Overlap of Grapevine and Cover-Crop Roots Enhances Interactions among Grapevines, Cover Crops, and Arbuscular Mycorrhizal Fungi

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Abstract: Cover crops are often planted in between vine rows to reduce soil erosion and improve soil fertility and structure. The roots of grapevines and most vineyard cover crops are colonized by beneficial root fungi known as arbuscular mycorrhizal fungi (AMF). Assuming grapevines and cover crops share AMF species in common, contact among grapevine and cover-crop roots may lead to development of a common mycorrhizal network that, in turn, may facilitate direct nutrient transfer from cover crops to grapevines. We quantified grapevine root biomass and mycorrhizal colonization, at different soil depths and distances between grapevines, in trenches (180 x 100 x 90 cm) excavated perpendicular to vine rows. Grapevine fine roots (<2 mm) were found at each sampling depth and distance. Root biomass was lower in the center of the vineyard middles (~90 cm from the base of the vine trunk) than in the area closest to the vine trunk, especially in the top soil depth (0 to 40 cm). Fine root biomass was significantly greater in the three lower soil depths (20 to 40 cm, 40 to 60 cm, 60 to 80 cm) than in the top soil depth (0 to 20 cm). Very fine roots (<1 mm) collected from each sampling point were found to be colonized by AMF. Mycorrhizal colonization was generally greater at soil depths from 0 to 40 cm than from 40 to 80 cm, but did not appear to differ with respect to distance from either vine trunk. Overlap of mycorrhizal grapevine and cover-crop roots may enhance below-ground interactions between grapevines and cover crops, such as nutrient transfer between the two crops. Future work is needed to study the effects of different cover-crop management practices on these interactions.

Key words: arbuscular mycorrhizae, cover crops, N transfer, root distribution, *Vitis vinifera*

Arbuscular mycorrhizal fungi (AMF) are an important group of beneficial soil microbes that form mutualistic symbioses with grapevines (Deal et al. 1972, Menge et al. 1983, Nappi et al. 1985, Possingham and Groot-Obbink 1971). Grapevines respond positively to inoculation with AMF. Greenhouse studies showed that inoculated grapevines have larger shoot and root biomass (Biricolti et al. 1997, Linderman and Davis 2001, Schubert et al. 1988), greater P concentrations (Biricolti et al. 1997), and more compact, highly branched roots than noninoculated grapevines (Schellenbaum et al. 1991). Field research showed that grapevines became severely stunted in fumigated soil because of the absence of AMF propagules (Menge et al. 1983). Soil fumigation may even have long-term effects on AMF species diversity several years after vineyard establishment (Cheng and Baumgartner 2004a).

Vineyard floor management practices, such as cover cropping, likely affect grapevine mycorrhizal colonization and the AMF community. AMF form mutualistic symbioses with vineyard cover crops, with the notable exception of *Brassica* species such as black mustard (*B. nigra* (L.) Koch) (Schreiner and Koide 1993). Dormant-season cover crops increase mycorrhizal colonization of subsequent crops of *Zea mays* L. (Boswell et al. 1998, Kabir and Koide 2000, 2002). Cover crops of *Secale cereale* L. cv. Merced rye and X *Triticosecale* Wittm. ex A. Camus cv. Trios 102 were found to increase AMF spore production in a California vineyard (Baumgartner et al. 2004). As host species is known to influence AMF species composition (Bever et al. 1996, Eom et al. 2000, Johnson et al. 1992), introducing different cover-crop species to a vineyard may change the indigenous AMF community and thereby influence the interactions between grapevines and AMF.

AMF can simultaneously colonize the roots of multiple plants to form a common mycorrhizal network through which nutrients can be transferred from one plant to another (Fitter et al. 1998, Johansen and Jensen 1996, Newman et al. 1994). To study nutrient transfer from cover crops to grapevines through mycorrhizal links, we grew grapevines and cover crops in specially designed containers in the greenhouse that restricted their root systems to separate compartments, but allowed AMF to colonize both crops (Cheng and Baumgartner 2004b). Our results showed evidence of AMF-mediated ^{15}N transfer from cover crops to grapevines five and ten days after labeling cover-crop leaves. N transfer was significantly greater from the grass

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cover crop (*Bromus hordeaceus* L. ssp. *molliformis* (Lloyd) Maire and Weiller cv. Blando) to the grapevine than from the legume cover crop (*Medicago polymorpha* L. cv. Santiago) to the grapevine, suggesting that grasses may be more generous N-donors than legumes.

Mycorrhizal cover crops affect colonization of grapevine roots, either through spore production or by colonization of grapevine roots by extraradical hyphae originating from cover crop roots. In vineyards, the proximity of grapevine roots to cover-crop roots likely affects the development of common mycorrhizal networks, given that the density of extraradical hyphae decreases with increasing distance from roots (Jakobsen et al. 1992). As the distance between grapevine and cover-crop roots increases, we might expect to see fewer mycorrhizal links between them. In order to test the hypothesis that roots of grapevines and cover crops coexist in the vineyard middle (the section of the vineyard floor in between vine rows), we examined grapevine fine root distribution and mycorrhizal colonization in a California vineyard.

Materials and Methods

In November 2002, we quantified grapevine root distribution in the soil profile in between vine rows in a commercial winegrape vineyard in Napa, California. The vineyard was established in 1996 with *Vitis vinifera* cv. Merlot (clone 314) on 110R rootstock (*V. berlandieri* Planch. X *V. rupestris* Scheele) to a 2 x 2 m vine spacing. Grapevines were drip-irrigated. An annual, no-till cover crop of *Vulpia myuros* var. *hirsuta* cv. Zorro (Zorro fescue) was maintained between every row since 1998. The soil was classified as fine-loamy, mixed, thermic, Cumulic Ultic Haploxerolls (USDA, Natural Resources Conservation Service). Soils contained 2.1% organic matter, 17.3 μg g^{-1} soil of Olsen-P, 259 μg g^{-1} soil of exchangeable K, and 389 μmol g^{-1} soil of cation exchange capacity. Petioles collected at full bloom had 8 mg g^{-1} of N, 7.2 mg g^{-1} of P, 13.1 mg g^{-1} of K, 40.6 μg g^{-1} of B, and 92.03 μg g^{-1} of Zn.

We used the trench profile method (Böhm 1979) to quantify roots in three trenches (180 cm length x 100 cm width x 90 cm depth) that were excavated perpendicular to vine rows from one grapevine trunk to the trunk of the adjacent grapevine. In each trench, nine consecutive soil monoliths (20 cm length x 10 cm width x 20 cm height) were collected at each of four soil depths (0 to 20 cm, 20 to 40 cm, 40 to 60 cm, and 60 to 80 cm). Grapevine roots were extracted by washing in a hydropneumatic root elutriator system (Gillison's Variety Fabrication, Benzonia, MI) and separated into fine roots (<2 mm) and coarse roots (>2 mm). Cover-crop roots were not collected, as the growing season of the cover crop begins in November, at the time of the excavations. Presence of dead roots from the cover crop of the previous winter were noted, but not quantified.

Grapevine fine roots were scanned using an Epson Expression 1600 scanner (Epson America, Long Beach, CA). Root images were analyzed for root diameter, surface area, and length using WinRHIZO (version 5.0a; Regent Instruments, Quebec, Canada). After scanning, roots were oven-dried at 60°C for one week and weighed.

Very fine roots (<1 mm) were separated from grapevine fine roots (<2 mm) and stained using the method of Koske and Gemma (1989). Stained roots (30 cm total root length per sample) were mounted in five parallel lines on microscope slides and examined for AMF structures under a compound microscope (100X magnification) using the grid-line intersect method, modified from Giovannetti and Mosse (1980). Mycorrhizal colonization was expressed as the percentage of intersects where AMF structures were present out of the total number of intersects examined (50 intersects) for an average of three grid rearrangements per subsample. Mycorrhizal colonization per 50 intersects was adjusted for percent root length, where root length was estimated from 50 intersect counts using the method of Newman (1966).

Data were analyzed using the GLM procedure in SAS (version 8.2; SAS Institute, Cary, NC). A two-way analysis of variance (ANOVA) was used to examine the effects of soil depth and distance from the vine trunk on grapevine root biomass. Very fine grapevine roots from one trench were too decomposed to examine mycorrhizal colonization; mycorrhizal colonization data are reported from two trenches.

Results and Discussion

Grapevine fine roots (<2 mm) were present in every soil monolith (20 cm length x 10 cm width x 20 cm height) collected between the vine rows to a depth of 80 cm. Root biomass was lower in the center of the vineyard middles (approximately 90 cm from the base of the vine trunk) than in the area closest to the vine trunk, especially in the top soil depth (0 to 20 cm) (Figure 1). Fine root biomass was significantly greater in the three lower soil depths (20 to 40 cm, 40 to 60 cm, and 60 to 80 cm) than in the top soil depth (0 to 20 cm). Fine root distribution did not differ significantly among the three lower soil depths (20 to 80 cm). The percentage of very fine grapevine roots (<1 mm) was highest in the top soil depth (0 to 20 cm, data not shown). More coarse roots were found in the three lower soil depths (20 to 80 cm) than in the top soil depth (0 to 20 cm), and close to the vine row than in the middle (data not shown). Coarse root distribution within the three lower soil depths (20 to 80 cm) was highly variable among trenches, which made it difficult to compare differences among soil depths.

A cover crop of Zorro fescue was maintained in the central 1.2 m (0.3 m from the vine trunk) of the 2-m-wide vineyard middles. When the trenches were excavated in November 2002, the growing season for Zorro fescue had just begun; a few, young cover-crop roots were found in the top 10 cm of soil. Based on the presence of dead Zorro fescue roots, from winter 2001 to 2002, Zorro fescue roots are concentrated in the upper 40 cm of soil.

Very fine grapevine roots (<1 mm) from every soil monolith (20 cm length x 10 cm width x 20 cm height) were found to be colonized by AMF. Mycorrhizal colonization was generally greater at soil depths from 0 to 40 cm than from 40 to 80 cm, but did not appear to differ with respect to distance from either vine trunk (Figure 2). Given that roots of the cover crop were concentrated in the upper 40 cm of soil, it is possible that Zorro fescue enhances mycorrhizal colonization of grapevine roots. Sources of inoculum to initiate colonization of new roots for most plants include spores, colonized root fragments, and hyphae (Smith and Read 1997). In the vineyard middle, AMF hyphae originating from cover crops may be able to colonize grapevine roots and form mycorrhizal links between these two crops.

Many factors affect grapevine root distribution, including soil physical properties, vine spacing, rootstock (Morano and Kliewer 1994), and irrigation (Araujo et al. 1995). It was not surprising to find fine roots in the upper 20 cm of soil and in the center of the vineyard middle, since the examined vineyard has deep and fertile soil with a high water table in spring. Higher fine root biomass at the vine trunk than in the vineyard middle is likely due to drip irrigation.

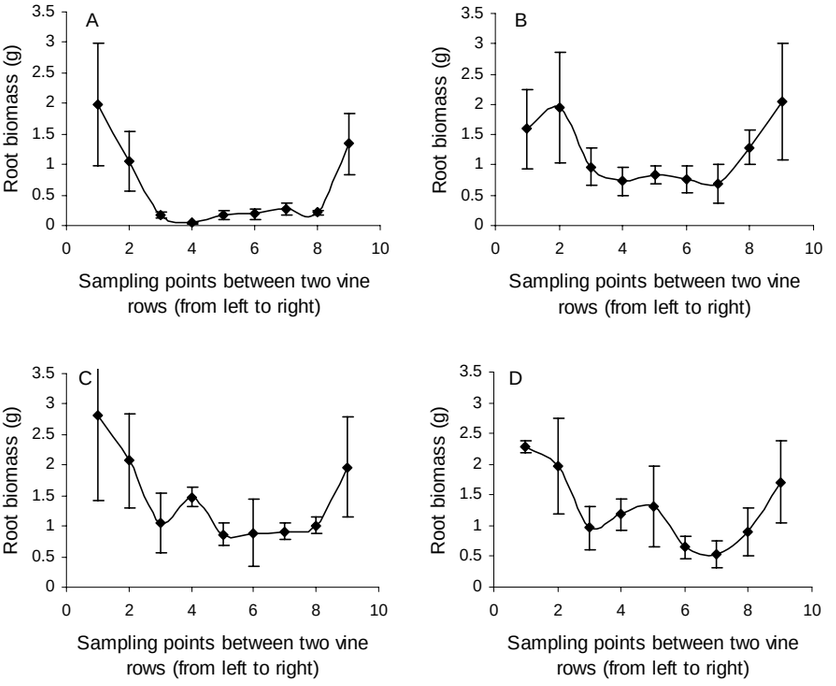

Figure 1 Grapevine fine root (<2 mm) distribution at nine sampling points (20 cm between points) between two grapevines in adjacent vine rows at soil depths of (**A)** 0 to 20 cm, (**B)** 20 to 40 cm, (**C)** 40 to 60 cm, and (**D)** 60 to 80 cm. Grapevine trunks were present at sampling points 0 and 10. Error bars represent standard errors (n = 3).

In the vineyard we examined, overlap of grapevine and cover-crop roots may enhance the formation of a common mycorrhizal network between the two crops. In a greenhouse study, we demonstrated that nutrients can be transferred from cover crops to grapevines through AMF links (Cheng and Baumgartner 2004b). We found that cover-crop species affects the extent of N transfer to grapevines. Cover-crop management practices (tilling, mowing, and so on) may also affect AMF-mediated nutrient transfer. For example, mowing cover crops in spring, a practice carried out in frost-prone vineyards, may stimulate nutrient transfer from decomposing cover-crop roots to grapevines through an existing common mycorrhizal network. AMF have been shown to accelerate decomposition and acquire N directly from complex organic materials, such as grass shoots (Hodge et al. 2001). In a tilled system, AMF may facilitate decomposition of incorporated cover crops, and increase nutrient transfer from decomposing cover crops to grapevines.

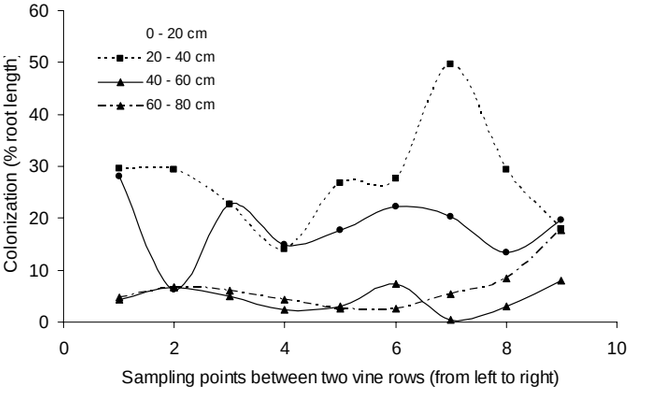

Figure 2 Mycorrhizal colonization at nine sampling points (20 cm between points) between two grapevines in adjacent vine rows at soil depths of 0 to 20 cm, 20 to 40 cm, 40 to 60 cm, and 60 to 80 cm. Grapevine trunks were present at sampling points 0 and 10.

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