

## Delayed response to ring nematode (*Mesocriconema xenoplax*) feeding on grape roots linked to vine carbohydrate reserves and nematode feeding pressure<sup>☆</sup>

R. Paul Schreiner\*, John N. Pinkerton, Inga A. Zasada

USDA-ARS Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330, USA

### ARTICLE INFO

#### Article history:

Received 14 June 2011

Received in revised form

19 October 2011

Accepted 23 October 2011

Available online 7 November 2011

#### Keywords:

Arbuscular mycorrhizal fungi

Carbohydrate reserves

Nutrient reserves

*Vitis vinifera*

### ABSTRACT

The chronic impact of ring nematode (*Mesocriconema xenoplax*) feeding on grapevine (*Vitis vinifera*) was studied under controlled conditions. 'Pinot noir' grapevines were exposed to ring nematode or kept nematode-free for three growing seasons and vines were either grown in full sunlight, 15% of full sun, or partially defoliated to manipulate vine carbohydrate status. Whole plants were destructively sampled to assess the impact of ring nematode on whole plant biomass, carbohydrate, and mineral nutrient accumulation. Vine shoot growth and total biomass was unaffected by ring nematode in the first growing season, although reserves of nonstructural carbohydrates (NSC), P, K, and Ca in the roots and wood were reduced in all canopy management treatments. Vine shoot growth and total biomass were reduced by ring nematode in Year 2, and greater declines in reserve NSC and most mineral nutrients had occurred. Reserves of NSC were affected more than biomass or nutrients during the second year. During the third year of exposure to ring nematode, vines in the 15% sun treatment were dying (prompting an earlier destructive harvest), even though these vines had similar biomass and NSC reserves as the partially defoliated vines at the end of the second year. The demise of the 15% sun vines was associated with higher ring nematodes per unit of root mass, as compared to either full sun or defoliated vines. Therefore, predicting plant response to this nematode requires an understanding of nematode density per quantity of roots, not nematodes per unit of soil which is how plant parasitic nematodes are currently enumerated.

Published by Elsevier Ltd.

### 1. Introduction

The ring nematode (*Mesocriconema xenoplax*) is a widespread parasite on numerous perennial crops and is the most numerous nematode pest in western Oregon vineyards (Pinkerton et al., 1999). This nematode was shown to reduce root growth, colonization of roots by arbuscular mycorrhizal fungi (AMF), and above-ground vine productivity in field microplots by the third year after planting new grapevines (Pinkerton et al., 2004). In an effort to understand how ring nematode feeding alters vine physiology and AMF colonization of roots, we recently showed that ring nematode rapidly reproduced in potted grapevines, reducing fine root starch concentrations by 39%, arbuscular colonization of roots by 40%, and vine uptake of P, K, and S by about 20% each (Schreiner and

Pinkerton, 2008). However, total vine biomass was not affected by ring nematode even though populations were above 40 nematodes  $g^{-1}$  soil, indicating that vines can tolerate high numbers of ring nematode for at least a single growing season. We suggested that the loss of root starch would lead to reduced survival of roots over the winter and reduced vine growth in later years. Understanding the chronic impact of ring nematode on grapevine was the goal of the multi-year experiment undertaken here. Since our previous research focused on the fine feeder roots of vines (where AMF colonization occurs) over a single growing season, we could not directly compare the effects of ring nematode on total plant carbohydrate versus mineral nutrient status and subsequent use of stored reserves in following seasons. Understanding if ring nematode has a greater impact on whole vine carbohydrate status and storage as opposed to mineral nutrient uptake and storage will aid in the development of appropriate management methods to mitigate damage caused by this important root pest.

It is well known that early season growth of grapevines is dependent on the use of stored reserves of carbon and minerals that are remobilized from the permanent parts of the vine (Yang and Hori, 1979; Mullins et al., 1992; Creasy and Creasy, 2009).

<sup>☆</sup> Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

\* Corresponding author.

E-mail address: [paul.schreiner@ars.usda.gov](mailto:paul.schreiner@ars.usda.gov) (R.P. Schreiner).

Indeed, recent studies have shown that starch and N and P reserves in the roots (Bates et al., 2002; Schreiner et al., 2006; Zapata et al., 2004) were important to support the newly growing canopy of grapevines in the spring and early summer. Nutrient (particularly K, Ca, and Mg) and starch reserves stored in the permanent vine parts (trunk and/or cordons) may also be important for subsequent growth of vines (Bates et al., 2002; Schreiner et al., 2006). Accordingly, we focused our findings here on the impact of ring nematode on the reserves of carbohydrates and nutrients stored in the permanent vine parts and roots.

We employed two canopy treatments (shading and defoliation) in the present study to manipulate the carbohydrate and nutrient status within vines to gain further insight into how carbohydrate or nutrient reserves influence vine response to ring nematode. We tested the hypothesis that added carbon stress brought about by limiting the plants capacity to fix carbon via shading or defoliation would increase the severity and rapidity of nematode damage to vines. Understanding how ring nematode affects plant carbon and nutrient balance could be important in real world vineyards, as various canopy management options that manipulate vegetative and reproductive growth of vines (number of buds retained, leaf pulling, and crop thinning) influences carbon available to roots (Comas et al., 2005; Smith and Holzappel, 2009). We examined the long term impact of ring nematode on whole vine carbohydrate and mineral nutrient reserves using potted grapevines grown over three growing seasons.

## 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 × 3 × 2 factorial experiment with three destructive harvests (originally planned to occur at the end of three consecutive growing seasons), three canopy treatments (full sun, 75% defoliation in full sun, 15% of full sun), and two levels of ring nematodes (0 and 1.0 nematodes g<sup>-1</sup> dry soil initial density) was applied to self-rooted 'Pinot noir' grapevines (*Vitis vinifera* L.) grown in 4 L white plastic pots. Each treatment combination was replicated 6 times for a total of 108 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<sub>3</sub>, 40% MgCO<sub>3</sub>) was added at a rate of 35 g kg<sup>-1</sup> 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<sup>-1</sup> (Trident Inc., Vancouver, WA) to kill resident nematodes. Soil was stored for three months at room temperature after fumigation before use and available nutrients were determined using standard methods for western Oregon soils by the Oregon State University, Central Analytical Laboratory. Available soil nutrients (mg kg<sup>-1</sup>) and pH were: NO<sub>3</sub>-N, 5.3; P (Bray 1), 14; K, 121; Ca, 1158; Mg, 285; SO<sub>4</sub>-S, 77; Fe, 62; Mn, 70; B, 0.20; Zn, 1.3; Cu, 1.0; pH, 5.9.

Five kg of the dry soil mix was placed into each pot and inoculated with a mixture of three species of AMF: *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders INVAM #OR219, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe INVAM #OR218, and *Glomus* sp. INVAM #OR215, all of which had been previously isolated from Jory soil at the OSU-WRV. Each AMF species was isolated and propagated by hand-picking spores from trap cultures and re-culturing on *Sorghum bicolor* L. in a low P, sandy loam soil. A pre-rooted, three-node cutting of 'Pinot noir' (Pommard clone, FPS

91) was transplanted into each pot and thinned to a single shoot per vine. Ring nematodes were introduced into the infested pots two days after planting, 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 1.0 nematodes g<sup>-1</sup> soil (5000 individuals pot<sup>-1</sup>). 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 125 nematodes mL<sup>-1</sup> prior to inoculation.

### 2.2. Growth conditions

All plants were grown under full sun conditions in a greenhouse for 21 d before applying different light levels or defoliation treatments intended to manipulate vine carbohydrate status. At this time, one-third of the pots in each nematode treatment (0 and 1.0 nematodes g<sup>-1</sup> soil initial density) were moved under shade cloth, which reduced incident photosynthetically active radiation (PAR) by ~85%. Typical midday light levels (PAR) in the canopy of the full sun plants averaged ~1200 μmol m<sup>-2</sup> s<sup>-1</sup> and this was reduced to ~175 μmol m<sup>-2</sup> s<sup>-1</sup> in the 15% sun treatment. Another set of vines was partially defoliated by removing three of every four leaves on these vines by detaching the petiole at its base. Leaves were periodically removed from defoliated treatment vines ~ every 10–14 d leaving only every fourth new leaf born on the shoot. The remaining set of vines served as the full sun treatment and were grown in full sunlight without further manipulation.

Plants were grown during the spring and summer of 2005, 2006, and 2007 (~early April to mid September) in a greenhouse. Automatic controls maintained greenhouse temperatures between 10 and 30 °C. During the growing season, plants were watered as needed by monitoring soil surface wetness. Each pot was watered to field capacity only when the surface soil began to appear dry and all pots were checked three times daily. A one-half strength Hoagland's solution (with P) was applied to all pots once every two weeks to maintain a moderate level of soil fertility (Hoagland and Arnon, 1950). Daytime soil temperatures in the different canopy treatments were periodically measured by inserting thermometers in randomly selected pots and were not found to differ. The full sun vines were watered more often than the defoliated and shaded plants and hence experienced greater fluctuations in soil moisture content. However, no vines showed signs of water stress (wilted tips or leaves) at any time during the experiment.

In September of 2005 and 2006 when plants started to show early signs of leaf senescence (yellowing) on basal leaves, a set of plants ( $n = 6$  per nematode and canopy treatment combination) were destructively harvested, and remaining vines (scheduled for later harvests) were moved outdoors under a clear plexiglass cover to overwinter. On two occasions when heavy frosts were predicted during the dormant season, plants were moved to a cold room and held at 4 °C for ~1 week. Plants were moved back into the greenhouse whenever ~50% of buds had burst to start spring growth.

### 2.3. Sampling and assays

Periodic measurements of shoot length were made throughout each growing season using a flexible tape measure. Leaf area was determined periodically by directly comparing each leaf to a series of concentric circles of known area (10, 25, 50, 75, 100, 125, 150, 175, 200, 250, and 300 cm<sup>2</sup>) and adding this value for all leaves per vine. Measurements using this non-destructive approach were highly correlated ( $r = 0.997$ , slope = 0.988,  $n = 25$  vines) to destructive

assessment of leaf area using a calibrated area meter (LI-3000, LiCor Instruments, Lincoln, NE).

Plants were destructively harvested near the end of each growing season in Years 1 and 2 and at the end of rapid shoot growth phase in Year 3. We originally intended to carry out all harvests at the end of each growing season, but it was apparent that few, if any, of the 15% sun vines with ring nematode would survive through the entire 2007 summer. Vines were harvested on September 19, 2005, September 1, 2006, or June 11, 2007, corresponding to 5, 16, and 26 months after initial planting, respectively. At all sampling dates, harvested vines were separated into leaves (blades and petioles), shoots, wood (original cutting), and roots. Roots were washed free from all soil over a large (20 L) container in ~12 L of cold water leaving all the soil in the container. All plant parts were washed with distilled water, oven dried at 70 °C for 4 d, and weighed.

For the 2005 and 2006 sampling dates, all dried plant material was ground to pass through a 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). Total soluble sugars (hereafter referred to as sugars) and starch were determined in each tissue after extraction/precipitation in 80% ethanol according to Chow and Landhäusser (2004), except that glucose released from starch hydrolysis was determined with the GOPOD enzymatic glucose assay (Megazyme International, Bray, Ireland) (Edwards et al., 2011). All carbohydrate fractions were determined in duplicate and are expressed as glucose equivalents kg<sup>-1</sup> dry matter. Reserves of nonstructural carbohydrates (NSC, sugar + starch) and individual mineral nutrients were also determined and are defined as the total content (concentration × biomass) of each nutrient in the permanent parts of the vines (roots + wood), since the shoots were pruned back every year during winter dormancy.

A subsample of fine roots (primary roots with an intact cortex), ~0.5 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 and the proportion of roots that were black in color were determined using the grid line intercept method (Newman, 1966). The proportion of root length colonized by any AMF structure (total colonization = aseptate hyphae + vesicles or arbuscules) and by arbuscules alone was determined on root fragments that were mounted and squashed between microscope slides (McGonigle et al., 1990).

At all sampling dates, nematodes were extracted from the soil-water slurry used to initially wash root samples. The volume of the soil water slurry was brought to a total of 15 L, mixed thoroughly, and duplicate 150 mL subsamples were removed from the mid area of the container immediately after stirring the slurry vigorously (by inserting an inverted 0.15 L beaker halfway below the surface and turning it upright to fill). The subsamples were 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× and expressed as individuals pot<sup>-1</sup> or g root dry matter<sup>-1</sup>.

#### 2.4. Statistical analysis

Data were analyzed by ANOVA and differences were considered significant at  $p < 0.05$ . Each year was analyzed separately, as yearly

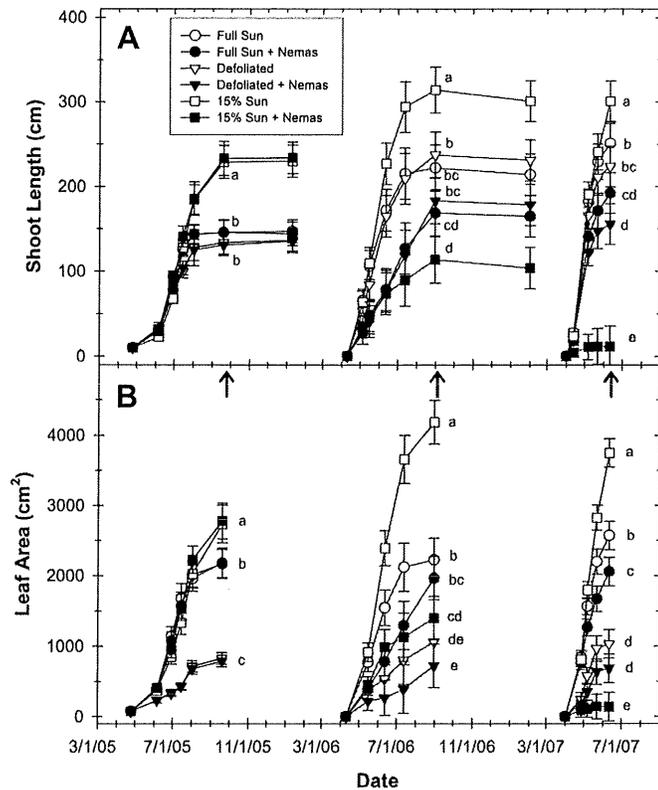
differences in variance which violated assumptions (Cochran's test) could not be overcome for many variables using transformations. Shoot length and leaf area were analyzed by repeated measure (sample time) within each year using canopy treatment and nematode treatment as factors. All other data were analyzed using canopy treatment and nematode treatment as factors. Transformations of raw data were used to satisfy assumptions of variance for some variables. Shoot length and leaf area in 2005, nematode populations per pot and per unit root mass at all dates, Fe and B reserves at the end of year 1, and Fe reserves at the end of Year 2 were log-transformed; leaf mass data in 2005 were square root-transformed prior to ANOVA. The proportion of fine roots that was black in color at each harvest was analyzed using Kruskal–Wallis nonparametric test, as these data could not be transformed to overcome variance violations. Data presented in tables are the means and standard error of the mean, while data shown in figures represent means and 95% least square confidence limits which were back-transformed when a transformation was used. All statistical analyses were carried out using Statistica software (version 8.0, Statsoft Inc., Tulsa, OK).

### 3. Results

#### 3.1. Plant growth and nematode populations

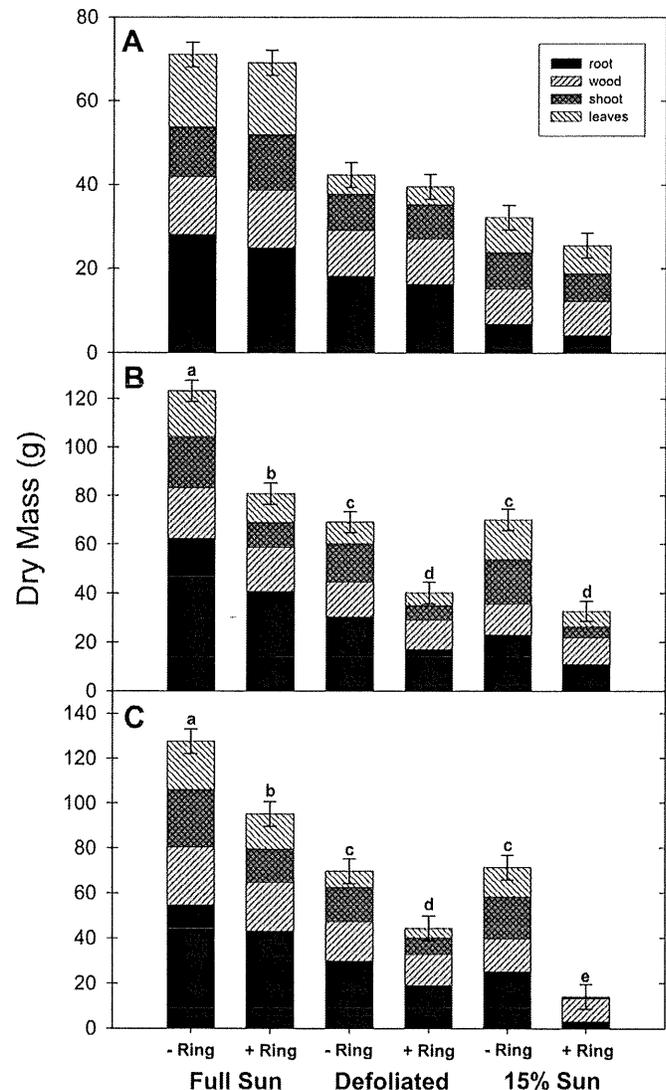
Plant growth was altered by the different canopy treatments in an expected manner in Year 1 ( $p < 0.001$ ). Shoots were longer in the 15% sun vines than either the full sun or defoliated vines on the last three sampling dates in 2005 (Fig. 1A). Shoot length was not affected by nematodes or an interaction between canopy and nematode treatments in Year 1 ( $p > 0.05$ ). However, in Year 2, shoot length was driven by the interaction between canopy and nematode treatments ( $p < 0.001$ ) over time. By the end of the season, the 15% sun vines without nematodes had longer shoots than either full sun or defoliated vines without nematodes (similar to Year 1), but all the nematode-infested vines had reduced shoot lengths compared to the corresponding nematode-free vines within each canopy treatment. The reduction in shoot growth due to ring nematode was greatest in the 15% sun vines, being reduced by more than 50%, compared to the full sun or defoliated vines which were reduced about 25% when infested with nematodes. This same effect was even more pronounced in Year 3, where the 15% sun vines with ring nematode barely grew at all. Effects of canopy and nematode treatments on vine leaf area were comparable to the impact on shoot length. Only canopy treatment affected leaf area of vines in Year 1, but both canopy and nematode treatments and their interaction affected leaf area in Years 2 and 3 over time. The defoliated vines (by design) had the lowest leaf area in Years 1 and 2 (Fig. 1B), but by Year 3, the 15% sun vines with ring nematode had the lowest leaf area. The defoliated vines without nematodes had roughly 30% of the leaf area that the full sun vines had, even though we removed 75% of the leaves, suggesting that some compensation had occurred in the remaining leaves. It should be noted that leaves from the 15% sun vines, which had greatly increased specific leaf area due to shading, were noticeably thinner than leaves from full sun or defoliated vines.

Similar to shoot length and leaf area, each plant part and total plant mass were significantly affected by canopy treatments at the end of Year 1 ( $p < 0.001$ ) such that: leaf mass was lowest in the defoliated vines, intermediate in the 15% sun vines, and highest in full sun vines; shoot mass was lowest in both the defoliated and 15% sun vines compared to the full sun vines, and; wood, root, and total plant mass was lowest in the 15% sun vines, intermediate in defoliated vines, and highest in full sun vines (Fig. 2A). Across all canopy treatments, leaf ( $p = 0.024$ ) and root ( $p < 0.001$ ) mass were reduced



**Fig. 1.** Impact of ring nematode (*Mesocriconeema xenoplax*) and canopy manipulation on shoot length (A) and leaf area (B) of 'Pinot noir' grapevines (*Vitis vinifera*) over three growing seasons. Data represent means and 95% least square confidence limits (back-transformed in 2005) from six vines per treatment combination. Letters designate significant groups (Tukey's HSD at 95% confidence) at the end of each growing season based on canopy treatment (2005) or the interaction between nematode and canopy treatments (2006, 2007). Arrows indicate the approximate date of destructive harvests.

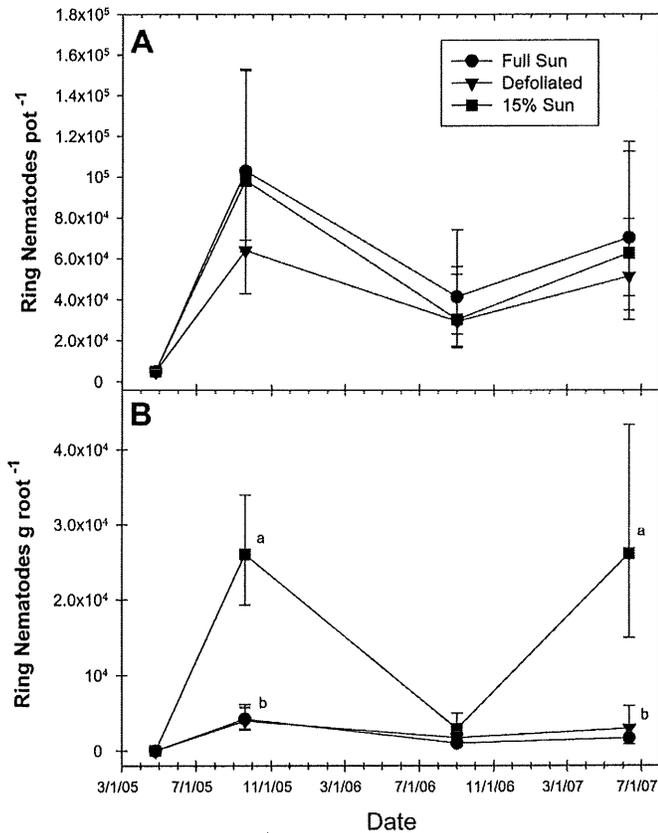
by nematodes at the end of Year 1, but whole plant ( $p = 0.242$ ) mass was not affected by nematodes. No plant part or total plant biomass was significantly affected by an interaction between canopy and nematode treatment in Year 1. By the end of Year 2, both canopy and nematode treatments affected biomass of all plant parts and an interaction between them occurred for leaf ( $p < 0.001$ ), root ( $p = 0.002$ ), and whole plant mass ( $p = 0.012$ ). Whole plant mass was significantly lower in the defoliated and 15% sun vines compared to full sun vines (Fig. 2B). Leaf mass was lower in the defoliated vines than in 15% sun vines, but root mass was lower in 15% sun vines than in defoliated vines, so that overall plant mass was the same in the defoliated and 15% sun vines. Interactions between canopy and nematode treatments that occurred at the end of Year 2 were the result of differences in the magnitude of change within each canopy treatment caused by the nematode. For example, a greater reduction in leaf mass had occurred in the 15% sun vines in response to ring nematode (10.1 g) than in the full sun (7.1 g) or defoliated (3.9 g) vines. However, more total root mass or total plant mass was lost in response to nematode in the full sun vines (21.6 g roots, 42.5 g total) than in either defoliated (13.3 g roots, 28.8 g total) or 15% sun (12.1 g roots, 37.3 g total) vines. Expressed in relative terms there was a 34%, 42%, and 53% loss of total plant biomass in the full sun, defoliated, and 15% sun vines, respectively, in response to nematodes at the end of Year 2. Total plant biomass of the defoliated and 15% sun vines with or without nematodes was similar at this time (Fig. 2B). Early in Year 3, the impact of ring nematode was now even more pronounced in the 15% sun vines (Fig. 2C). Indeed, we had to harvest plants earlier



**Fig. 2.** Impact of ring nematode (*Mesocriconeema xenoplax*) and canopy manipulation on plant biomass of 'Pinot noir' grapevines (*Vitis vinifera*) at the end of Year 1 (A) and Year 2 (B), and at the end of the rapid shoot growth phase in Year 3 (C). Data represent means and 95% least square confidence limits for total plant mass from six vines per treatment combination. Letters designate significant groups (Tukey's HSD at 95% confidence) for total plant mass based on the interaction between nematode and canopy treatments.

than planned because nematode-infested 15% sun vines were nearly dead and had barely pushed a new shoot. With the exception of the wood, all plant parts and total plant biomass were now lower in the 15% sun vines with nematodes than in the defoliated vines with nematodes resulting in significant interactions ( $p < 0.001$ ) between canopy and nematode treatments for all plant parts except wood.

All of the non-infested pots remained free of nematodes throughout all years of the study, and all nematode-infested plots had high population densities by the end of Year 1 (Fig. 3A). Nematode population densities in soil remained high throughout all years. Canopy treatment did not have an impact on nematode population densities on a per pot basis ( $p = 0.151$ ), however when nematode data were expressed per unit root mass, the 15% sun plants had much greater nematode densities (5–10 fold higher) in Year 1 and Year 3 as compared to either full sun or defoliated vines ( $p < 0.001$ ) (Fig. 3B).



**Fig. 3.** Impact of canopy manipulation on ring nematode (*Mesocriconema xenoplax*) populations expressed per pot (A) or per unit root dry mass (B) in pots with 'Pinot noir' grapevines (*Vitis vinifera*) over three growing season. Data are back-transformed means and 95% confidence limits from six vines per treatment combination. Letters designate significant groups (Tukey's HSD at 95% confidence) at each sampling date based on canopy treatment.

It should be noted that we did observe a greater proportion of fine roots that were black in color in the nematode-infested vines in Year 2 and three (data not shown), similar to previous observations in 'Concord' grapevines (Santo and Bolander, 1977). However, no blackened 'Pinot noir' roots were apparent in Year 1. The proportion of fine roots that were black in color in Year 2 was greater in infested vines across all canopy management treatments compared

to non-infested vines ( $p < 0.01$ ). In addition, the nematode-infested, 15% sun vines had more black roots than the nematode-infested, full sun vines at the end of Year 2. By our early harvest in Year 3, nearly all roots in 15% sun vines were black as vines were clearly dying.

**3.2. AMF, carbohydrates, and nutrients at end of Year 1**

Colonization of roots by AMF at the end of Year 1 was affected by both nematode and canopy treatments, without significant interactions (Table 1). The percentage of root length with any AMF structures (total colonization) or with arbuscules alone were both reduced in the presence of ring nematode, while percentage total colonization was lower in full sun vines compared to the defoliated vines and percentage arbuscules was lower in the full sun vines than in 15% sun vines. It should be noted that while the percentage of root length with arbuscules was indeed greater in the 15% sun vines, the intensity of arbuscules and hyphae (the density of AMF structures at root cross sections) in the 15% sun roots was often noted to be low compared to the other canopy treatments. Sugar concentrations in roots were not affected by nematodes, but were reduced sequentially (full sun > defoliated > 15% sun) by canopy treatments. Root starch concentrations were not altered by nematodes, but were reduced by nematodes. There was no interaction between nematode and canopy treatments for root nutrient concentrations. Nutrient concentrations in roots were largely influenced by canopy treatment, such that N, P, K, Ca, Mg, S, Mn, B, and Cu were at higher concentrations in the roots of 15% sun vines than the roots of full sun vines with the roots of defoliated vines often intermediate to these two canopy treatments. However, when nutrient concentrations were evaluated relative to biomass this difference can be interpreted as growth-induced nutrient dilution in the full sun vines that had about three times more root mass (and about two times more total plant mass) than the 15% sun vines (Fig. 2A). Root concentrations of N, Mg, Fe, Mn, and Cu were increased by ring nematode at the end of Year 1 (Table 1).

The impact of treatments on the concentration of nutrients in other plant parts generally reflected what was observed in the roots in Year 1. Nutrient concentrations in leaves and in the whole plant were largely driven by canopy treatments with few effects due to nematodes (data not shown). Concentrations of all nutrients in leaves and in whole vines (except whole vine Mg and Fe concentrations), were altered by canopy treatment. Similar to roots, nutrient concentrations were higher in the 15% sun vines than full

**Table 1**

Impact of ring nematode (*Mesocriconema xenoplax*) and canopy manipulation on root colonization by arbuscular mycorrhizal fungi (AMF) and root carbohydrate and nutrient concentrations at the end of Year 1 (5 months). Data represent means (standard error of mean) for each main factor ( $n = 18$  for nematode treatments,  $n = 12$  for canopy treatments). Bold simply indicates significant  $p$  values of either nematode or canopy treatments or their interaction on given variables.

Variable	Nematode treatment		Canopy treatment			Significance level ( $p$ -values)		
	-	+	Full Sun	Defoliated	15% Sun	Nematode	Canopy	N × C
% AMF	90 (2) a <sup>a</sup>	77 (2) b	80 (2) y	87 (2) x	82 (3) xy	<b>&lt;0.001</b>	<b>0.039</b>	0.964
% Arbuscules	19 (1) a	14 (1) b	13 (1) y	17 (1) xy	19 (2) x	<b>0.024</b>	<b>0.037</b>	0.963
Sugars (g kg <sup>-1</sup> )	79 (7)	79 (7)	110 (5) x	80 (3) y	47 (3) z	0.963	<b>&lt;0.001</b>	0.985
Starch (g kg <sup>-1</sup> )	149 (6) a	120 (6) b	134 (7)	133 (7)	137 (10)	<b>0.002</b>	0.924	0.990
N (g kg <sup>-1</sup> )	11.5 (0.9) b	13.0 (1.1) a	8.8 (0.3) y	9.9 (0.3) y	18.0 (0.5) x	<b>0.001</b>	<b>&lt;0.001</b>	0.096
P (g kg <sup>-1</sup> )	1.5 (0.08)	1.6 (0.08)	1.2 (0.03) y	1.7 (0.04) x	1.8 (0.05) x	0.784	<b>&lt;0.001</b>	0.676
K (g kg <sup>-1</sup> )	12.3 (0.6)	11.9 (0.5)	9.8 (0.1) z	11.9 (0.2) y	14.5 (0.2) x	0.427	<b>&lt;0.001</b>	0.327
Ca (g kg <sup>-1</sup> )	10.8 (0.4)	10.8 (0.4)	9.5 (0.2) y	10.3 (0.2) y	12.6 (0.3) x	0.932	<b>&lt;0.001</b>	0.742
Mg (g kg <sup>-1</sup> )	3.0 (0.1) b	3.8 (0.1) a	3.0 (0.2) y	3.4 (0.1) xy	3.8 (0.2) x	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.697
S (g kg <sup>-1</sup> )	3.4 (0.2)	3.7 (0.2)	3.0 (0.2) y	3.4 (0.1) y	4.3 (0.2) x	0.146	<b>&lt;0.001</b>	0.064
Fe (g kg <sup>-1</sup> )	1.6 (0.2) b	2.6 (0.3) a	1.6 (0.3)	2.2 (0.4)	2.4 (0.4)	<b>0.020</b>	0.189	0.404
Mn (mg kg <sup>-1</sup> )	60 (6) b	80 (7) a	54 (7) y	74 (9) xy	82 (7) x	<b>0.030</b>	<b>0.041</b>	0.188
B (mg kg <sup>-1</sup> )	24 (2)	30 (3)	18 (2) y	23 (2) y	40 (3) x	0.055	<b>&lt;0.001</b>	0.842
Zn (mg kg <sup>-1</sup> )	13 (1)	15 (1)	13 (1)	15 (1)	15 (1)	0.115	0.189	0.887
Cu (mg kg <sup>-1</sup> )	15 (1) b	17 (1) a	13 (1) y	17 (1) x	18 (1) x	<b>0.012</b>	<b>&lt;0.001</b>	0.640

<sup>a</sup> Means within a row followed by the same letter are not significantly different (based on Tukey's HSD test at 95% confidence).

**Table 2**

Impact of ring nematode (*Mesocriconema xenoplax*) and canopy manipulation on carbohydrate and nutrient reserves (total content in roots + trunks) of vines at end of Year 1 (5 months). Data represent means (standard error of mean) for each main factor ( $n = 18$  for nematode treatments,  $n = 12$  for canopy treatments). Bold simply indicates significant  $p$  values of either nematode or canopy treatments or their interaction on given variables.

Variable	Nematode treatment		Canopy treatment			Significance level ( $p$ -values)		
	–	+	Full Sun	Defoliated	15% Sun	Nematode	Canopy	N × C
NSC (g)	5.8 (0.7) <sup>a</sup>	4.8 (0.6) <sup>b</sup>	8.7 (0.3) x	5.2 (0.2) y	2.1 (0.2) z	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.641
N (mg)	246 (15)	227 (17)	315 (6) x	237 (6) y	158 (9) z	0.051	<b>&lt;0.001</b>	0.425
P (mg)	31 (2) a	26 (3) b	36 (1) x	33 (1) x	15 (2) y	<b>0.001</b>	<b>&lt;0.001</b>	0.530
K (mg)	240 (20) a	205 (22) b	311 (7) x	246 (9) y	111 (10) z	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.599
Ca (mg)	231 (21) a	209 (22) b	317 (6) x	233 (7) y	110 (8) z	<b>0.009</b>	<b>&lt;0.001</b>	0.599
Mg (mg)	66 (6)	68 (8)	97 (3) x	74 (3) y	30 (2) z	0.412	<b>&lt;0.001</b>	0.074
S (mg)	60 (5)	61 (7)	84 (4) x	66 (3) y	30 (3) z	0.800	<b>&lt;0.001</b>	0.067
Fe (mg) <sup>b</sup>	23	29	39 x	36 x	12 y	0.266	<b>&lt;0.001</b>	0.112
Mn (mg)	1.3 (0.1)	1.5 (0.2)	1.8 (0.2) x	1.6 (0.2) x	0.7 (0.1) y	0.126	<b>&lt;0.001</b>	0.117
B (μg) <sup>b</sup>	520	539	694 x	592 x	361 y	0.316	<b>&lt;0.001</b>	0.362
Zn (μg)	298 (27) b	417 (38) a	459 (30) x	414 (37) x	200 (18) y	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.287
Cu (μg)	322 (26)	308 (32)	417 (14) x	368 (14) x	159 (17) y	0.435	<b>&lt;0.001</b>	0.295

<sup>a</sup> Means within a row followed by the same letter are not significantly different (based on Tukey's HSD test at 95% confidence).

<sup>b</sup> Log transformed to satisfy assumptions of ANOVA, standard errors are not shown.

sun vines, with defoliated vines most often intermediate. Nematodes influenced whole plant concentrations of Ca, B, and Zn, and leaf concentrations of P, K, Ca, and Zn, such that higher concentrations occurred in nematode-infested vines (data not shown). The average (all vines) leaf macronutrient concentrations found at the end of Year 1 were [reported as  $\text{g kg}^{-1}$  (sem)]: N = 21.0 (1.4); P = 1.7 (0.1); K = 12.0 (1.0); Ca = 20.5 (0.7); Mg = 5.5 (0.2); and S = 7.3 (1.1). Leaf micronutrient concentrations at the end of Year 1 were [reported as  $\text{mg kg}^{-1}$  (sem)]: Fe = 90 (6); Mn = 85 (3); B = 31 (1); Zn = 14 (0.1); and Cu = 5.2 (0.4). Nutrient concentrations found in leaves generally were considered adequate (Gärtel, 1996; Robinson, 1992), but P and Zn concentrations were low. No nutrient deficiency symptoms of any kind had appeared on leaves in any of the vines.

The reserves (total content) of various nutrients and NSC in wood and roots at the end of Year 1 were altered foremost by canopy treatment resulting in greater reserves of NSC and all nutrients in full sun vines compared to 15% sun vines (Table 2). In addition, NSC, N, K, Ca, Mg, and S reserves were greater in full sun vines than defoliated vines, and greater in defoliated vines than 15% sun vines (Table 2). Nematodes reduced NSC, P, K, and Ca reserves in vines, but increased the content of Zn in vines. NSC, P, and K reserves were reduced the most 17%, 16%, and 15%, respectively, by ring nematode. N reserves were not reduced by nematodes even though root biomass was lower in nematode-infested vines

(Fig. 2A), because N concentrations in roots were actually higher in the infested vines (Table 1). There was no interaction between nematodes and canopy treatments on nutrient reserves at the end of Year 1. Whole vine nutrient and NSC contents (including the shoots, leaves, and petioles) at this time showed the same trends as were found for the root + wood reserves, except that whole vine N content was lowest in the defoliated vines because we were removing leaves from this treatment, where most vine N was located (data not shown).

### 3.3. AMF, carbohydrates, and nutrients at end of Year 2

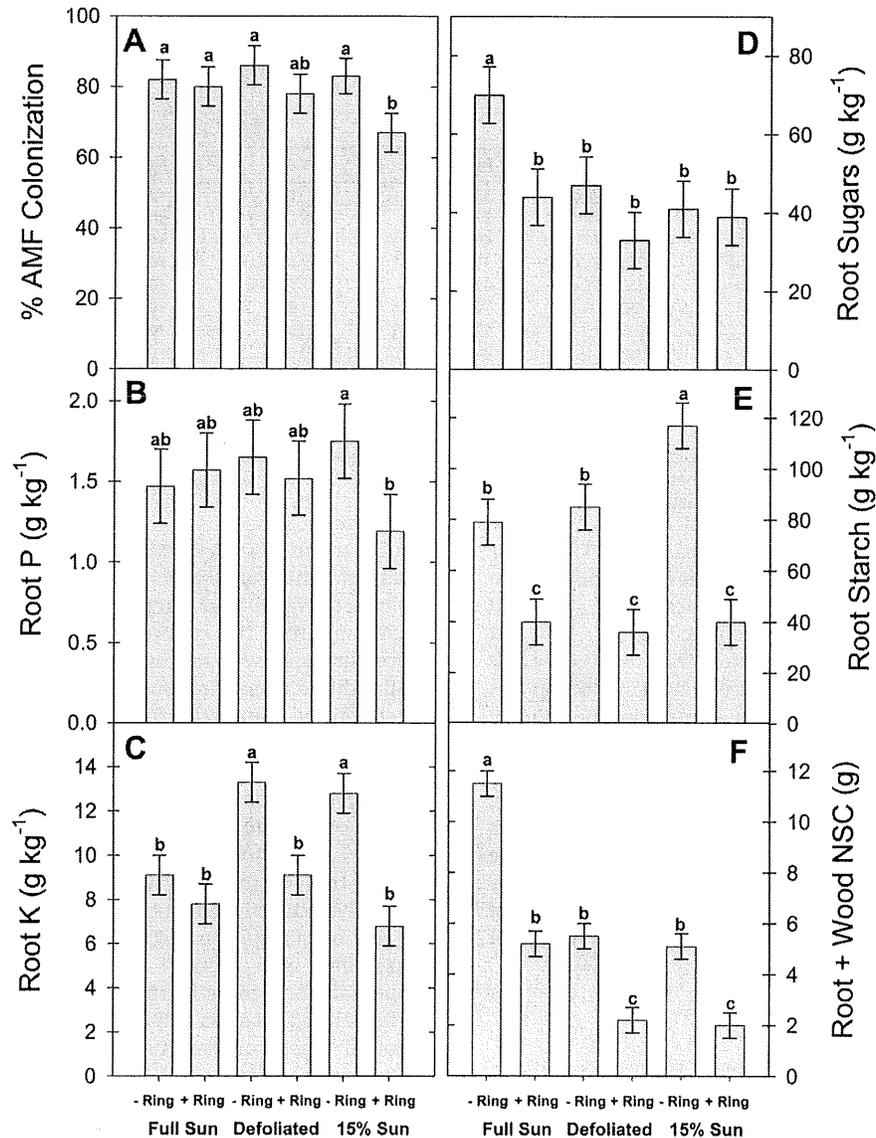
At the end of Year 2, AMF colonization of roots was still reduced by ring nematode, although root length with arbuscules was no longer altered by the nematode (Table 3). Canopy treatments marginally affected percentage AMF colonization, however means assessed by Tukey's were not different. An interaction between nematode and canopy treatments occurred, because AMF colonization was reduced by ring nematode only in the 15% sun vines (Fig. 4A). Root P concentrations reflected this change on AMF colonization such that root P concentration was also lower in nematode-infested 15% sun vines (Fig. 4B). Root concentrations of K, sugars, and starch were also affected by the interaction between nematode and canopy treatments (Table 3); K concentrations were lower in nematode-infested defoliated and 15% sun vines, but not in

**Table 3**

Impact of ring nematode (*Mesocriconema xenoplax*) and canopy manipulation on root colonization by AMF and root carbohydrate and nutrient concentrations at end of Year 2 (16 months). Data represent means (standard error of mean) for each main factor ( $n = 18$  for nematode treatments,  $n = 12$  for canopy treatments). Bold simply indicates significant  $p$  values of either nematode or canopy treatments or their interaction on given variables.

Variable	Nematode treatment		Canopy treatment			Significance level ( $p$ -values)		
	–	+	Full Sun	Defoliated	15% Sun	Nematode	Canopy	N × C
% AMF	84 (1) <sup>a</sup>	75 (2) b	81 (2)	82 (2)	76 (3)	<b>&lt;0.001</b>	<b>0.043</b>	<b>0.038</b>
% Arbuscules	13 (1)	11 (2)	11 (2)	14 (2)	11 (1)	0.173	0.229	0.171
Sugars ( $\text{g kg}^{-1}$ )	53 (4) a	40 (3) b	57 (5) x	41 (3) y	42 (4) y	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.009</b>
Starch ( $\text{g kg}^{-1}$ )	94 (4) a	39 (3) b	60 (7) y	60 (8) y	83 (11) x	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.002</b>
N ( $\text{g kg}^{-1}$ )	12.3 (0.6) b	13.6 (0.7) a	10.3 (0.4) z	12.3 (0.3) y	16.5 (0.5) x	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.934
P ( $\text{g kg}^{-1}$ )	1.6 (0.1)	1.5 (0.1)	1.5 (0.1)	1.6 (0.1)	1.5 (0.1)	0.113	0.384	<b>0.020</b>
K ( $\text{g kg}^{-1}$ )	11.7 (0.5) a	8.0 (0.5) b	8.4 (0.5) y	11.2 (0.8) x	10.1 (1.0) xy	<b>&lt;0.001</b>	<b>0.002</b>	<b>0.009</b>
Ca ( $\text{g kg}^{-1}$ )	12.8 (0.3) b	14.9 (0.4) a	13.6 (0.5) xy	14.7 (0.5) x	13.1 (0.5) y	<b>&lt;0.001</b>	<b>0.048</b>	0.905
Mg ( $\text{g kg}^{-1}$ )	2.5 (0.1)	2.4 (0.1)	2.4 (0.1)	2.5 (0.1)	2.4 (0.1)	0.595	0.335	0.128
S ( $\text{g kg}^{-1}$ )	2.1 (0.1)	2.0 (0.1)	1.8 (0.1) y	2.1 (0.1) x	2.2 (0.1) x	0.738	<b>&lt;0.001</b>	0.074
Fe ( $\text{g kg}^{-1}$ )	1.5 (0.2) b	2.7 (0.3) a	2.4 (0.3)	2.0 (0.3)	1.8 (0.4)	<b>&lt;0.001</b>	0.298	0.464
Mn ( $\text{mg kg}^{-1}$ )	47 (3) b	73 (5) a	67 (4)	55 (5)	56 (7)	<b>&lt;0.001</b>	0.165	0.314
B ( $\text{mg kg}^{-1}$ )	20 (1) b	31 (2) a	27 (2)	25 (2)	24 (3)	<b>&lt;0.001</b>	0.656	0.184
Zn ( $\text{mg kg}^{-1}$ )	9 (1) b	13 (1) a	11(1)	12 (1)	10 (1)	<b>&lt;0.001</b>	0.258	0.516
Cu ( $\text{mg kg}^{-1}$ )	12 (1) b	18 (1) a	14 (1)	16 (1)	14 (1)	<b>&lt;0.001</b>	0.103	0.665

<sup>a</sup> Means within a row followed by the same letter are not significantly different (based on Tukey's HSD test at 95% confidence).



**Fig. 4.** Impact of ring nematode (*Mesocricconema xenoplax*) and canopy manipulation on root colonization by arbuscular mycorrhizal fungi (A), root P concentrations (B), root K concentrations (C), root sugar concentrations (D), root starch concentrations (E), and total carbohydrate reserves (NSC) in roots and wood (F) at the end of Year 2 in 'Pinot noir' grapevines (*Vitis vinifera*). Data are means and SEM of six vines per treatment combination. Letters designate significant groups (Tukey's HSD at 95% confidence) for each variable based on the interaction between nematode and canopy treatments.

full sun vines (Fig. 4C), sugars were reduced by ring nematode only in full sun vines (Fig. 4D), and starch was reduced by ring nematode in all canopy treatments but was at higher concentration in roots of the non-infested 15% sun vines (Fig. 4E). N concentrations in roots were higher in nematode-infested vines, and higher in the 15% sun vines similar to Year 1 (Table 3). Root Ca, Fe, Mn, B, Zn, and Cu concentrations were higher also in nematode-infested vines at the end of Year 2.

At the end of Year two, the impact of treatments on nutrient concentrations in other plant parts was not similar to what was observed in roots (data not shown). Many leaf nutrients (N, P, K, S, and B) were altered by the interaction between nematode and canopy treatments because concentrations were greater in the nematode-infested full sun and defoliated vines, but lower in nematode-infested 15% sun vines. In a similar fashion, whole vine N, P, and K concentrations were significantly lower only in the 15% sun vines in response to nematodes, while nematodes did not reduce the concentrations of these nutrients in either full sun or

defoliated vines (data not shown). Clearly the manner in which nematodes were impacting growth and nutrient uptake and partitioning now differed in the 15% sun vines. Nematodes reduced leaf Ca and Mg concentrations, and increased leaf Zn concentrations. Across all treatments, leaf macronutrient concentrations at the end of Year 2 were [reported as g kg<sup>-1</sup> (sem)]: N = 22.4 (0.7); P = 2.5 (0.1); K = 11.5 (0.6); Ca = 29.6 (0.8); Mg = 7.4 (0.3); and S = 2.2 (0.1). Leaf micronutrient concentrations at the end of Year 2 were [reported as mg kg<sup>-1</sup> (sem)]: Fe = 64 (3); Mn = 78 (4); B = 56 (3); Zn = 15 (0.4); and Cu = 5.2 (0.3). Thus, leaf nutrients were at similar concentrations as were found in Year 1, except that P concentrations in leaves increased in Year 2.

Nutrient reserves in roots and wood at end of Year 2 were affected by both nematode and canopy treatments (Table 4). Similar to the end of Year 1, reserves of all nutrients were greater in full sun vines compared to 15% sun vines, but now full sun vines had more reserves for all nutrients than the defoliated vines as well. Defoliated vines also had more K, Ca, Mg, B, Zn, and Cu contents than the

**Table 4**

Impact of ring nematode (*Mesocriconema xenoplax*) and canopy manipulation on carbohydrate and nutrient reserves (total content in roots and trunks) of vines at end of Year 2 (16 months). Data represent means (standard error of mean) for each main factor ( $n = 18$  for nematode treatments,  $n = 12$  for canopy treatments). Bold simply indicates significant  $p$  values of either nematode or canopy treatments or their interaction on given variables.

Variable	Nematode treatment		Canopy treatment			Significance level ( $p$ -values)		
	–	+	Full Sun	Defoliated	15% Sun	Nematode	Canopy	N × C
NSC (g)	7.3 (0.7) a <sup>a</sup>	3.2 (0.4) b	8.3 (0.9) x	3.8 (0.5) y	3.7 (0.5) y	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
N (mg)	545 (33) a	374 (33) b	638 (30) x	373 (24) y	368 (34) y	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.272
P (mg)	71 (5) a	44 (7) b	86 (6) x	49 (5) y	37 (5) y	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.555
K (mg)	476 (32) a	237 (32) b	511 (45) x	327 (40) y	232 (38) z	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.864
Ca (mg)	596 (58) a	435 (53) b	815 (33) x	428 (29) y	291 (25) z	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.276
Mg (mg)	115 (10) a	73 (10) b	148 (9) x	77 (7) y	54 (6) z	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.681
S (mg)	82 (6) a	54 (7) b	99 (5) x	57 (6) y	47 (5) y	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.405
Fe (mg) <sup>b</sup>	69	69	128 x	48 y	29 y	0.718	<b>&lt;0.001</b>	0.597
Mn (mg)	2.4 (0.2)	2.1 (0.2)	3.8 (0.2) x	1.6 (0.1) y	1.2 (0.1) y	0.058	<b>&lt;0.001</b>	0.731
B (mg)	1.1 (0.1) a	0.9 (0.1) b	1.6 (0.1) x	0.8 (0.1) y	0.6 (0.1) z	<b>0.023</b>	<b>&lt;0.001</b>	0.670
Zn (μg)	564 (51) a	469 (54) b	774 (25) x	480 (33) y	282 (22) z	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.280
Cu (μg)	529 (50)	483 (59)	774 (27) x	441 (31) y	287 (22) z	0.065	<b>&lt;0.001</b>	0.446

<sup>a</sup> Means within a row followed by the same letter are not significantly different (95% confidence).

<sup>b</sup> Log transformed to satisfy assumptions of ANOVA, standard errors are not shown.

15% sun vines (Table 4). Nematodes affected reserves of most nutrients (except Fe, Mn, and Cu) such that lower reserves were found in the nematode-infested vines. Reserves of NSC, K, and P were affected most and reduced by ring nematode by 56%, 50%, and 38%, respectively. Root + wood biomass alone was reduced by 33% (Fig. 2B) while reserve N was reduced 31% by ring nematode at this time (Table 4). An interaction between nematode and canopy treatments occurred only for NSC reserves at the end of Year 2 because the content of NSC in full sun vines without nematodes was higher than all other treatments, and was reduced by ring nematode to the same level found in non-infested defoliated vines or 15% sun vines (Fig. 4F). NSC reserves were at the same level in the absence of nematodes and were reduced to a similar lower level by nematodes in both defoliated vines and 15% sun vines. Reserves of N and P were also the same in the defoliated and 15% sun vines at the end of Year 2 (Table 4).

#### 4. Discussion

Results from this study have increased our understanding of the chronic impact of ring nematode feeding on grape roots suggesting that the primary response leading to plant decline is the loss of carbohydrate reserves. We also confirmed a number of previous findings regarding the short term responses of grapevines to ring nematode that occurred within the first growing season (Schreiner and Pinkerton, 2008). Similar to prior findings, results here showed that ring nematode did not alter whole plant biomass, nor did it affect shoot growth or leaf area development within the first year, despite high nematode populations. Shoot growth and biomass of all vine parts were later reduced by ring nematode in Years 2 and 3 of this study. Reduced starch concentrations in roots, lower AMF colonization of roots, and reduced vine uptake of P and K in response to ring nematode in the first year also confirmed previous observations (Schreiner and Pinkerton, 2008). We did not observe the same increase in whole plant Fe uptake in ring nematode-infested vines as before, although Fe concentration in roots (as well as N, Mg, Mn, and Cu) were actually higher in nematode-infested vines at the end of Year 1.

The impact of low light levels on vine growth and allocation of biomass to shoots and roots was in accordance with previous findings (Schreiner and Pinkerton, 2008), even though we used a much lower light level in the current study (15% of full sunlight, compared to 50% of full sunlight previously). Reduced light levels in both studies increased vine shoot length and decreased root mass after a single season, but root mass was decreased more in this study (70% compared to 30% previously). Total vine biomass at the

end of Year 1 in 15% sun vines was also reduced by ~50% compared to full sun vines here, whereas the 50% sun vines grown in the previous study had total biomass reduced by only ~10% compared to full sun vines. The concentration of sugars in roots was also reduced in response to low light in both studies, but previously we found reduced concentrations of root starch too. Therefore, while we reproduced many prior results when vines were grown at 50% sun (Schreiner and Pinkerton, 2008), vines grown here at 15% sun shifted even more resources to above-ground structures at greater cost to root development and nutrient uptake. It was our intent to have a more severe reduction in root growth in the 15% sun and defoliated vines, and we expected this added stress on roots to increase the sensitivity of vines to ring nematode. While this eventually occurred (in Years 2 and 3), it did not happen within the first year of exposure to ring nematode, indicating how well even young vines with reduced capacity to fix carbon can initially compensate for feeding by ring nematode.

The delayed biomass response to ring nematode observed here in potted vines confirms previous findings in field microplots, where biomass of 'Pinot noir' (assessed via dormant season pruning weights) was not reduced until the third year after exposure to ring nematode (Pinkerton et al., 2004). Ring nematode populations expressed per gram of soil reached a maximum or plateau (indicative of the carrying capacity) in field microplots at the end of Year 2, while nematode populations in the potted vines studied here reached a maximum by the end of Year 1. In both cases, vine biomass was not reduced by ring nematode until the following growing season after the nematode population had peaked within each experimental system. The peak population density reached in our potted vines here (~20 nematodes g soil<sup>-1</sup>) was about two to three times higher than the population density attained in field microplots previously (Pinkerton et al., 2004) and about ten times higher than maximum values found in western Oregon vineyards (Pinkerton et al., 1999). McKenry (1992) suggested that populations of ~0.5 ring nematodes g<sup>-1</sup> soil is the damage threshold in California vineyards. A damage threshold was not apparent in the vineyards of western Oregon, as populations as high as 2.0 ring nematodes g<sup>-1</sup> soil did not appear to cause any noticeable harm or loss of productivity of vines (Pinkerton et al., 1999). However, if vines are under added stressors, such as limited access to soil water or nutrients, then ring nematode may interact with other constraints and exceed the capacity of vines to compensate.

To better understand if carbohydrates or mineral nutrients were primarily responsible for the delayed growth response of vines to ring nematode in this study, we focused our analysis on the total quantity (or reserves) of carbohydrates or mineral nutrients

present in roots and trunks of vines at the end of each growing season, known to be critical for growth in subsequent years (Mullins et al., 1992; Creasy and Creasy, 2009). We also focused on the concentrations of carbohydrates or minerals in roots alone, because higher concentrations of nutrients on a single root basis may be linked to a greater ability for roots to survive over the winter (i.e. a smaller root system with high concentrations of nutrients may survive better than a large root system with low concentrations). Examination of these data together implicates carbohydrates as the key factor altered by ring nematode in grapevines. For example, only root starch concentrations were reduced by ring nematode at the end of Year 1, while the concentrations of all other mineral nutrients in roots were not (Table 1). Indeed, root N, Mg, Fe, Mn, and Cu concentrations were actually higher in the nematode-infested vines at the end of Year 1. At the same time total NSC contents in roots and wood were reduced by ring nematode by 18%, while most mineral nutrient contents, including N, were unaffected by ring nematode (Table 2).

At the end of Year 2, reserves of NSC were reduced by 56%, while root and wood biomass was only 31% lower and most mineral nutrients were about 30% lower in nematode-infested vines. Reserves of K were the mineral nutrient affected most by ring nematode in Year 2, being reduced by about 50%. These findings contrast with those in peach, where N uptake was the nutrient most affected by ring nematode (Cao et al., 2006). However, carbohydrate reserves in our vines, as well as root concentrations of starch were always more strongly impacted by ring nematode than either biomass or any mineral nutrient. These findings are consistent with the well known reduction of root and trunk carbohydrates in various *Prunus* trees after exposure to ring nematode (Nyczepir et al., 1987; Olien et al., 1995; Ferris et al., 2004), known to increase tree susceptibility to bacterial canker.

The 15% sun treatment employed in our experiment increased vine sensitivity to ring nematode, and this became evident in Year 2 and more pronounced in Year 3 (prompting us to harvest vines earlier than planned). One might have expected the defoliated vines to be equally sensitive to ring nematode in Year 3, since the defoliated and 15% sun vines had the same quantity of NSC reserves in roots and wood at the end of Year 2 and similar root and wood biomass. However, the defoliated vines were still able to compensate for ring nematode feeding and support reasonable canopy growth in Year 3, while the 15% sun vines began to die. The most likely reason for this difference was that the density of nematodes feeding per unit of roots was much higher in the 15% sun vines than the other canopy treatments. The number of nematodes per unit of root biomass was more than eight times higher in 15% sun vines at the final harvest in Year 3 compared to full sun or defoliated vines. This finding supports the notion that nematode populations should be expressed in relation to root mass (or possibly some other measure of root growth) instead of the number of nematodes per volume of soil, which is a common practice used by testing labs. Expressing ring nematode number per unit root mass has improved the evaluation of rootstock resistance to ring nematode for *Prunus* (Nyczepir et al., 2009). Soil populations of ring nematode without a corresponding measure of root development may misrepresent the potential impact of the population on vine health.

In conclusion, we have shown that ring nematode parasitism on grape roots alters vine physiology primarily by reducing carbohydrate reserves in the roots and wood needed to support growth in future years. Our results also show that grapevines have a fairly robust capacity to tolerate feeding by ring nematode, since it took three years under a harsh treatment of only 15% sunlight exposure, along with high nematode numbers per unit root mass to cause enough damage to kill vines. Even vines with three quarters of their leaves removed were able to compensate for ring nematode

parasitism through at least three growing seasons. These findings suggest that as long as we don't tip the balance too far, ring nematode-infested vineyards may continue to be productive. Management options that will increase reserves of carbohydrates in the roots and trunks of vines should extend the life and economic return of infested vineyards.

## References

- Ayoub, S.M., 1977. Plant Nematology: An Agricultural Training Aid. California Department of Food and Agriculture, Sacramento, 157 p.
- Bates, T.R., Dunst, R.M., Joy, P., 2002. Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. *HortScience* 37, 313–316.
- Cao, T., McKenry, M.V., Duncan, R.A., DeJong, T.M., Kirkpatrick, B.C., Shackel, K.A., 2006. Influence of ring nematode infestation and calcium, nitrogen, and indoleacetic acid applications on peach susceptibility to *Pseudomonas syringae* pv. *syringae*. *Phytopathology* 96, 608–615.
- Chow, P.S., Landhäusser, S.M., 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology* 24, 1129–1136.
- Comas, L.H., Anderson, L.J., Dunst, R.M., Lakso, A.N., Eissenstat, D.M., 2005. Canopy and environmental control of root dynamics in a long term study of Concord grape. *New Phytologist* 167, 829–840.
- Creasy, G.L., Creasy, L.L., 2009. *Grapes*. CAB International, Oxfordshire, 295 p.
- Edwards, E.J., Downie, A.F., Clingeffer, P.R., 2011. A simple microplate assay to assess nonstructural carbohydrates of grapevine tissues. *American Journal of Enology and Viticulture* 62, 133–137.
- Ferris, H., McKenry, M.V., Jaffee, B.A., Anderson, C.E., Jurma, A., 2004. Population characteristics and dosage trajectory analysis for *Mesocriconema xenoplax* in California *Prunus* orchards. *Journal of Nematology* 36, 505–516.
- Gärtel, W., 1996. *Grapes*. In: Bennett, W.F. (Ed.), *Nutrient Deficiencies and Toxicities in Crop Plants*. American Phytopathological Society, St. Paul, MN, pp. 77–183.
- Hoagland, D.R., Arnon, D.I., 1950. *The Water-culture Method for Growing Plants without Soil*, vol. 347. California Agricultural Experiment Station Circular, Berkeley, CA, 32 p.
- Jones, J.B., Case, V.W., 1990. Sampling, handling, and analyzing plant tissue samples. In: Westerman, R.L. (Ed.), *Soil Testing and Plant Analysis*, third ed. Soil Science Society of America, Madison, WI, pp. 389–427.
- McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115, 495–501.
- McKenry, M.V., 1992. Nematodes. In: Flaherty, D.L., Christensen, L.P., Lanini, W.T., Marois, J.J., Phillips, P.A., Wilson, L.T. (Eds.), *Grape Pest Management*, second ed. University of California Division of Agricultural Science, Oakland, pp. 281–285.
- Mullins, M.G., Bouquet, A., Williams, L.E., 1992. *Biology of the Grapevine*. Cambridge University Press, Cambridge, UK, 239 p.
- Newman, E.I., 1966. A method of estimating the total length of root in a sample. *Journal of Applied Ecology* 3, 139–145.
- Nyczepir, A.P., Reilly, C.C., Okie, W.R., 1987. Effects of initial population density of *Criconebella xenoplax* on reducing sugars, free amino acids and survival of peach seedlings over time. *Journal of Nematology* 19, 296–303.
- Nyczepir, A.P., Nagel, A.K., Schnabel, G., 2009. Host status of three transgenic plum lines to *Mesocriconema xenoplax*. *HortScience* 44, 1932–1935.
- Olien, W.C., Graham, C.J., Hardin, M.E., Bridges, W.C., 1995. Peach rootstock differences in ring nematode tolerance related to effects on tree dry weight, carbohydrates, and prunasin content. *Physiologia Plantarum* 94, 117–123.
- Pinkerton, J.N., Forge, T.A., Ivors, K.L., Ingham, R.E., 1999. Plant-parasitic nematode associated with grapevines, *Vitis vinifera*, in Oregon vineyards. *Journal of Nematology* 31, 624–634.
- Pinkerton, J.N., Schreiner, R.P., Ivors, K.L., Vasconcelos, M.C., 2004. Effects of *Mesocriconema xenoplax* on *Vitis vinifera* and associated mycorrhizal fungi. *Journal of Nematology* 36, 193–201.
- Robinson, J.B., 1992. Grapevine nutrition. In: Coombe, B.G., Dry, P.R. (Eds.), *Viticulture. Practices*, vol. 2. Winetitles, Adelaide, pp. 178–208.
- Santo, G.S., Bolander, W.J., 1977. Effects of *Macroposthonia xenoplax* on the growth of Concord grape. *Journal of Nematology* 9, 215–217.
- Schreiner, R.P., 2003. Mycorrhizal colonization of grapevine rootstocks under field conditions. *American Journal of Enology and Viticulture* 54, 143–149.
- Schreiner, R.P., Scagel, C.F., Baham, J., 2006. Nutrient uptake and distribution in a mature 'Pinot noir' vineyard. *HortScience* 41, 336–345.
- Schreiner, R.P., Pinkerton, J.N., 2008. Ring nematodes (*Mesocriconema xenoplax*) alter root colonization and function of arbuscular mycorrhizal fungi in grape roots in a low P soil. *Soil Biology and Biochemistry* 40, 1870–1877.
- Smith, J.P., Holzapfel, B.P., 2009. Cumulative responses of Semillon grapevines to late season perturbation of carbohydrate reserve status. *American Journal of Enology and Viticulture* 60, 461–470.
- Yang, Y.-S., Hori, Y., 1979. Studies on retranslocation of accumulated assimilates in 'Delaware' grapevines I. Retranslocation of <sup>14</sup>C-assimilates in the following spring after <sup>14</sup>C feeding in summer and autumn. *Tohoku Journal of Agricultural Research* 30, 43–55.
- Zapata, C., Deleens, E., Chaillou, S., Magne, C., 2004. Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). *Journal of Plant Physiology* 161, 1031–1040.

