected by light, but total plant dry mass and dry mass of the wood were slightly lower in the vines grown under low light. Nematodes reduced leaf mass at the highest nematode density, but other plant tissues and total plant biomass were not affected by nematodes. Indeed, nematodes did not significantly reduce leaf mass when a separate ANOVA was conducted on the +AMF vines only (data not shown).

The lack of growth of the nonAMF vines in this soil was clearly a result of P deficiency. The P deficiency symptoms noted above in the nonAMF 'Pinot noir' leaves were confirmed by analysis of plant nutrients. Leaf P concentrations in the nonAMF vines were $0.36 \pm 0.04 \text{ g kg}^{-1}$, while leaf P concentrations in +AMF vines were $1.91 \pm 0.21 \text{ g kg}^{-1}$. Similarly, root P concentrations were only $0.38 \pm 0.05 \text{ g kg}^{-1}$ in nonAMF vines and $1.32 \pm 0.23 \text{ g kg}^{-1}$ in +AMF vines. Concentrations of other nutrients in the nonAMF vine leaves

were above levels known to be critical for grapevines (Robinson, 1992; Gärtel, 1996).

3.2. Nematodes, AMF, and root carbohydrates

The remaining results will focus on +AMF vines only, since the nonAMF vines were so small and unresponsive to nematode or light treatments and nematodes did not significantly increase in population in the nonAMF soils (data not shown). Ring nematode and light treatments altered root variables in the +AMF vines in general accordance with our expectations (Table 2). However, there was no interaction between nematode and light treatments as we suspected based on our hypothesis. Sample date also had a significant effect on most root variables, but did not interact significantly with the nematode or light treatments. Nematodes increased over time from 78 to 121 DAP, but no further increase was apparent between 121 and 161 DAP. Total colonization of roots by AMF did not change after 78 DAP, but the frequency of arbuscules showed a steady decline over time after 78 DAP. Reducing sugars and sucrose in roots increased between 78 and 121 DAP and remained the same thereafter until 161 DAP. The nematode treatment affected nematode populations in soil as expected, with the number of nematodes found per pot (data not shown) or per meter of root (Table 2) reaching higher levels as the initial infestation density increased. The zero nematode control pots remained free of nematodes throughout the experiment. The final ring nematode populations (161 DAP) were 12 and 45 nematodes g^{-1} soil in the low (0.1) and high (1.0) infestation density, respectively. Nematodes did not alter total AMF colonization of roots nor did they alter sugar concentrations in roots. However, nematodes depressed arbuscule frequency in roots with increasing effect at the higher nematode density and nematodes reduced root starch concentrations. Arbuscular colonization of roots was negatively correlated ($r = -0.87$, Spearman's rank) to the final number of nematodes per unit of root length and positively correlated ($r = 0.72$, Pearson's product moment) to root starch concentrations at 161 DAP (Fig. 2). Arbuscule frequency was not correlated ($p > 0.05$) to reducing sugars or sucrose concentrations in roots at any sampling date. Low light increased the total root colonization by AMF, but decreased

Table 1
Effects of arbuscular mycorrhizal fungi, light, and ring nematodes on dry matter production of 'Pinot noir' grapevines

Treatments			Dry Mass (g)				
AMF	Light	Nematode density (g^{-1})	Leaf	Stem	Wood	Root	Total
nonAMF	Full sun	0	0.95 (0.10)	0.99 (0.12)	9.65 (0.75)	2.54 (0.30)	14.1 (1.2)
		0.1	1.12 (0.20)	1.11 (0.21)	9.69 (1.02)	3.22 (0.62)	15.1 (2.1)
		1.0	0.72 (0.09)	1.10 (0.61)	9.85 (0.75)	2.94 (0.34)	14.6 (1.3)
	50% Sun	0	0.90 (0.11)	0.91 (0.17)	8.66 (0.60)	2.56 (0.41)	13.1 (1.1)
		0.1	0.72 (0.15)	1.14 (0.11)	10.11 (0.82)	2.89 (0.42)	14.9 (1.3)
		1.0	0.52 (0.04)	0.93 (0.12)	9.11 (0.73)	2.54 (0.29)	13.1 (1.0)
+AMF	Full sun	0	5.48 (0.44)	2.62 (0.44)	11.27 (0.97)	11.92 (1.29)	31.7 (2.2)
		0.1	5.78 (0.19)	2.99 (0.40)	11.32 (0.62)	12.49 (0.77)	32.9 (1.0)
		1.0	4.93 (0.27)	2.36 (0.36)	9.98 (0.61)	12.83 (0.77)	30.3 (1.4)
	50% Sun	0	5.76 (0.30)	5.45 (0.46)	10.19 (0.27)	7.12 (0.64)	28.9 (1.1)
		0.1	5.38 (0.33)	4.37 (0.65)	9.25 (0.34)	7.46 (0.94)	26.9 (1.6)
		1.0	4.92 (0.51)	4.75 (0.59)	8.91 (0.47)	8.19 (1.06)	27.5 (1.3)
ANOVA significance levels							
		AMF	<0.001	<0.001	0.119	<0.001	<0.001
		Light	0.077	<0.001	0.021	<0.001	0.024
		Nematode	0.001	0.684	0.409	0.388	0.548
		A × L	0.112	<0.001	0.253	<0.001	0.415
		A × N	0.637	0.405	0.225	0.597	0.521
		L × N	0.249	0.590	0.970	0.946	0.969
		A × L × N	0.894	0.273	0.391	0.824	0.569

Data represent back-transformed means (\pm standard error of the arithmetic mean, $n = 8$). Boldface indicates significant effect.

Table 2
Main effects of sample date, ring nematodes or light on fine root variables in mycorrhizal (+AMF) 'Pinot noir' grapevines

Factor		Nematodes (#m ⁻¹ root) ^a	AMF colonization (% root length)		Glucose equivalents (mg g ⁻¹ root fr. wt.)		
			Total	Arbuscules	Reducing sugars	Sucrose ^a	Starch ^a
Sample date (n = 16)	78 DAP	34 (53) b ^b	81 (2)	46 (3) a	15 (1) b	9 (1) b	n.d. ^c
	121 DAP	343 (114) a	86 (2)	37 (2) b	20 (1) a	14 (1) a	5.6 (0.5)
	161 DAP	359 (200) a	84 (2)	31 (2) c	22 (1) a	12 (1) a	5.9 (0.8)
ANOVA sig. level		<0.001	0.298	<0.001	<0.001	<0.001	0.501
Initial nematode density (n = 16)	0 g ⁻¹	0 ^d	85 (3)	48 (2) a	19 (1)	11 (1)	7.8 (1.0) a
	0.1 g ⁻¹	72 (27) b	84 (2)	36 (2) b	20 (1)	13 (1)	5.1 (0.5) b
	1.0 g ⁻¹	416 (146) a	83 (2)	29 (2) c	18 (1)	12 (1)	4.8 (0.5) b
ANOVA sig. level		<0.001	0.820	<0.001	0.242	0.324	<0.001
Light (n = 24)	Full Sun	141 (67)	81 (2) b	40 (2)	21 (1) a	12 (1)	7.6 (0.8) a
	50% Sun	217 (161)	87 (1) a	35 (2)	17 (1) b	12 (1)	4.3 (0.3) b
ANOVA sig. level		0.331	0.034	0.055	<0.001	0.593	<0.001

Interactions among factors were not significant at 95% confidence. Data represent means (\pm standard error of the arithmetic mean). Boldface indicates significant effect.

^a Data are back-transformed means.

^b Means followed by the same letter for a given factor in a column are not significantly different based on Tukey's HSD test at 95% confidence.

^c Not determined.

^d 0 nematode treatment was excluded from analysis.

root reducing sugars and starch concentrations (Table 2). Light did not significantly affect arbuscular colonization, but plants grown at 50% sun had slightly lower ($p = 0.055$) levels of arbuscules in roots.

3.3. Plant nutrients

Nutrient concentrations and contents (N, P, K, Ca, Mg, S, Fe, Mn, B, Zn, Cu) were examined in all plant tissues in +AMF vines, and significant main effects of either nematode or light treatment were numerous (although interactions were not significant). The concentration or the content of every mineral nutrient was affected by either nematode or light treatments in one of the four tissues that we examined. However, these effects were primarily tied to the biomass changes (for example, more than 50% of the significant effects of light treatment on nutrients occurred for content changes in stems or roots and these mirrored the biomass changes that were caused by light). So, to summarize effects on plant nutrition, we show only those effects that significantly altered whole plant uptake (total plant content) in response to our treatments. Nematodes reduced P uptake by 'Pinot noir' with the lowest values in plants infested at the high nematode density and intermediate values at the low infestation density (Table 3). Nematodes reduced K and S uptake at the highest nematode density, although the effect on S was not supported by mean contrasts (Tukey's at 95% confidence). Nematodes increased vine Fe uptake with the greatest values occurring at the high nematode density and intermediate values at the low infestation density. Low light reduced the uptake of Mg by 'Pinot noir' vines.

4. Discussion

The most significant findings from this study were that ring nematodes suppressed arbuscules and reduced starch in fine roots without altering total AMF colonization; ring nematodes reduced P and K uptake of +AMF vines; but ultimately ring nematodes had no effect on plant dry matter accumulation of +AMF vines after a single growing season. The fact that 'Pinot noir' required AMF in order to achieve significant growth in this soil was no surprise, since this was shown previously using heat (as opposed to fumigation) to kill the resident AMF (Schreiner, 2007). As in the prior study, the growth dependence of 'Pinot noir' on AMF in this

study was clearly a phosphorus response. We had hoped to encourage growth of nonAMF vines in the present study by providing P in the fertilizer so we could assess whether AMF would improve grapevine tolerance to ring nematodes, but the level of P provided in Hoagland's solution given once every 2 weeks was insufficient. Whether AMF may affect ring nematode development or influence their impact on grapevine physiology in soils with greater available P remains to be examined.

4.1. Nematode effects on AMF and root carbohydrates

Ring nematodes specifically reduced arbuscules in grape roots (without affecting total AMF colonization) confirming our prior observations with 'Pinot noir' and 'Chardonnay' in field microplots (Pinkerton et al., 2004). Reduced AMF colonization of roots in response to plant parasitic nematodes (such as root knot or root lesion) is fairly common (Pinochet et al., 1996), although this is not always the case (Pinochet et al., 1997; de la Peña et al., 2006). Our work has been the first to show that the presence of plant parasitic nematodes specifically reduces arbuscules in roots. Whether or not this finding is restricted to ring nematodes or even to ectoparasitic nematodes is unclear, because of the lack of published work with ectoparasites and AMF, and because quantification of arbuscule frequency in roots has only recently become a standard practice in AMF research. However, others have observed arbuscules in root cortical cells adjacent to cells containing eggs of root-lesion nematodes (Lopez et al., 1997) and adjacent to galls produced by root knot nematodes (Pinochet et al., 1997). These observations suggest that endoparasitic nematodes may not suppress arbuscules in roots and may impact AMF development in a different manner than the ectoparasitic, ring nematode. Previous observational studies indicated that endoparasitic, citrus or root-lesion nematodes reduced vesicles but not arbuscules in roots (O'Bannon et al., 1979; Calvet et al., 1995).

Ring nematodes reduced fine root starch concentrations in our study, lending support to the hypothesis that nematodes may compete with AMF for carbohydrates in roots and reduce arbuscules. Indeed, at both 121 and 161 DAP (where we had nematode, starch and arbuscular colonization data) arbuscule frequency in fine roots was negatively correlated to nematodes and positively correlated to starch concentrations ($p < 0.01$, Fig. 2). A reduction in

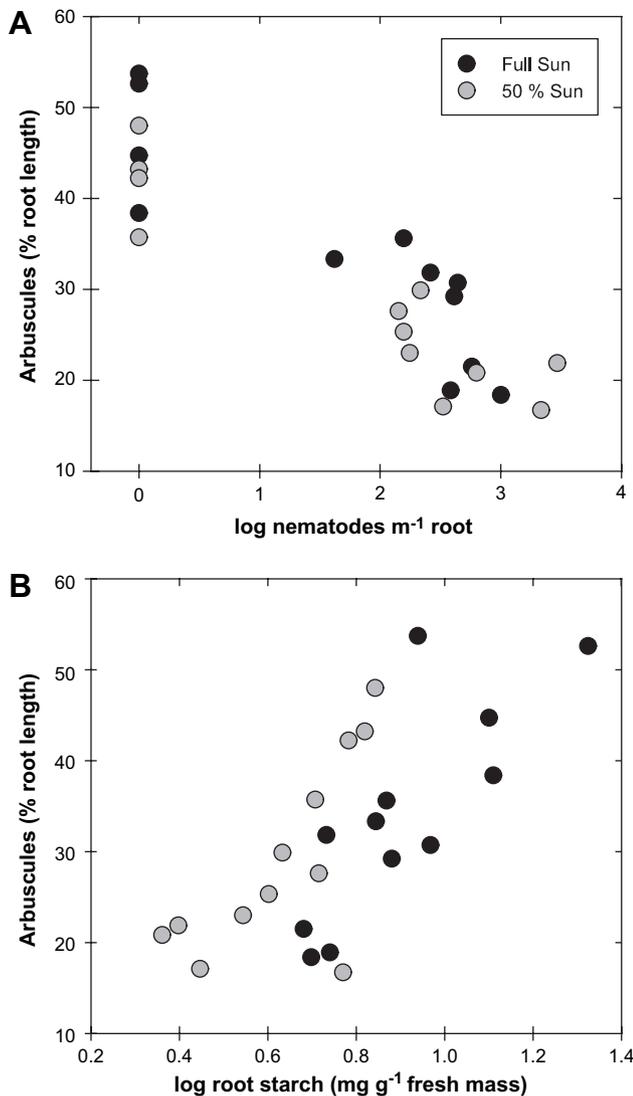


Fig. 2. Relationship between arbuscular colonization and ring nematode density (A) or starch concentration (B) in 'Pinot noir' roots at the final harvest (161 DAP). Data are from core samples from one-half of the experimental units.

both arbuscules and starch was observed in roots of lemon (*Citrus volkameriana*) exposed to periodic drought as compared to well-watered plants (Fidelibus et al., 2000). Other observations of a link between root starch concentrations and arbuscules could not be found in our survey of the literature. However, this is largely

a reflection of the past research focussing on soluble carbohydrates (sugars) and AMF colonization, which are sometimes but not always correlated in roots (see Amijee et al., 1993). Root sugars may be too dynamic to consistently show a relationship with AMF colonization, but starch may represent an integrated measure of root carbon status that better relates to the timescale of changes in AMF colonization. While our data are consistent with the carbohydrate competition hypothesis, it is by no means causal. Indeed, the lack of a significant interaction between light and nematode treatments on arbuscules or root carbohydrate concentrations indicates that carbohydrates may only be part of the mechanism responsible for nematode suppression of arbuscules. We expected to see a greater impact of nematodes on arbuscules in plants grown under low light, since the low light treatment was expected to reduce root carbohydrates. This was the result (i.e. low light treatment decreased starch and reducing sugars in roots), but the effect of the nematodes on arbuscular colonization was no worse in low light plants.

Nematodes reached the same population in either light treatment, whether data were expressed on a soil (data not shown) or root (Table 2) basis. There were, however, less roots in low light plants. Therefore, ring nematodes should have consumed a greater proportion of root carbon under low light and should have had a greater impact on arbuscules, if the effect was solely due to carbohydrate competition. However, we cannot be certain that nematodes consumed a similar quantity of carbohydrates per individual under the low and high light conditions. It is possible that fewer nematodes in the low light treatment were actively feeding on roots. Nematodes may have had to spend more time foraging because of the lower density of roots in our low light treatment. Other mechanisms by which ring nematodes could have reduced arbuscules in roots include the stimulation of plant defense responses (such as the production of anti-microbial, secondary metabolites or pathogenesis-related (PR) proteins) that are known to be upregulated in plants exposed to other plant parasitic nematodes (Gheysen and Fenoll, 2002).

Another consideration when comparing treatment effects on AMF root colonization is that the proportion of root length colonized by arbuscules or other fungal structures is a function of both root growth rate and fungal growth rate (or spread) within the roots. Examination of the total length of roots colonized by AMF can therefore be useful to tease apart effects on roots versus fungi. In this study, the response of the total length of roots with arbuscules (arbuscular root length) to nematodes and light was similar to the response we found for percentage arbuscular colonization. Arbuscular root length was reduced by nematodes with a greater effect at the highest nematode density, and it was reduced by low light (owing primarily to the reduction of root length), but there was no interaction between nematode and light treatments (data not shown). So, similar to the

Table 3
Main effects of ring nematodes or light on nutrient uptake by mycorrhizal 'Pinot noir' grapevines

Factor	Level	Nutrient Content (mg plant ⁻¹)				
		P	K	Mg	S	Fe
Nematodes (n = 8)	0	35.8 (1.8) a ^a	281 (13) a	92.4 (5.7)	68.3 (5.8) a	8.10 (0.69) b
	0.1	32.8 (1.9) ab	289 (13) a	93.9 (3.7)	68.2 (4.5) a	9.06 (0.55) ab
	1.0	27.8 (1.0) b	231 (9) b	87.7 (5.5)	53.5 (4.7) a	10.82 (0.76) a
ANOVA sig. level		0.011	0.008	0.610	0.045	0.041
Light (n = 12)	Full sun	33.7 (1.6)	274 (12)	98.3 (3.6) a	58.9 (3.2)	9.46 (0.75)
	50% Sun	30.6 (1.5)	260 (12)	84.3 (3.4) b	67.8 (5.2)	9.18 (0.48)
ANOVA sig. level		0.119	0.366	0.015	0.101	0.734

Interactions between nematodes and light were not significant at 95% confidence. Data represent means (\pm standard errors). Boldface indicates significant effect.

^a Means followed by the same letter for a given factor in a column are not significantly different based on Tukey's HSD test at 95% confidence.

effects on percentage arbuscules in roots, nematodes did not have a greater effect on arbuscular root length under low light as compared to full sun. Of course, arbuscular root length was affected in the opposite manner with respect to time because the total length of roots with arbuscules increased over time, while % arbuscules decreased (because root length increased over time).

4.2. Nematode effects on nutrients

While our hypothesis that competition for carbohydrates between nematodes and AMF may not entirely account for how nematodes reduced arbuscule development, our results show that the impact of ring nematode parasitism on root function can occur through both direct effects on root growth and indirect effects on AMF. The ultimate result for plant performance would then be expected to manifest itself in different ways depending on what factor or factors (water or nutrients) might be limiting plant growth in a given soil system. Since our experiment was conducted in a low P soil and plants were maintained under well-watered conditions, the impact of ring nematodes appeared to be most significant for their indirect effect on AMF-mediated P uptake.

Ring nematodes reduced P uptake of +AMF vines and the decline in P uptake as nematode density increased closely matched the decline in arbuscules that we observed in roots. This nematode-induced suppression of P uptake occurred presumably because the capacity of nutrient transfer was reduced by reducing the proportion of root cells with arbuscules. This finding is important for two reasons. First, it justifies taking the extra time to carefully assess arbuscular colonization (McGonigle et al., 1990) when studying multi-trophic interactions in roots, which can be related to AMF function (i.e. P transfer to the host). Had we examined total root colonization by AMF using the more traditional approach we would have concluded that nematodes had no influence on AMF in this study. Second, the relationship between reduced arbuscules and reduced starch in fine roots exposed to ring nematodes suggests that arbuscule development is related (at least to some degree) to root carbohydrate availability. While it has become increasingly clear that P transfer between host and fungus occurs in arbuscule-containing cells (Harrison et al., 2002; Isayenkov et al., 2004), there is debate as to whether carbohydrate transfer also occurs in arbusculated cells (Blee and Anderson, 1998; Fitter, 2006).

We suspect that the effect of nematodes on K uptake may have been a direct effect of nematodes on root function unrelated to AMF, since K uptake was reduced only at the highest infestation density with no intermediate effects at the lower nematode density. Ring nematodes have been shown to reduce N, P, and K uptake in peach and plum seedlings in steam-pasteurized soils (Mojtahedi and Lownsbery, 1975; Sharpe et al., 1988). So it is apparent that ring nematodes can influence uptake of N, P, and K independent of their effect on AMF. However, we are certain that the suppression of P uptake caused by ring nematodes in this study was due to their impact on AMF, since nonAMF plants were unable to get P from this soil. Therefore, we believe that the primary negative effect of ring nematodes on plant nutrient acquisition in most Oregon vineyards will be their indirect effect on P uptake, since vines are so reliant on AMF to obtain P from red hill soils. The primary effect of ring nematodes in other agrosystems may be related to their direct effect on root nutrient uptake, such as with N in peach orchards of central California (Cao et al., 2006). The increase in Fe uptake by 'Pinot noir' exposed to ring nematodes in our study confirms similar findings in peach seedlings (Sharpe et al., 1988) believed to be caused by the depression of soil pH by ring nematodes. We did not measure soil pH at the end of our experiment, but we also did not see an effect of nematodes on Mn uptake (data not shown), which is typically more indicative of low soil pH (Marschner, 1996).

4.3. Nematode effects on plant growth

The fact that ring nematodes did not affect plant dry matter accumulation in our experiment shows that grapevines can compensate for nematode feeding for at least the first growing season (~5 months) in pots. Ring nematodes also had no influence on plant vigor of 'Pinot noir' grapevines in field microplots during the first and second growing season (Pinkerton et al., 2004). These findings are in agreement with numerous studies in peach from both field and greenhouse settings (Lownsbery et al., 1977; Nyczepir et al., 1987; Cao et al., 2006) where a negative impact of ring nematodes on plant growth may take 6 months to 2 years to develop. However, a significant reduction on plant dry weight has been found in as short as 11 weeks in plum seedlings grown in pots where ring nematodes reached a similar population as in our study (Mojtahedi and Lownsbery, 1975). Whether these differences are due to different levels of tolerance to nematode parasitism in different plant species or to other factors is not known. Since root starch was reduced by ring nematodes in our study, we suspect this would lead to an overall weakening of roots and lower survival over the dormant season, ultimately affecting shoot growth the next year. This scenario seems particularly likely in lieu of the findings by Nyczepir et al. (1987) in potted peach, where ring nematodes began to affect root growth and reducing sugars in peach at the end of the second, 3-month growing cycle (i.e. 6 months after exposure to nematodes) where shoots were pruned back at the end of each 3-month cycle.

A careful examination of fine roots collected from our core samples at 78, 121 and 161 DAP for necrosis or other possible signs of nematode damage under a stereomicroscope was undertaken, but revealed no apparent damage to roots caused by ring nematodes. Roots ranged in color from light brown to nearly white and there was no difference in the proportion of roots that were brown in color in different treatments. These observations do not support earlier findings of blackened roots found in concord grapevines (*Vitis labrusca*; Santo and Bolander, 1977) or the fairly rapid development of root necrosis observed in peach roots (Nyczepir and Pusey, 1986) exposed to ring nematodes. These findings suggest the possibility of different levels of tolerance to ring nematodes occurring in different woody perennials, but we suspect that similar symptoms on roots may develop after a longer exposure time to ring nematodes in *Vitis vinifera*.

5. Conclusions

We have shown that ring nematodes can reduce arbuscules in roots under controlled conditions similar to our prior observations from field microplots, and that there is an associated decrease in fine root starch and the functioning of AMF for P uptake. While these findings are consistent with the hypothesis that nematodes alter AMF in roots by competing for carbohydrates, the fact that ring nematodes did not have a more severe impact on AMF under conditions of low light (that independently reduced sugars and starch in roots) suggests that other factors (such as plant defense responses) may also contribute to AMF–ring nematode interactions in grape roots.

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