Managing Arbuscular Mycorrhizal Fungi in Arid Columbia Basin Vineyards of the Pacific Northwest United States

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Abstract
Background and goals
Arbuscular mycorrhizal fungi (AMF) are root symbionts that help grapevines acquire nutrients from soil. Growers in many regions, including the Columbia River basin of Oregon and Washington, lack information needed to best manage AMF. Such lacking information includes understanding whether natural AMF populations are sufficient to ensure healthy colonization in new plantings, and what factors influence AMF in established plantings.

Methods and key findings
AMF colonization of grapevine fine roots was determined in 32 commercial vineyards and in a vineyard that was heavily infested by the northern root-knot nematode (NRKN, Meloidogyne hapla). Root colonization by AMF was as extensive in young vines (one- to two-years-old) as in older vines (>15-years-old) examined in the survey, and was high throughout the season in the single vineyard trial. AMF colonization was greater overall in red wine grape cultivars than in white wine grape cultivars. AMF colonization correlated negatively with soil nitrate and leaf nitrogen (N) concentrations across vineyards, while arbuscules in roots correlated negatively with NRKN populations in soil. Both AMF and arbuscules in roots correlated negatively with NRKN populations per unit of fine root length at the beginning and end of the growing season in the heavily infested vineyard.

Conclusions and significance
Results suggest that AMF populations in soils of the Columbia River basin are ample to ensure high levels of grapevine root colonization, so inoculation is likely not needed when planting or replanting vineyards in the region. AMF colonization was reduced by high N and high populations of NRKN in soil.

Key words: inoculation, Meloidogyne hapla, nitrogen, root-knot nematode, soil amendment, Vitis vinifera

Introduction
The fine roots of grapevines (Vitis sp.) are colonized extensively by arbuscular mycorrhizal fungi (AMF) when available soil phosphorus (P) levels are moderate or low (Karagianidissis and Nikolaoiu 1999, Schreiner and Linderman 2005) and vines grown in low-P soils appear to be completely reliant on AMF to obtain P (Schreiner 2005b, 2007). The extent that AMF enhance uptake of other soil nutrients by grapevines, including nitrogen (N), is less clear (Schreiner 2005b, Cheng et al. 2008, Trouvelot et al. 2015, Tian 2020). Little is known about how well grapevine roots are colonized by AMF in the irrigated vineyards of the Columbia River basin of Oregon and Washington, but we expect grapevines to be dependent on AMF given the very low levels of available P in local soils (Lauer 1988). When assessing AMF in plant roots, it is useful to include colonization by arbuscules as a separate measure from the overall root length colonized by any AMF structures. Arbuscules are finely branched, tree-like structures that occur within individual root cortical cells. They are short-lived, highly regulated, and their frequency provides an indication of active nutrient transfer between AMF and plants, such as carbon (C) exchange for P or other nutrients (Smith and Read 2008, Schreiner and Scagel 2016). However, some nutrient exchange may occur across hyphal walls (Manjarrez et al. 2010).

When considering AMF in perennial crops like European winegrapes (Vitis vinifera L.), it is logical to separate pre-planting management considerations from post-planting concerns (Schreiner 2019). The most important question viticulturists face about AMF prior to planting is whether the existing AMF population in the soil is adequate to ensure that grapevine roots are colonized quickly after planting, or whether the vines must be inoculated with AMF at planting (Holland et al. 2018).
Inoculation with AMF is necessary if the vineyard has been fumigated to kill soil-borne pests or pathogens (Menge et al. 1983). Since AMF cannot complete their life cycle outside of host plant roots, the recent presence of host plants is required to maintain AMF propagules in soil (Smith and Read 2008).

Post-planting, there are numerous factors to consider to maintain or enhance AMF in vineyards. Chief among these are fertilizer and water inputs, and tillage. High soil and plant P status typically reduces AMF colonization (particularly arbuscules) since increased P uptake is the most common benefit plants gain from AMF symbiosis (Smith and Read 2008). In vineyards, root colonization by AMF was depressed as the available soil P levels increased and was also lower in vines receiving foliar P fertilizers (Karagiannidis and Nikolaou 1999, Schreiner and Linderman 2005, Schreiner and Scagel 2016). AMF can also improve N uptake in some plants, and high N supply to plants can similarly depress AMF colonization of roots (Smith and Read 2008, Chen et al. 2018). Indeed, the combination of high P and high N in soil is often highly detrimental to AMF colonization and/or the size and frequency of arbuscules in roots (Smith and Read 2008, Javot et al. 2011, Bonneau et al. 2013). The form of P and N may also be important: more soluble, inorganic forms of N and P appear to be more detrimental to AMF than organic sources, including mulches (Gryndler et al. 2006, Smith and Read 2008). This may explain why greater AMF root colonization was found in organic vineyards than in conventional vineyards (Radić et al. 2014). Reduced irrigation inputs or drier areas within vineyards also have increased AMF colonization of grape roots, which might be attributed to lower availability of P in drier soils (Schreiner et al. 2007, Donkó et al. 2014). Tillage operations reduce AMF propagules in soil by destroying hyphal networks. The destruction worsens as the extent of soil disturbance increases, although some species of AMF may be more tolerant to tillage (Lekberg and Koide 2005, Bowles et al. 2017). Compared to non-cultivated vineyards, cultivation has reduced grape root colonization by AMF and the number of AMF spores in soil (Schreiner and Linderman 2005, Oehl and Koch 2018).

Many other soil organisms, including bacteria, fungi, nematodes, and other soil fauna, may positively or negatively affect AMF propagules in soil and root colonization (Johansson et al. 2004, Raaijmakers et al. 2009). Plant parasitic nematodes are root pests in many crops and compete with AMF for C in roots, sometimes suppressing root colonization by AMF, while AMF may suppress nematode development and enhance plant tolerance to damage caused by nematode feeding (Campos 2020, Bell et al. 2022). The most widespread plant parasitic nematode pest in vineyards of the Columbia River basin is the northern root-knot nematode (NRKN, Meloidogyne hapla) (Zasada et al. 2012).

The studies presented here were designed to capture information on AMF colonization of grapevine roots in commercial vineyards of the irrigated Columbia River basin of the inland Pacific Northwest of the United States. We examined root colonization across numerous winegrape vineyards varying in age, cultivar, soil type, and management factors and compared AMF colonization data with soil and plant nutrient status, and with populations of plant parasitic nematodes in the soil. To better understand relationships between NRKN, fine roots, and AMF, we also examined patterns of vine root growth, AMF colonization, and NRKN population densities in a single vineyard infested by this nematode at different times during the growing season. Finally, using a greenhouse study, we compared different doses of AMF inoculum supplied to Pinot noir cuttings and Zinnia seedlings and examined the subsequent root colonization of those plants to test the hypothesis that grapevines require fewer AMF propagules in soil to become well-colonized relative to other plants.

Materials and Methods

Vineyard survey of AMF

Root colonization by AMF was examined in 32 winegrape (Vitis vinifera) vineyards from the Columbia River basin region in eastern Washington, including a few sites in eastern Oregon, near the time of veraison. All sites were within the level IV ecoregions known as the ‘Pleistocene lake basins’ or the ‘Yakima folds’ that have low annual rainfall (175 to 300 mm) and were dominated by sagebrush and bunchgrass native plant communities prior to intensive agricultural development (EPA 2022). All sites require supplemental irrigation for grape production and all vines examined were planted on their own roots (non-grafted, V. vinifera roots). Each vineyard was sampled over three days in late August 2000. Soil cores were taken at each site within the wetting zone beneath drip emitters (for those sites with drip), but not right under the droplet point (10 cm from actual drop point, which was clear in most cases), and a minimum of 20 cm from vine trunks. In sites with overhead irrigation, cores were taken from within the vine row at least 20 cm from trunks. A set of five soil cores containing roots was obtained using a large diameter (3 cm) corer from 0 to 45 cm depth within each replicate sampling zone in each vineyard. The replicate sampling locations in each vineyard were determined by splitting the area into thirds and sampling from two rows of vines located in the approximate middle of each third. These large cores resulted in samples of ~2.5 kg wet soil per replicate sampling zone. These were placed in sealable plastic bags, kept cool in coolers with ice packs during the day, then stored at 4°C at the end of each day and thereafter. Separate leaf blade samples were collected from each of the three replicate zones congruent with the root core samples. Ten pairs of leaves, comprised of one recently fully-expanded and one opposite-cluster leaf, were collected from the same vines where cores were taken, the petioles were removed, and the leaf blade was stored in sealable bags and also placed in coolers. Smaller diameter (1.8 cm) soil cores were used to obtain samples from 0 to 30 cm depth for nematodes and included ~30 cores from each replicate location, as per large root cores. However, all replicates were pooled into a single composite nematode sample.
for each vineyard. This was required because the nematode survey included about three times more sites than the AMF survey and replication within each vineyard was not feasible for so many samples.

The large soil core samples collected for AMF root colonization were mixed thoroughly, and a 75 g subsample was removed to determine soil water content, then air-dried for subsequent soil nutrient analysis. The remaining soil was washed with cold water to retrieve roots. Roots were collected on a 500 μm sieve (U.S. mesh no. 35) and only the fine feeder roots with an intact cortex were kept to measure AMF colonization. The fine roots were stored in an acetic acid:alcohol (AA, 5%:50% v/v) preservative prior to clearing and staining to reveal AMF colonization. Roots were cleared and stained by the following treatment: incubated at 90°C in 5% (w/v) potassium hydroxide for 30 min, rinsed two times with water, incubated at room temperature in 3% hydrochloric acid for 30 min, rinsed with water, incubated in 1% hydrochloric acid at 90°C for 10 min, incubated in stain solution (0.5% w/v trypan blue, 5% v/v lactic acid, 50% v/v glycerol) at 90°C for 30 min, and incubated in de-stain solution (5% lactic acid, 50% glycerol) overnight at room temperature. The extent of root length (RL) colonized by any AMF structures (hyphae, arbuscules, or vesicles) and a separate count of only arbuscules was determined after squash-mounting roots between two microscope slides and examining a minimum of 150 root intersections with grid lines per sample.

Nutrients in leaf blades and in soil were analyzed at 17 of the sites (see Supplemental Table 1). The analyses were limited to these sites as they represented a wide range of AMF colonization levels; the number of tested sites was limited due to cost constraints (we could not afford to test all sites). Leaf blades were rinsed with distilled water, oven-dried at 65°C, and ground to a fine powder. N was determined by combustion and conversion to N₂ via the Dumas method, and P, K, Ca, Mg, S, Fe, Mn, B, Zn, and Cu were determined by inductively coupled plasma-optical emission spectrometry after microwave digestion in nitric acid (Jones and Case 1962). The fine grape roots with an intact cortex retained on a 500 μm sieve (U.S. mesh no. 35) and only the fine feeder roots with an intact cortex were kept to measure AMF colonization as described above. At each time period in each vineyard, the two sample dates nearest to budbreak, nearest to fruit set, and nearest to harvest were pooled to provide 10 root samples per time period.

AMF colonization parameters and leaf blade nutrients using all replicates from the 17 sites (n = 51) were analyzed using Pearson's correlation analysis. Relationships between the mean AMF colonization per vineyard site and soil nutrients from pooled samples (n = 17), or each plant-parasitic nematode type encountered from pooled samples (n = 51; one site was lost) were analyzed using Pearson's correlation analysis.

### Seasonal dynamics of NRKN and AMF colonization

The vineyard used for the two-year seasonal study was a 30-year-old V. vinifera cv. Riesling vineyard with a high population density of NRKN located near Mattawa, WA. This vineyard was one of three sites used in 2015 and 2016 to generate a developmental model for NRKN (East et al. 2019). Ten soil core samples were collected and pooled at weekly intervals during the growing season from five replicate sampling locations consisting of 10 vines each. We focused this analysis on three time periods in each year, corresponding to budbreak, the peak of root growth in mid-summer, and fruit maturity (harvest). We chose the mid-summer time period when root growth was at its peak, as we suspected that root colonization by AMF might be lowest during this time due to rapid root growth potentially outpacing spread of AMF within roots. A 250 g fresh weight subsample was used to extract NRKN second-stage juveniles (J2) and grape roots from the soil using a semi-automatic elutriator (Seininhorst 1962). The fine grape roots with an intact cortex retained on a 500-μm sieve were treated with 10% bleach solution for three min, with shaking, to release NRKN eggs, rinsed with tap water, and stored in AA preservative. The fixed grape roots were later rinsed with water and examined under a microscope, where only those fine roots with an intact cortex were cut away from older, woody roots and retained for analysis. The number of fine root tips in each sample was counted and the total length of fine roots (within an intact cortex) was determined using the grid line intercept method (Newman 1966). Roots were then cleared, stained, and mounted between microscope slides to assess AMF root colonization as described above. At each time period in each year, the two sample dates nearest to budbreak, nearest to the maximum RL in soil during summer, and nearest to fruit harvest were pooled to provide 10 root samples per time period per year.

Data were analyzed by two-factor ANOVA using year and time period in the model. RL data were square-root transformed and NRKN densities per mm RL were log-transformed to satisfy variance assumptions. Correlations...
between NRKN and AMF colonization parameters were examined by pooling data at each time period from both years, after showing that the year × time period interaction was not significant for AMF colonization parameters or NRKN density data (n = 20).

Impact of AMF inoculum dose on root colonization of grape and Zinnia

An experiment was conducted to test the hypothesis that grapevines require fewer AMF propagules in soils to become well-colonized than other host plants. This trial was conducted in a greenhouse using four-week-old, pre-rooted V. vinifera cv. Pinot noir cuttings and one-week-old Zinnia elegans cv. Peter Pan Plum seedlings. Zinnia was chosen for comparison to grapevines because it is well-colonized by AMF, particularly by one of the fungi used in this test (Funneliformis mosseae) (Long et al. 2010). Plants were transplanted into 1.5-L pots with a peat:pumice 80:20 growing medium, to which different doses of AMF inoculum were added. This growing medium was chosen because it was shown to contain no AMF propagules when grapevines were previously grown in it (RP Schreiner, unpublished data). The experiment was factorial in design, with three doses of AMF (0, 10, or 50 mL inoculum soil per pot) × two plant species with five replicates, for 30 experimental units. The AMF inoculum was a mixture of three AMF species, F. mosseae, Scutellospora calospora, and Rhizophagus irregularis, that were isolated originally from the Oregon State University Research Vineyard, located near Alpine, OR. The same mix of AMF was used previously in experiments with V. vinifera cv. Pinot noir (Schreiner 2007). Plants were fertilized once per week with half-strength Hoagland’s solution with quarter-strength P (Hoagland and Arnon 1950). Plants were grown for 12 weeks, after which they were removed from pots and roots were shaken and washed free of the growing medium. The plants were partitioned into shoots and roots. Shoots were dried for five days at 65°C and dry weight was determined. A subsample of roots was removed to determine AMF colonization and processed as described above, but omitting the peroxide bleaching step during staining for the Zinnia roots. RL was also measured (as described above). The remainder of the roots were dried like the shoots to obtain their dry weight. Data were analyzed using two-factor ANOVA with plant species and AMF dose in the model. RL and % RL with AMF were log-transformed to satisfy variance assumptions prior to ANOVA.

Results

Vineyard survey of AMF

Fine roots of grapevines (V. vinifera) growing in commercial vineyards in the Columbia River basin of eastern Washington and Oregon were well-colonized by AMF (Figure 1A). Colonization by AMF ranged from 50 to 90% near the time of veraison, with an average across all 32 vineyards of 75% of fine RL colonized. Arbuscules in roots were much less prevalent, averaging only 19% of fine RL colonized (Figure 1B). Vine age had no influence on the extent of AMF in roots (Figure 2A), nor did it alter the frequency of roots with arbuscules (data not shown). AMF colonization was greater in red wine cultivars than in white wine cultivars (Figure 2B). This was true also for arbuscules, with red wine cultivars averaging 22% of RL with arbuscules and white wine cultivars only 16%. In addition, those vineyards that were overhead-irrigated had less root colonization by AMF than vineyards that were drip-irrigated (Figure 2C). Irrigation type did not alter the level of arbuscules in roots (data not shown). Lower AMF colonization in the overhead-irrigated vines may have been confounded by the fact that five of the six overhead-irrigated sites were white wine cultivars.

The relationships between root colonization by AMF and other data showed that colonization correlated most closely to the inverse of soil nitrate levels, followed by the inverse of soil P, Fe, and Mn levels (Table 1). AMF colonization also correlated negatively with leaf blade N concentrations, but positively with leaf blade Fe. Arbuscules in roots correlated inversely to leaf blade N levels, but not to any other plant or soil nutrients. Root colonization by AMF did not correlate with any plant-parasitic nematode type encountered in soil. However, the extent of roots with arbuscules correlated inversely with the density of NRKN J2 in soil.

Seasonal dynamics of NRKN and AMF colonization

The length of fine roots in soil was lowest at budbreak, increased by about four-fold in mid-summer (late July in 2015, early August in 2016), and declined again by harvest (Figure 3A). The density of NRKN J2 in soil showed the opposite response as fine roots, consistent with a previous report (East et al. 2019), with the greatest densities at budbreak, very low densities in mid-summer, and increasing again at the end of season (Figure 3B). The extent of roots with AMF did not change over the course of the growing season (Figure 3C), but arbuscules in roots were most abundant at budbreak, declined slightly by mid-summer, and were least abundant at the end of the season (Figure 3D). Correlation analysis revealed that AMF colonization parameters correlated inversely to NRKN J2 density per length of roots in soil at both budbreak and fruit harvest, but not in mid-summer when NRKN J2 densities were low but RL was high (Table 2).

Impact of AMF inoculum dose on root colonization of grape and Zinnia

Although Zinnia produced two to three times more RL per pot, vastly increasing the chance of root encounters with AMF propagules in soil, AMF colonization of Pinot noir roots was about three times greater than in Zinnia roots in those treatments that received AMF (Figure 4). Root colonization in grape reached the same high level (~75%) when supplied with either 10 or 50 mL of AMF inoculum, but colonization in Zinnia was only ~10% RL at the low AMF dose and increased to ~30% RL at the high AMF dose. The dry weight of shoots and roots was not altered by AMF treatments and was similar in both grape and Zinnia (data not shown). These findings...
Figure 1  Proportion of fine root length with A) arbuscular mycorrhizal fungi (AMF) and B) arbuscules in 32 own-rooted winegrape (Vitis vinifera) vineyards in eastern Washington and Oregon at veraison in 2000. “Red” and “white” indicate red and white wine cultivars, respectively. Data are means (± sem) and data for B) was square-root transformed prior to analysis of variance to satisfy variance assumptions (n = 3).

Figure 2  Impact of A) vine age, B) winegrape (Vitis vinifera) type (“red” and “white” indicate red and white wine cultivars, respectively), and C) irrigation method on the proportion of fine root length with arbuscular mycorrhizal fungi (AMF) from 32 vineyards in eastern Washington and Oregon at veraison in 2000. Data are means (± sem) and letters above bars within a plot indicate significant groups based on Tukey’s honest significant difference test at 95% confidence. N.S., not significant.
confirm that grape is a more receptive host for AMF than Zinnia, and that a lower quantity of AMF inoculum in soil is needed to obtain high levels of root colonization in grape roots.

### Discussion

Our findings from the vineyard survey showed that young vines in their first or second growing season planted in the Columbia River basin region were just as well-colonized by AMF as much older vines (Figure 2A). While this survey only focused on a single time point near veraison in a small number of vineyards, this result suggests that natural populations of AMF in the soils of the region are ample to ensure healthy root colonization without the need to inoculate vines. None of the growers had knowingly inoculated vines with AMF prior to planting, nor did they grow winter cover crops prior to planting the vineyards to boost natural AMF populations in soil. All the young vineyards in our survey were planted using bare-root vines produced by nurseries in natural soils, typically in fields where grapevines were not grown previously. These bare-root vines were highly likely to have been colonized by AMF, bringing inoculum within their roots from the nursery fields. Prior findings in Oregon from three grapevine nurseries showed that bare-root vines were colonized by AMF at low rates (16 to 22% RL colonized) (RP Schreiner, unpublished data). In addition, Cabernet Sauvignon roots sampled from a grapevine nursery near Paterson, WA, at the same time as the vineyard survey in 2000, had 78% RL colonized by AMF. While we cannot know whether the indigenous AMF at each vineyard site or AMF brought in on roots from nurseries was the most important source of AMF at any given site, it is likely that both sources of AMF inoculum contributed to colonization in the young

<table>
<thead>
<tr>
<th><strong>Variable (range)</strong></th>
<th><strong>Leaf nutrients (n = 51; 17 sites)</strong></th>
<th><strong>Correlation coefficient (p value)</strong></th>
<th><strong>% root length with AMF</strong></th>
<th><strong>% root length with arbuscules</strong></th>
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<td><strong>Leaf nutrients</strong></td>
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<td>N (1.67 - 3.41% DW)</td>
<td>-0.535 (&lt;0.001)</td>
<td>-0.291 (0.033)</td>
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<td>P (0.12 - 0.48% DW)</td>
<td>-0.092 (n.s.)</td>
<td>-0.138 (n.s.)</td>
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<td>K (0.51 - 1.26% DW)</td>
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<td>-0.099 (n.s.)</td>
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<td>Ca (1.81 - 3.48% DW)</td>
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<td>0.223 (n.s.)</td>
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<td>Mg (0.20 - 0.52% DW)</td>
<td>0.001 (n.s.)</td>
<td>-0.049 (n.s.)</td>
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<td>Fe (81 - 316 mg/L)</td>
<td>0.384 (0.004)</td>
<td>-0.073 (n.s.)</td>
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<td>Mn (30 - 260 mg/L)</td>
<td>-0.043 (n.s.)</td>
<td>-0.136 (n.s.)</td>
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<td>B (23 - 138 mg/L)</td>
<td>0.262 (n.s.)</td>
<td>-0.008 (n.s.)</td>
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<td>Zn (6 - 220 mg/L)</td>
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<td>-0.243 (n.s.)</td>
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<td>Cu (3 - 34 mg/L)</td>
<td>-0.127 (n.s.)</td>
<td>0.070 (n.s.)</td>
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<td><strong>Soil Nutrients</strong></td>
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<td>NO3-N (0.7 - 18.6 mg/L)</td>
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<td>NH4-N (0.8 - 3.1 mg/L)</td>
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<td>0.116 (n.s.)</td>
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<td>P (Olsen, 3 - 42 mg/L)</td>
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<td>-0.309 (n.s.)</td>
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<td>K (170 - 513 mg/L)</td>
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<td>Ca (7.4 - 29.8 meq/100 g)</td>
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<td>0.375 (n.s.)</td>
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<td>Mg (1.3 - 6.7 meq/100 g)</td>
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<td>0.238 (n.s.)</td>
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<td>Fe (3.8 - 47.9 mg/L)</td>
<td>-0.532 (0.028)</td>
<td>-0.357 (n.s.)</td>
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<td>Mn (1.5 - 14.3 mg/L)</td>
<td>-0.556 (0.021)</td>
<td>-0.382 (n.s.)</td>
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<td>B (hot water, 0.1 - 0.5 mg/L)</td>
<td>-0.199 (n.s.)</td>
<td>0.121 (n.s.)</td>
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<td>Zn (0.6 - 8.0 mg/L)</td>
<td>-0.212 (n.s.)</td>
<td>-0.102 (n.s.)</td>
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<td>Cu (0.3 - 1.6 mg/L)</td>
<td>-0.195 (n.s.)</td>
<td>0.036 (n.s.)</td>
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<td><strong>Nematodes (per 250 g, n = 31)</strong></td>
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<td>Paratylennchus spp. (0 - 814)</td>
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<td>0.094 (n.s.)</td>
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<td>Xiphinema spp. (0 - 330)</td>
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<td>0.109 (n.s.)</td>
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<td>Pratylenchus spp. (0 - 132)</td>
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<td>0.108 (n.s.)</td>
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<td>Meloidogyne hapla (0 - 363)</td>
<td>-0.098 (n.s.)</td>
<td>-0.510 (0.003)</td>
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</table>
vines. The land use history for all the vineyards in our survey was not known; however, the prior crops grown at each of the eight young sites (one- to two-year-old vineyards) were identified from interviews with growers. Two of these young vineyards were most recently in grass pasture, two in alfalfa, two were planted into virgin ground (sagebrush steppe), and one each was in mint or apple production prior to establishing the vines. All these prior crops and the native sagebrush and bunchgrass vegetation are host plants known to form associations with AMF (Smith and Read 2008, Brundrett 2009) and would be expected to maintain AMF propagules in the soil. In addition, the inoculum dose experiment conducted here (Figure 4) showed that Pinot noir grapevines were far more receptive hosts than Zinnia, needing a lower dose of AMF propagules in soil to achieve a high level of root colonization. This finding, while based upon only one alternative host plant comparison, indicates that grapevines may not require as many AMF propagules in soil to become highly colonized.

While our results showed that grapevine roots become well-colonized with fewer AMF propagules and that colonization of AMF of roots in the region’s vineyards was high even in young vineyards, we do not imply that inoculating vines with AMF will not further enhance root colonization and potentially benefit vine health. Adding AMF inoculum even to older vines has increased root colonization in some cases (Nicolás et al. 2015, Berdeja et al. 2023). Inoculating 10-year-old V. vinifera Crimson Seedless vines in a vineyard in Spain with a lab-produced AMF inoculum increased root colonization to 60% of RL, rather than only 10 to 20% RL colonized in the existing, uninoculated, control vines (Nicolás et al. 2015). Boosting root colonization in that trial is not surprising, given such a low level of natural root colonization, although why it was so low to begin with is unknown. The inoculum dose experiment demonstrated that Pinot noir grapevines were far more receptive hosts than Zinnia, requiring a lower inoculum dose to achieve a high level of root colonization. This finding, while based upon only one alternative host plant comparison, indicates that grapevines may not require as many AMF propagules in soil to become highly colonized.

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with is not clear. Inoculation with commercial AMF products also enhanced root colonization from ~60% in uninoculated controls to 70% RL in 10-year-old Pinot noir vines on 3309C rootstock, and from ~50% in the controls to 60% of RL in 20-year-old Riesling vines on 3309C or SO4 rootstocks in New York vineyards (Berdeja et al. 2023). Others have reported increased AMF colonization after inoculating vines with commercial products in young vineyards, where a greater response might be expected due to loss of AMF propagules after tilling and preparing the soil, and a potential lack of AMF in planting stock (Mikiciuk et al. 2019, Torres et al. 2021). However, we only observed increased root colonization after inoculating newly-planted vines in western Oregon in one of five separate vineyard experiments using lab-produced AMF inoculum (summarized in Schreiner 2019). Therefore, predicting when AMF inoculation will be beneficial in both older and younger vines is not possible at this time. More work is needed to better understand what factors are most influential in controlling responses to AMF inoculum in vineyards. Progress in this area will be hampered by the complex nature of the plant-soil-AMF interface and the myriad of physical, chemical, and biological factors that influence AMF symbiosis (Schreiner 2005b, Smith and Read 2008, Trouvelot et al. 2015, Holland et al. 2018).

The average RL colonized by AMF across the 32 vineyards (75%) in this study was considerably greater than in a survey of 45 vineyards in Greece that averaged only 40% (Karagiannidis and Nikolau 1999), but similar to a survey from western Oregon, where colonization averaged 73% at bloom and 69% at veraison (Schreiner and Linderman 2005). In Greece, root colonization by AMF near veraison correlated inversely with available soil P levels, which were about two times greater than soil P levels in this study. While root colonization correlated inversely with soil P levels here as well, soil nitrate levels correlated more closely with AMF. Leaf blade N, but not leaf blade P, levels also correlated negatively with AMF (Table 1). Karagiannidis and Nikolau (1999) did not measure soil N levels. In the acid soils of western Oregon, AMF colonization in vine roots did not correlate with either soil P or soil N levels, but did correlate negatively with leaf blade N and P concentrations (Schreiner and Linderman 2005). While it is well known that high P levels in plants or soils can suppress AMF, owing to the clear role that AMF play to help plants obtain P (Smith and Read 2008, Schreiner and Scagel 2016), it appears that high N plays a greater role in altering AMF colonization in Columbia River basin vineyards. Greater soil and vine N status appears to reduce AMF colonization in grapevine roots in this region, possibly interacting with high P status. A rationale for the inverse correlations between AMF colonization and soil Fe and Mn levels in this study is not clear, based on past studies of Fe or Mn uptake by AMF in grapevines (Schreiner 2005b, Trouvelot et al. 2015).

### Table 2

<table>
<thead>
<tr>
<th>Time period</th>
<th>Correlation coefficient (p value) of M. hapla/mm fine root length to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% root length with AMF</td>
</tr>
<tr>
<td>Budbreak</td>
<td>-0.481 (0.032)</td>
</tr>
<tr>
<td>Mid-summer (peak root density)</td>
<td>-0.285 (n.s.)</td>
</tr>
<tr>
<td>Harvest</td>
<td>-0.637 (0.003)</td>
</tr>
</tbody>
</table>

*M. hapla at lowest seasonal density in soil (see Figure 3).* 

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**Figure 4** Effect of arbuscular mycorrhizal fungal (AMF) inoculum dose on **A** total root length per plant and **B** the proportion of fine root length with AMF in own-rooted *Vitis vinifera* cv. Pinot noir and *Zinnia elegans* grown in the greenhouse for 12 weeks in a peat-based growing medium. Data are means ± sem. Data for **A** and **B** were log-transformed prior to analysis of variance to satisfy variance assumptions (*n* = 4). Letters in **B** indicate significant groups based on Tukey’s honest significant difference test at 95% confidence.
al. 2015), although both of these micronutrients correlated strongly with soil P levels within the data set, which may explain their negative correlation with AMF colonization.

The reduced root colonization in overhead-irrigated vines in this study may be because five of the six sites using overhead irrigation in the survey were also white wine grapes. While we did not obtain irrigation input records from the sites we sampled, producers of white wine grapes are known to irrigate more heavily than red wine grapes in the region, as greater water deficits are deemed more favorable to improve red wine quality (Moyer et al. 2013). These findings are consistent with observations of increased arbuscules in roots when less water was supplied to Cabernet Sauvignon in the Columbia River basin (Schreiner et al. 2007), or when a lower frequency of irrigation, albeit at the same level of water supply, was given to Merlot and Syrah vines in the Okanagan Valley, Canada (Holland et al. 2014). Since AMF may enhance vine tolerance to water stress, further work to understand their role under different grape production practices is an important area for future research.

The level of arbuscules in grape roots at veraison in this vineyard survey was low, at only 19%, compared to ~47% of RL with arbuscules observed at veraison in studies from western Oregon (Schreiner 2005a, Schreiner and Linderman 2005). We suspect that this may reflect differences in crop level typically produced in these different regions. At the time that these surveys were conducted, vineyards in western Oregon, with more abundant arbuscules in roots, were cropped to low tonnage (4500 to 6800 kg/ha), while vineyards in the Columbia River basin were cropped to ~11,000 to 15,000 kg/ha. The greater crop carried on vines in eastern Washington and Oregon would have been a larger sink for fixed carbon and would be expected to reduce sugars available to roots and limit arbuscule development or maintenance. A reduction in sugars going to roots quickly results in a loss of arbuscules in roots, while colonization by other AMF structures is far less affected (Smith and Read 2008, Baier et al. 2010, Schreiner and Scagel 2016). The lower levels of AMF and arbuscules in the white wine grape cultivars in the survey may also be related to crop load, as white wine cultivars are typically cropped heavier than red wine cultivars. Future research to better understand how crop load alters C transport to roots and AMF that can drive nutrient uptake may be beneficial to define more sustainable crop load and nutrient management goals for future vineyard production.

The seasonal pattern of arbuscules in roots that occurred in the NRKN-infested Riesling vineyard was unexpected based on prior studies. Arbuscules in roots were highest at budbreak in both years and declined by mid-summer and again at the end of the season (Figure 3D). Previously, arbuscular colonization was lowest at budbreak and increased dramatically by mid-summer in both Cabernet Sauvignon in the Columbia River basin and in Pinot noir in western Oregon (Schreiner 2005a, Schreiner et al. 2007). While the reason for this is unknown, it appears that NRKN parasitism in the Riesling vineyard altered the normal seasonal pattern of when arbuscules are most prevalent in roots. Since high levels of arbuscules occur when sugar transport to roots is high, this finding suggests that the NRKN may stimulate an early season shift of C transport to roots when vines are heavily infested. Further study to confirm these observations is needed.

The negative relationships between NRKN populations and arbuscules in roots from the survey (Table 1), and between NRKN per mm of fine RL and colonization levels from the seasonal study (Table 2) suggest that NRKN competes with AMF in roots and may interfere in nutrient exchange between AMF and vines. While these findings are not causal, they are consistent with previous work on ring nematode (Mesocrictonema xenoplax) and grapevines. High populations of ring nematode suppressed arbuscules in grape roots in field microplots (Pinkerton et al. 2004), which was later shown to be due, in part, to a drain on root carbohydrates needed to support AMF (Schreiner and Pinkerton 2008). It is clear from numerous studies with root-knot nematodes (Meloidogyne) and various plant species that AMF compete with this group of nematodes, but the outcomes of such interactions are complex and depend on many factors (Campos 2020). The vast majority of AMF-Meloidogyne studies also focused on AMF suppression of the nematode and often ignored the effect of the nematode on AMF development. More work to understand how root-knot nematodes alter the timing of fine root production and how root C supply might be directed away from nutrient uptake functions performed by both roots and AMF is needed.

Conclusions

Our results indicate that AMF populations in the arid soils of the Columbia River basin are ample to ensure high levels of root colonization by AMF, so inoculation with AMF is likely not necessary when planting or replanting vineyards in this region to ensure reasonable root colonization. One cannot say with full confidence, however, whether adding AMF inoculum will not have some benefit to further boost root colonization and potentially enhance vine nutrient uptake. The data from the survey of vineyards showed that AMF colonization of roots may be reduced by high soil nitrate and plant N status. Therefore, keeping N inputs to the minimum needed to meet production goals is an important practice to maintain AMF and ensure that the other benefits they provide are not compromised. Finally, reducing population densities of NRKN in vineyards should improve the functioning of AMF in vines. The NRKN appears to compete with AMF for root C, reducing arbuscules that are the primary site of nutrient exchange between vines and AMF.
Supplemental Data
The following supplemental materials are available for this article at ajevonline.org:

Supplemental Table 1 Geographic and production information for 32 wine grape (Vitis vinifera) vineyards sampled at veraison in 2000 in eastern Washington and Oregon.

Acknowledgments
The authors would like to dedicate this paper in memory of Dr. Jack Pinkerton. Jack organized the survey of vineyards conducted in 2000 and enumerated plant-parasitic nematodes in soil samples. We also thank all the participating growers who gave us access to their vineyards to collect soil samples and provided records of their farming operations. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Citation

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