Commercial Bioinoculants Increase Root Length Colonization and Improve Petiole Nutrient Concentration of Field-grown Grapevines

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Abstract. **Agricultural bioinoculants containing arbuscular mycorrhizal fungi represent a potential opportunity to reduce the dependence of grapevines (*Vitis*) on agrochemicals. This field study assessed the ability of four commercial bioinoculants to colonize grapevine roots and their effects on petiole nutrient concentration, berry composition, and root morphology of 'Pinot noir' (*Vitis vinifera*) grafted onto rootstock 'Couderc 3309' (*Vitis riparia* × *Vitis rupestris*) and 'Riesling' (*V. vinifera*) grafted onto 'Couderc 3309' and Selection Oppenheim four (*Vitis berlandieri* × *V. riparia*). Three bioinoculants increased root mycorrhizal colonization; however, regardless of the treatment, mycorrhizal fungal structures were enhanced. Grapevine petiole nutrient concentration was improved by bioinoculants. Root diameter, root length density, and specific root length increased with greater mycorrhizal root colonization. Using bioinoculants to reduce chemical fertilizers may be a good strategy to improve grapevine productivity and health in cool climates; however, the impact of mycorrhizal bioinoculants in the vineyard may differ among scion–rootstocks, edaphoclimatic conditions, and vineyard soil microbiomes.**

Commercial vineyards often partially depend on the application of synthetic fertilizers to stimulate plant growth as well as the use of chemical pesticides to control plant pathogens and fungal diseases. These practices adversely affect beneficial soil organisms (Cesarano et al. 2017) and enhance greenhouse gas emissions (Hamilton et al. 2016), ground and surface water pollution (Herrero-Hernández et al. 2016), and soil quality degradation (Hamilton et al. 2016). Because of the consequences of climate change, the use of more eco-friendly practices to increase grapevine (*Vitis*) production may be considered as a suitable mitigation option for the grape and wine industry.

Bioinoculants are soil additives that are composed of beneficial fungal and/or bacterial organisms and algae, and they may contain abiotic amendments such as humic acids, nutrients, softwood biochar, worm castings (family Lumbricidae), and carriers. They are applied to soil or plants to improve crop nutrition, productivity, and soil fertility (Diagne et al. 2020). Among these microorganisms, arbuscular mycorrhizal fungi (AMF) from the phylum Glomeromycota are ubiquitous and form mutualistic symbiosis with roots of ≈72% of terrestrial plants (Brundrett and Tedersoo 2018). AMF transfer water and nutrients to their host plant in exchange for carbon (Smith and Smith 2012), contribute to soil aggregation processes (Powell and Rillig 2018), and increase resistance to various stressors (Begum et al. 2019). Because of these ecological benefits, AMF have been produced and applied as bioinoculants for several decades, mostly as a practice to improve horticulture and grain crop productivity (Basiru et al. 2021) while reducing environmental costs (Berruti et al. 2016). Furthermore, to be considered successful, the bioinoculant producers should provide the appropriate carrier material to prevent the decline of the microorganisms and maintain their effectiveness during storage and transport and after introduction into the soil (Basiru et al. 2021; Raimi et al. 2021). However, adoption and acceptance of bioinoculants by agronomical and perennial crop farmers have been slow because of their poor quality attributable to their inappropriate formulations, poor packaging techniques (Raimi et al. 2021), as well as inconsistent results associated with improving plant performance under greenhouse and field conditions (Holland et al. 2018; Mikiciuk et al. 2018; Rosa et al. 2020).

Grapevines generally exhibit low root density and have few root hairs (Smart et al. 2006). As a result, AMF grapevine symbiosis is considered a

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
0.0108	ft/oz	$m \cdot g^{-1}$	93.0102
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.1550	inch/inch^3	$cm \cdot cm^{-3}$	6.4516
28.3495	oz	g	0.0353
0.001	ppm	$g \cdot kg^{-1}$	1000
0.001	ppm	$g \cdot L^{-1}$	1000
1	ppm	$mg \cdot L^{-1}$	1
1	ppm	$\mu g \cdot g^{-1}$	1
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

key component of the vineyard system (Torres et al. 2021; Trouvelot et al. 2015). It is recognized that under controlled conditions, bioinoculants containing AMF enhance grapevine growth (Krishna et al. 2006; Linderman and Davis 2001), mineral nutrient concentration (Khalil 2013; Schreiner 2007), and resistance to various stresses, including drought (Nikolaou et al. 2003; Valentine et al. 2006), salinity (Belew et al. 2010), metals (Nogales et al. 2019), viruses (Hao et al. 2019), and pathogens (Cruz-Silva et al. 2021). Similarly, under controlled conditions, AMF inoculation may improve grape berry parameters related to both primary and secondary metabolism under increased temperatures and water deficits (Antolín et al. 2020; Torres et al. 2019).

Despite AMF impacts on grapevine productivity, studies of the efficacy of commercial bioinoculants to colonize grapevine rootstocks and influence aboveground and belowground growth and development under field conditions remain scarce (Aguín et al. 2004; Karoglan et al. 2021; Rosa et al. 2020; Sas-Paszt et al. 2020; Torres et al. 2021). A recent study by Torres et al. (2021) performed under field conditions found that commercial AMF inoculation successfully colonized vine roots and enhanced vegetative growth, photosynthetic activity, water status, and flavonoid accumulation in 'Merlot' (*Vitis vinifera*). Aguín et al. (2004) reported that AMF *Rhizophagus aggregatum* altered root morphology of cuttings from three different rootstocks by increasing branching of first-order lateral roots. Moreover, rootstock trait interactions with different AMF species in various environment scenarios may differ among rootstock cultivars because of their intensive breeding and genetic background (Gautier et al. 2020) and could influence the AMF symbioses establishment and the impact they have on plant physiology (Holland et al. 2018; Torres et al. 2021).

It is important to highlight that most AMF studies involving grapevines were conducted in regions where the climate, soil conditions, and rootstocks differ substantially from those of the northeastern United States. Because bioinoculants containing AMF are expected to be an important component in sustainable agriculture, assessment of their potential in viticulture must consider their adaptability to local soil and the interactions between scion cultivar and rootstocks under different growing conditions and environments. The objective of these studies was to evaluate the effectiveness of various commercially available bioinoculants for colonizing grapevine roots and their potential effects on nutrient concentration, berry composition, and root morphological characteristics of 'Pinot noir' and 'Riesling' (both *V. vinifera*) grapevines.

Materials and methods

Plant material and bioinoculant. From 2018 to 2019, this field study was conducted in three and four adjacent vineyard rows for 'Riesling' and 'Pinot noir', respectively, at a commercial vineyard in Hector, NY (lat. 42.50°N, long. 76.88°W). The vineyard is located on a slope with 3% to 8% inclination, Howard gravelly loam soil (loamy-skeletal, mixed, active, medic Glossic Hapludalfs), low organic matter content and fertility, moderate soil susceptibility to compaction, and medium to neutral acid surface layers and upper part of the subsoil (Puglia 1979). 'Pinot noir' grafted onto rootstock 'Couderc 3309' (*Vitis riparia* × *Vitis rupestris*) (Pinot noir/3309C) was planted in 1987, whereas 'Riesling' grafted onto rootstock 'Couderc 3309' (Riesling/3309C) and Selection Oppenheim four (*Vitis berlandieri* × *V. riparia*) (Riesling/SO4) were planted in 1999. The three vineyard blocks all have a north–south row orientation with spacing of 5.9 ft between vines and 8.8 ft between rows. All vines were vertically shoot-positioned and managed using recommended management practices for *V. vinifera* cultivars according to regional guidelines (Wolf 2008), including herbicide use under vines, regular fungicide applications, and occasional pesticide applications if required.

The inoculation treatments were noninoculated plants irrigated with water as a control and four commercial bioinoculant as treatments (Table 1); however, the soil in all plots presumably contained native AMF species (native AMF species were not identified in this study). Treatments were widely available commercial bioinoculants from large companies that were purchased anonymously through consumer channels. Nine AMF species (six from the Glomeraceae family, one from the Claroideoglomeraceae family, one from the Gigasporaceae family, and one from the Paraglomeraceae family) were declared by the manufacturers to be present in the bioinoculants used in this study. The commercial bioinoculants were designated as product 1, which contained four AMF species, product 2, which contained nine AMF species, product 3, which contained nine AM species, and product 4, which contained nine AMF species. According to the manufacturer's specifications, AMF species propagules concentrations varied among inoculants as well as among the presence of ectomycorrhizal fungi species, beneficial bacteria (phylum Bacillota), and special formulated amendments, including biochar, earthworm (*Eisenia foetida*) castings, kelp (phylum Ochrophyta), and humic acid. For example, products 3 and 4 contained ectomycorrhizal species (*Rhizopogon* and *Pisolithus*) and active bacteria species. Products 1, 3, and 4 contained additional additives for nutrition (i.e., fertilizers and biostimulants such as humic acid and algae extract). Product 2 contained only AMF species and clay as a carrier material. Grapevines were inoculated on 1 Jun 2018, at the beginning of flowering (E-L 19), according to the modified Eichhorn and Lorenz developmental scale (Coombe 1995). All bioinoculants were applied according to the manufacturer's specifications as follows: 14 g of powder bioinoculant was mixed with 1 gal of water and applied directly to the root zone of every two vines for products 1 and 3, and 28 g of granular bioinoculant was applied directly to the root zone of every two vines for products 2 and 4.

Pinot noir/3309C was inoculated with products 1, 2, 3, and 4; however, Riesling/3309C and Riesling/SO4 were inoculated with products 2, 3, and 4. The experiment (Fig. 1) had a randomized complete block design, with four adjacent vineyard rows of the experimental site for Pinot noir/3309C (n = 80) and three adjacent vineyard rows for Riesling/3309C and Riesling/SO4 (n = 48), respectively. Vineyard rows were used as blocks and treatments, and control were randomly assigned to replicates. Each experimental unit consisted of 10 contiguous

Table 1. Characteristics of commercial bioinoculants applied on 1 Jun 2018 to grapevine roots of 'Pinot noir' grafted onto rootstock 'Couderc 3309' (Pinot noir/3309C) and 'Riesling' grafted onto rootstock 'Couderc 3309 '(Riesling/3309C) and rootstock Selection Oppenheim four (Riesling/SO4) grown under field conditions in Hector, NY.

Product no.	AMF species[i]	ECM species[ii]	Other components	Propagule density[iii]
1	*Funneliformis mosseae* *Rhizophagus aggregatum* *Rhizophagus irregularis* *Claroideoglomus etunicatum*		Nitrogen, phosphorus, and potassium Humic acids Softwood biochar Worm castings	20 propagules/g for each species of the phylum Glomeromycota
2	*F. mosseae* *Funneliformis monosporum* *Rhizophagus clarus* *R. aggregatum* *R. irregularis* *Septoglomus deserticola* *C. etunicatum* *Gigaspora margarita* *Paraglomus brasilianum*		Clay	50 propagules/g for each species of the phylum Glomeromycota
3	*F. mosseae* *F. monosporum* *R. irregularis* *R. aggregatum* *R. clarus* *C. etunicatum* *S. deserticola* *P. brasilianum* *G.* margarita	*Rhisopogon villosolus* *Rhizopogon fulvigleba* *Pisolithus tinctorius* *Laccaria bicolor* *Laccaria laccata* *Scleroderma cepa* *Scleroderma citrinum*	*Bacillus licheniformis* *Bacillus azotoformans* *Bacillus megaterium* *Bacillus coagulans* *Bacillus pumilus* *Trichoderma harzianum* Kelp Humic acids	34 propagules/g for *R. aggregatum*, *R. irregularis, F. mosseae*, and *C. etunicatum* 13 propagules/g for *F. monosporum*, *S. deserticola*, *R. clarum*, *P. brasilianum*, and *G. margarita*
4	*F. mosseae* *F. monosporum* *R. irregularis* *R. aggregatum* *R. clarus* *S. deserticola* *G.* margarita *C. etunicatum* *P. brasilianum*	*Rhizopogon luteolus* *R. villosolus* *Rhizopogon amylopogon* *R. fulvigleba* *P. tinctorius* *S. cepa* *S. citrinum*	*Bacillus subtilis* *B. licheniformis* *B. pumilus* *B. megaterium* Clay Nitrogen, phosphorus, and potassium	50 propagules/g for each species of the phylum Glomeromycota

[i] Arbuscular mycorrhizal fungi species from the phylum Glomeromycota.
[ii] Ectomycorrhizal fungi species from the phylum Basidiomycota.
[iii] Manufacturer's information: 1 propagule/g = 28.3495 propagules/oz.

vines. Four vines with similar sizes of each experimental unit were designated as treatments for data collection. Five months (Pinot noir/3309C) and 17 months (Riesling/3309C and Riesling/SO4) after inoculation, two soil/root core samples per vine (diameter, 2.5 cm; depth, 60 cm) perpendicular to the row and at a distance of 25 cm per vine were taken from four separated data collection vines in each experimental unit for root morphology and mycorrhizal colonization analyses. The Pinot noir/3309C block was removed by the grower after the 2018 growing season; therefore, it could not be sampled 17 months after inoculation.

Root morphology. Grapevine roots were removed from soil and grass roots by washing cores through a 2-mm sieve. They were classified and separated according to Guo et al. (2008) and McCormack et al. (2015) by branching order classes. The first class comprised first- and second-order fine absorptive roots. The second class was composed of third-order roots and higher. Only absorptive roots (first- and second-order) for Riesling/3309C and Riesling/SO4 were scanned for subsequent image analysis (Winrhizo; Regent Instruments Inc., Québec City, QC, Canada). The average root diameter (RD; millimeters) and total root length (RL; centimeters) of each sample were measured. Root length density (RLD) was determined as the ratio of the root length and soil volume (centimeters per cubic centimeter of soil). Then, root samples were oven-dried at 60 °C for 48 h and weighed to calculate specific root length (SRL) as the ratio of the total root length and root dry weight (meters per gram of root). After scanning, first-class roots were preserved in 75% (by volume) ethanol for later mycorrhizal colonization determination.

Quantification of mycorrhizal colonization. To quantify the proportion of the total root length colonized (RLC) by AMF, the trypan blue method of Koske and Gemma (1989) was used, with some modifications. Briefly, absorptive roots were cut into 2-cm fragments and incubated for 20 min at 90 °C in 10% potassium hydroxide, bleached for 30 min with alkaline hydrogen peroxide solution, acidified in 1% hydrochloric acid for 30 min, and stained for 25 min at 90 °C in 0.05% trypan blue (Sigma-Aldrich, St. Louis, MO) in acidic glycerol solution. Stained root samples were stored in an acidic glycerol solution for 72 h before being mounted in the same solution on a microscope slide. Stained 2-cm roots

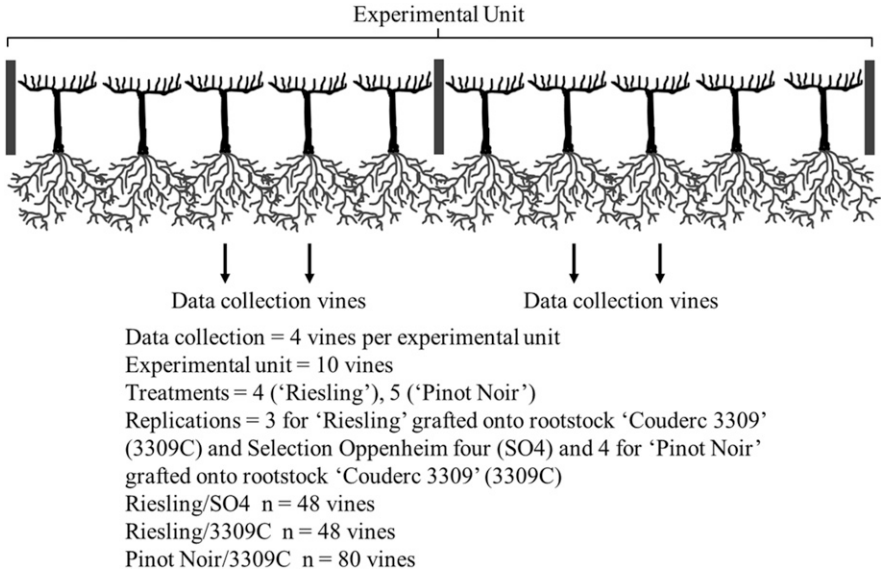

Fig. 1. Experimental setup of one replication for 'Pinot noir' grapevine grafted onto rootstock 'Couderc 3309' (Pinot noir/3309C) and 'Riesling' grapevine grafted onto rootstock 'Couderc 3309' (Riesling/3309C) and rootstock Selection Oppenheim four (Riesling/SO4) under field conditions. This image was created with graphic design software (BioRender, Toronto, ON, Canada).

were cut into 1-cm segments. A total of 100 stained root segments per sample (10 root fragments per microscope slide) were quantified under a compound microscope using the intersection method (McGonigle et al. 1990). Mycorrhizal arbuscules, vesicles, and hyphae were quantified as evidence of root colonization.

Petiole nutrient analysis. Petiole nutrient concentrations of Pinot noir/3309C in 2018, of Riesling/3309C in 2019, and of Riesling/SO4 at veraison in 2019 (E-L 36) were measured according to the modified Eichhorn and Lorenz developmental scale (Coombe 1995). Eighty petiole samples per experimental unit were taken from young fully expanded leaves (fourth and fifth leaves from the growing tips). The petioles were washed with mild soap and rinsed with distilled water. The plant material was sent to the Cornell Nutrient Analysis Laboratory to determine the petiole concentration of macronutrients and micronutrients [phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), boron (B), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), and sodium (Na)] using inductively coupled plasma-optical emission spectrometry (Spectro Analytical Instruments GmbH, Kleve, Germany) for the dry ash extraction method (Campbell and Plank 1998). Total carbon (C) and nitrogen (N) were quantified using a combustion analysis with a total C and total N analyzer (Primacs; Skalar, Inc., Buford, GA). Both analyses were performed for Pinot noir/3309C; however only C and N analysis was conducted for Riesling/3309C and Riesling/SO4.

Grape berry composition. In 2019, 20 clusters were randomly collected from each experimental unit for juice composition analysis of Riesling/3309C and Riesling/SO4. Juice soluble solids (percent) were quantified with a temperature-compensating refractometer (model PA203X; Misco, Solon, OH,). Titratable acidity (g/L) was determined by titration using 50 mL of juice with 0.10 M of sodium hydroxide (NaOH) with pH 8.2 using a pH meter (848 Titrino Plus; Metrohm, Riverview, FL). The pH was measured with a calibrated pH meter (Accument Basic AB15; Fisher Scientific, Pittsburgh, PA). Yeast assimilable nitrogen (YAN) (mg/L) was calculated using an enzymatic analysis (model RS-232; Randox Monaco RX, Kearneysville, WV) and combining the contents of ammonia and primary amino N.

Data analysis. Statistical analyses were performed with the open-source R statistical computing environment (R Development Core team 2010). The data were checked for normality and homogeneity of variance with the built-in package stats for R functions (Ihaka and Gentleman 1996). The mycorrhizal root length colonization percentage and fungal structures (arbuscules, vesicles, and hyphae), plant nutrient parameters, root morphology parameters (root diameter, root length, root length density, and specific root length), and berry composition (soluble solids, titratable acidity, pH, and YAN) were analyzed using a mixed-model analysis of variance in which bioinoculant treatments were classified as fixed effects and blocks were classified as random effects. Differences between means were determined using the Tukey multiple comparison test at $P \leq 0.05$. A matrix of Pearson correlation coefficients was used to explore the relationships among mycorrhizal fungal structures, micronutrient and macronutrient concentration, and root morphology and berry composition parameters; only selected data for Pearson's correlation are presented. Graphs were made using statistical software (JMP Pro version 16.0; SAS Institute Inc., Cary, NC).

Results

Performance of bioinoculants on grapevine roots. Inoculation increased the RLC, vesicles, arbuscules, and hyphae in all three scion/rootstock combinations when compared with control plants (Table 2, Fig. 2), except for the RLC of Riesling/3309C plants inoculated with product 3. All vines, but particularly those in the control treatment group, likely benefited from indigenous AMF in the soil. The evaluation of AMF colonization showed that products 4 and 2 increased RLC ≈14% and ≈9%, respectively, for Pinot noir/3309C, ≈14% and ≈11%, respectively, for Riesling/SO4, and ≈10% and ≈7%, respectively, for Riesling/3309C when compared with control plants (Fig. 2). Similarly, Pinot noir/3309C, Riesling/SO4, and Riesling/3309C plants inoculated with products 4 and 2 showed increased arbuscules, vesicles, and hyphae colonization when compared with control plants (Table 2).

Effects of AMF inoculants on petiole nutrient status. Petiole nutrient differences were found for inoculated Pinot noir/3309C plants (Fig. 3). Products 4 and 2 increased N ≈0.16% (1.6 $g \cdot kg^{-1}$) and ≈0.11% (1.1 $g \cdot kg^{-1}$), respectively, compared with control, P ≈0.16% (1.6 $g \cdot kg^{-1}$) and ≈0.09% (0.9 $g \cdot kg^{-1}$), respectively, compared with control, K ≈1.34% (13.4 $g \cdot kg^{-1}$) and ≈0.97% (9.7 $g \cdot kg^{-1}$), respectively, compared with control,

Table 2. Arbuscular mycorrhizal fungal structures observed in fine roots of 'Pinot noir' grapevine grafted onto rootstock 'Couderc 3309' (Pinot noir/3309C) and 'Riesling' grapevine grafted onto rootstock 'Couderc 3309' (Riesling/3309C) and rootstock Selection Oppenheim four (Riesling/SO4), inoculated or not on 1 Jun 2018 with commercial bioinoculants containing different arbuscular mycorrhizal fungi (AMF) species. Fine roots of Pinot noir/3309C were collected 5 months after inoculation, whereas fines roots of Riesling/SO4 and Riesling/3309C were collected 17 months after inoculation.

		Mean ± *SE* (%)		
Scion/rootstock	Treatment[i]	Vesicles[ii]	Arbuscules[ii]	Hyphae[ii]
Pinot noir/3309C	Control	18.23 ± 0.89 c[iii]	23.38 ± 1.10 b	17.37 ± 1.31 d
	Product 1	20.23 ± 0.89 bc	26.25 ± 1.10 ab	18.10 ± 1.31 cd
	Product 2	22.55 ± 0.89 ab	29.33 ± 1.10 a	21.35 ± 1.31 b
	Product 3	20.76 ± 0.89 bc	28.05 ± 1.10 a	21.31 ± 1.31 bc
	Product 4	25.42 ± 0.89 a	29.55 ± 1.10 a	28.55 ± 1.31 a
	P	<0.001	<0.001	<0.001
Riesling/S04	Control	17.89 ± 0.62 c	19.32 ± 0.35 d	20.17 ± 0.68 c
	Product 2	23.74 ± 0.62 a	24.20 ± 0.35 b	26.48 ± 0.68 ab
	Product 3	20.88 ± 0.62 b	21.15 ± 0.35 c	23.61 ± 0.68 b
	Product 4	25.17 ± 0.62 a	27.10 ± 0.35 a	28.60 ± 0.68 a
	P	<0.001	<0.001	<0.001
Riesling/3309C	Control	21.21 ± 0.89 b	22.13 ± 1.66 c	23.40 ± 1.19 b
	Product 2	27.04 ± 0.89 a	32.28 ± 1.66 ab	32.63 ± 1.19 a
	Product 3	21.99 ± 0.89 b	25.93 ± 1.66 bc	26.92 ± 1.19 b
	Product 4	27.65 ± 0.89 a	33.82 ± 1.66 a	33.44 ± 1.19 a
	P	0.001	0.003	0.001

[i] Treatment details are available in Table 1.
[ii] AMF structures.
[iii] Different letters within columns indicate significant differences among treatments within a rootstock according to the Tukey honestly significant difference test derived from the mixed-model analysis of variance at $P \leq 0.05$ (n = 4 for Pinot noir/3309C; n = 3 for Riesling/3309C and Riesling/SO4).

Mg ≈0.22% (2.2 g·kg^{-1}) and ≈0.11% (1.1 g·kg^{-1}), respectively, compared with control, and Ca ≈1.21% (12.1 g·kg^{-1}) and ≈0.67% (6.7 g·kg^{-1}), respectively, compared with control. However, no significant differences were found for macronutrient S (data not shown). Micronutrients (B, Zn, Fe, Mn, Cu, and Na) were also affected by inoculation when compared with control plants, except for Fe in plants inoculated with product 1 (Table 3). The C concentration and C/N ratio were reduced in inoculated plants when compared with control plants (Table 3), with greater significance observed for plants inoculated with products 4 and 2, respectively.

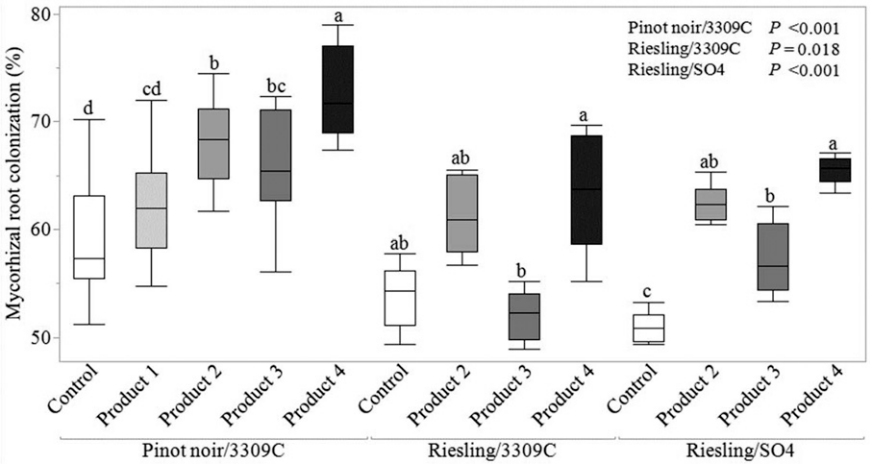

Fig. 2. Total percent of the root length colonized by arbuscular mycorrhizal fungi applied to 'Pinot noir' grapevine grafted onto rootstock 'Couderc 3309' (Pinot noir/3309C) and 'Riesling' grapevine grafted onto rootstock 'Couderc 3309' (Riesling/3309C) and rootstock Selection Oppenheim four (Riesling/SO4) inoculated or not with four bioinoculants on 1 Jun 2018. Fine roots for Pinot noir/3309C were collected 5 months after inoculation, whereas fines roots for Riesling/SO4 and Riesling/3309C were collected 17 months after inoculation. Detailed treatment information appears in Table 1. Boxplots show the third and first quartiles (box edges), median (middle line), and whiskers extending to the minimum and maximum data points. Boxplots with different letters indicate significance differences among treatments within a rootstock according to the Tukey honestly significant difference test (Pinot noir/3309C, n= 4; Riesling/3309C and Riesling/SO4, n=3) derived from the mixed-model analysis of variance at $P \leq 0.05$.

Regardless of the treatment, the petiole tissue N concentrations of Riesling/3309C and Riesling/SO4 were improved when compared with those of control plants. The increase in N was higher for plants inoculated with products 4 and 2 (Table 4) compared with product 3. The increase in the N, but not in the C, concentration in the leaf tissues resulted in significantly lower C/N ratios for inoculated plants when compared with control plants (Table 4), with a significant decrease observed in plants inoculated with products 4 and 2, respectively.

Effects of bioinoculants on root morphology. Fine root morphology was altered by inoculation, except for RL for Riesling/SO4 (Table 5). Plants inoculated with products 2 and 4 had a greater increase in

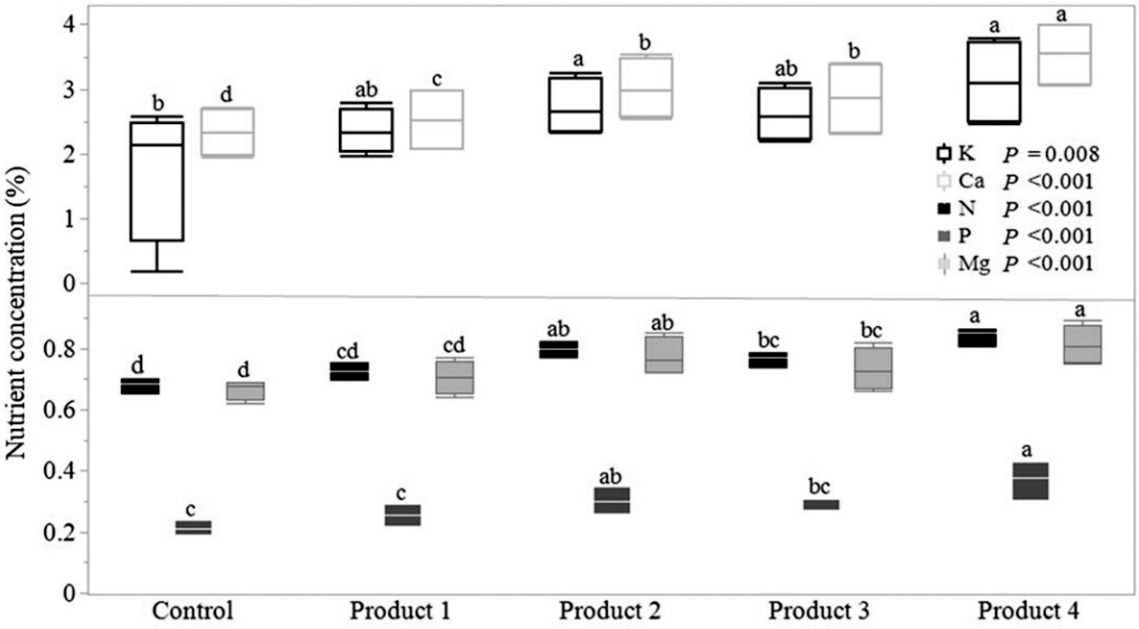

Fig. 3. Vine petiole potassium (K), calcium (Ca), nitrogen (N), phosphorus (P), and magnesium (Mg) nutrient concentrations of 'Pinot noir' grapevine grafted onto rootstock 'Couderc 3309' (Pinot noir/3309C) inoculated or not with four bioinoculants on 1 Jun 2018. Petiole tissues were collected 2 months after inoculation at veraison. Detailed treatment information is available in Table 1. Boxplots show the third and first quartiles (box edges), median (middle line), and whiskers extending to the minimum and maximum data points. Boxplots with different letters indicate significance differences among treatments within a nutrient according to the Tukey honestly significant difference test (n = 4) derived from the mixed-model analysis of variance at $P \leq 0.05$.

RD, ranging from 28% to 30%, respectively, for Riesling/SO4 and from 16% to 32%, respectively, for Riesling/3309C when compared with control. The average RL per core (n = 24) was significant only for Riesling/3309C, and it was increased by up to 33.88 and 24.45 cm for plants treated with products 4 and 2, respectively. The greatest RLD was observed in plants inoculated with products 2 and 4, ranging from 13.46 to 13.83 $cm \cdot cm^{-3}$, respectively, for Riesling/SO4 compared with control and from 19.57 to 27.11 $cm \cdot cm^{-3}$, respectively, for Riesling/3309C when compared with control. SRL was increased with products 2 and 4 by up to 17.63 and 30.09 $m \cdot g^{-1}$, respectively, for Riesling/SO4 compared with control and by up to 8.81 to 15.03 $m \cdot g^{-1}$, respectively, for Riesling/3309C when compared with control.

Effects of bioinoculants on berry composition. Berry soluble solids, titratable acidity, and pH for Riesling/SO4 and Riesling/3309C were not impacted by any of the treatments. However, regardless of treatment, bioinoculants improved YAN only for Riesling/3309C (data not shown). The increases in YAN were

Table 3. Vine petiole nutrient analysis of 'Pinot noir' grapevine grafted onto rootstock 'Couderc 3309' (Pinot noir/3309C) inoculated or not on 1 Jun 2018 with four commercial bioinoculants containing different arbuscular mycorrhizal fungi species. Petiole tissues were collected 2 months after inoculation at veraison.

	Pinot noir/3309C					
	Mean ± *SE*					
Petiole tissue nutrient[i]	Control[ii]	Product 1[ii]	Product 2[ii]	Product 3[ii]	Product 4[ii]	*P*
Total C (%)	33.23 ± 0.25 a[iii]	32.56 ± 0.25 ab	31.16 ± 0.25 c	31.85 ± 0.25 bc	30.08 ± 0.25 d	<0.001
C/N (ratio)	48.94 ± 1.03 a	45.00 ± 1.03 b	39.12 ± 1.03 cd	41.69 ± 1.03 bc	35.85 ± 1.03 d	<0.001
B (ppm)	28.75 ± 0.75 d	32.00 ± 0.75 c	37.00 ± 0.75 b	33.75 ± 0.75 c	40.75 ± 0.75 a	<0.001
Zn (ppm)	39.50 ± 1.96 d	41.75 ± 1.96 cd	49.25 ± 1.96 ab	46.00 ± 1.96 bc	52.50 ± 1.96 a	<0.001
Fe (ppm)	19.78 ± 1.03 c	20.27 ± 1.03 c	25.47 ± 1.03 ab	22.30 ± 1.03 c	25.93 ± 1.03 a	<0.001
Mn (ppm)	70.18 ± 8.63 b	86.05 ± 8.63 ab	102.70 ± 8.63 a	81.82 ± 8.63 ab	97.69 ± 8.63 a	0.016
Cu (ppm)	5.60 ± 0.38 c	6.26 ± 0.38 bc	8.13 ± 0.38 a	6.63 ± 0.38 abc	7.56 ± 0.38 ab	0.002
Na (ppm)	131.77 ± 9.48 c	142.41 ± 9.48 bc	180.48 ± 9.48 a	148.74 ± 9.48 abc	175.60 ± 9.48 ab	0.004

[i] C = carbon; C/N = carbon-to-nitrogen ratio; B = boron; Zn = zinc; Fe = iron; Mn = manganese; Cu = copper; Na = sodium. 1 ppm = 1 $\mu g \cdot g^{-1}$.
[ii] Treatments details are available in Table 1.
[iii] Different letters within columns indicate significant differences among treatments within a nutrient according to the Tukey honestly significant difference test derived from the mixed-model analysis. of variance at $P \leq 0.05$ (n = 4 for Pinot noir/3309C).

Table 4. Vine petiole carbon (C) and nitrogen (N) nutrient analysis and C-to-N ratio (C/N) of 'Riesling' grapevine grafted onto rootstock 'Couderc 3309' (Riesling/3309C) and rootstock Selection Oppenheim four (Riesling/SO4) inoculated or not on 1 Jun 2018 with three commercial bioinoculants containing different arbuscular mycorrhizal fungi species. Petiole tissues were collected 14 months after inoculation at veraison.

		Petiole tissue nutrient		
		Mean ± *SE*		
Scion/rootstock	Treatment[i]	Total N (%)	Total C (%)	C/N (ratio)
Riesling/SO4	Control	0.61 ± 0.03 b[ii]	40.38 ± 0.36	65.71 ± 2.46 a
	Product 2	0.75 ± 0.03 a	40.16 ± 0.36	53.35 ± 2.46 b
	Product 3	0.67 ± 0.03 ab	39.77 ± 0.36	61.08 ± 2.46 ab
	Product 4	0.76 ± 0.03 a	40.01 ± 0.36	55.10 ± 2.46 ab
	P	0.027	0.698	0.026
Riesling/3309C	Control	0.62 ± 0.01 c	40.61 ± 0.25	65.01 ± 1.31 a
	Product 2	0.71 ± 0.01 b	39.76 ± 0.25	55.29 ± 1.31 bc
	Product 3	0.69 ± 0.01 b	40.17 ± 0.25	58.05 ± 1.31 b
	Product 4	0.77 ± 0.01 a	40.24 ± 0.25	52.21 ± 1.31 c
	P	<0.001	0.123	<0.001

[i] Treatment details are available in Table 1.
[ii] Different letters within columns indicate significant differences among treatments within a rootstock according to the Tukey honestly significant difference test derived from the mixed-model analysis of variance at $P \leq 0.05$ (n = 3 for Riesling/3309C and Riesling/SO4).

22 and 32 mg·L^{-1} higher with products 4 and 2, respectively, than with control for Riesling/3309C.

Correlation analysis. Significant and positive correlations between nutrients and RLC for Pinot noir/3309C were found only for the N concentration of plants inoculated with product 4 and the P concentration of plants inoculated with products 4 and 2. Additionally, N, P, K, Mg, Fe, and Na were significantly and positively correlated with hyphae for plants inoculated with product 4, whereas significant and negative correlations between Mn and hyphae were observed for plants inoculated with product 4. Moreover, significant and positive correlations between P and K with hyphae were observed for plants inoculated with product 2 (Table 6).

Similarly, significant and positive correlations between N concentrations and hyphae were found for Riesling/SO4 and Riesling/3309C plants inoculated with products 4 and 2. Additionally, RD and RL exhibited significant and positive correlations with RLC for Riesling/SO4 inoculated with product 2 and for Riesling/3309C inoculated with products 4 and 2. However, the correlation was significant and negative between RLD and RLC for Riesling/SO4 inoculated with products 4 and 2, but it was positive between SRL and RLC for plants inoculated with product 4. Furthermore, YAN was significantly and positively correlated with RLC only for Riesling/3309C inoculated with products 4 and 2 (Table 7).

Discussion

During this study, regardless of the bioinoculant and cultivar/rootstock, the percentage of AMF structures was increased. The greatest RLC was obtained with products 4 and 2 (Fig. 2), suggesting that propagules (spores) of AMF species present in these bioinoculants were compatible with the host plants and the native AMF associated with grapevine roots. However, Riesling/3309C inoculated with product 3 revealed lower RLC than noninoculated plants; this product was specified as having a moderate to lower

Table 5. Root morphology parameters for 'Riesling' grapevine grafted onto rootstock 'Couderc 3309' (Riesling/3309C) and rootstock Selection Oppenheim four (Riesling/SO4) inoculated or not on 1 Jun 2018 with three commercial bioinoculants containing different arbuscular mycorrhizal fungi species. Fine roots were collected 17 months after inoculation.

		Mean ± *SE*			
Scion/rootstock	Treatment[i]	Root diam (mm)[ii]	Root length (cm)[ii]	Root length density (cm·cm^{-3} soil)[ii]	Specific root length (m·g^{-1} root)[ii]
Riesling/SO4	Control	0.46 ± 0.01 b[iii]	22.95 ± 7.03	30.88 ± 2.42 b	15.35 ± 2.84 c
	Product 2	0.59 ± 0.01 a	43.08 ± 7.03	44.34 ± 2.42 a	32.98 ± 2.84 ab
	Product 3	0.51 ± 0.01 b	34.07 ± 7.03	34.08 ± 2.42 ab	22.42 ± 2.84 b
	Product 4	0.60 ± 0.01 a	48.17 ± 7.03	44.71 ± 2.42 a	45.44 ± 2.84 a
	P	<0.001	0.135	0.007	<0.001
Riesling/3309C	Control	0.50 ± 0.01 b	17.12 ± 7.95 c	13.69 ± 7.15 b	11.91 ± 3.93 b
	Product 2	0.58 ± 0.01 ab	41.57 ± 7.95 ab	33.26 ± 7.15 ab	20.72 ± 3.93 ab
	Product 3	0.54 ± 0.01 b	31.73 ± 7.95 b	25.39 ± 7.15 ab	15.38 ± 3.93 ab
	Product 4	0.66 ± 0.01 a	51.00 ± 7.95 a	40.80 ± 7.15 a	26.94 ± 3.93 a
	P	0.007	<0.001	0.029	0.031

[i] Treatment details are available in Table 1.
[ii] 1 mm = 0.0394 inch; 1 cm = 0.3937 inch; 1 cm·cm^{-3} = 6.4516 inch/inch^3; 1 m·g^{-1} = 93.0102 ft/oz.
[iii] Different letters within columns indicate significant differences among treatments within a rootstock according to the Tukey honestly significant difference test derived from the mixed-model analysis of variance at $P \leq 0.05$ (n = 3 for Riesling/3309C and Riesling/SO4).

Table 6. Selected Pearson correlation coefficients among roots colonized by mycorrhizas and nitrogen, macronutrient, and micronutrient concentrations for each product for 'Pinot noir' grapevine grafted onto rootstock 'Couderc 3309' (Pinot noir/3309C) inoculated or not in Jun 2018 with four bioinoculants. Petiole tissues were collected 2 months after inoculation at veraison.

	Pinot noir/3309C				
Correlations[i]	Control[ii]	Product 1[ii]	Product 2[ii]	Product 3[ii]	Product 4[ii]
N vs H	−0.27	0.25	0.89	0.60	0.97*[iii]
P vs H	−0.17	0.21	0.95*	0.40	0.98**
K vs H	0.53	−0.67	0.95*	0.78	0.99**
Mg vs H	0.17	0.61	0.78	0.08	0.97**
Ca vs H	−0.23	0.32	0.72	−0.60	0.84
Zn vs H	0.55	0.42	0.85	0.37	0.90
Fe vs H	−0.36	−0.44	0.84	0.83	0.98**
Mn vs H	0.73	−0.13	0.83	0.18	−0.97**
Na vs H	0.17	0.73	0.87	0.16	0.94*
N vs RLC	0.29	0.42	0.89	0.73	0.98**
P vs RLC	0.25	0.33	0.94*	0.51	0.95*
K vs RLC	0.07	0.22	0.88	0.49	0.86
Mg vs RLC	0.04	0.45	0.72	−0.61	0.91

[i] H = hyphae (%); RLC = root length colonization (%); N = total nitrogen content (%); P = total phosphorus content (%); K = total potassium content (%); Mg = total magnesium content (%); Ca = total calcium content (%); Fe = total iron content (ppm); Mn = total manganese content (ppm); Na = total sodium content (ppm).
[ii] Treatment details are available in Table 1.
[iii] Significant correlations for Pinot noir/3309C (n = 4): $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

density of propagules, several AMF, ectomycorrhizae, and bacterial species, and specific amendments such us kelp and humic acids to increase plant growth. The lower percentage of RLC likely reflects the small amount of product applied according to the manufacturer's specifications, the variation in the plant response to a particular AMF species from the bioinoculant (Salomon et al. 2022), the presence of the native AMF community (Köhl et al. 2016), colonization strategies among species of AMF (Klironomos and Hart 2002), soil properties, and management practices that could influence the dynamic and intensity of the plant response to bioinoculants (Rosa et al. 2020).

To be considered a sustainable alternative to synthetic fertilizer, a bioinoculant must provide a measurable benefit to the host plant (e.g., increased petiole nutrient concentration). Our results revealed that inoculation with commercial bioinoculants resulted in not only greater root colonization by AMF but also increased macronutrients and micronutrients in petioles for Pinot noir/3309C (Fig. 3, Table 3) and N concentrations in petioles for Riesling/SO4 and Riesling/3309C (Table 4). The increased P, N, K, Ca, and Fe in grapevine petioles compared with control plants are in line with the results of previous studies of grapevine (Khalil 2013; Schreiner 2007). The predominance of positive and strong correlations of macronutrients and micronutrients with RLC and hyphae for Pinot noir/3309C (Table 6) and of N with RLC and hyphae for Riesling/3309C and Riesling/SO4 (Table 7) plants treated with products 4 and 2 support the hypothesis that the application of AMF enhances plant tissues

Table 7. Selected Pearson correlation coefficients among fine roots colonized by mycorrhizae, nitrogen and carbon concentrations, and root morphology and berry composition parameters for each product for 'Riesling' grapevine grafted onto rootstock 'Couderc 3309' (Riesling/3309C) and rootstock Selection Oppenheim four (Riesling/SO4) inoculated on 1 Jun 2018 with three bioinoculants. Fine roots and petiole tissues were collected 17 and 14 (at veraison) months, respectively, after inoculation.

	Correlations[i]						
Riesling/SO4	N vs RLC	RD vs RLC	RL vs RLC	RLD vs RLC	SRL vs RLC	YAN vs RLC	N vs H
Control[ii]	−0.22	−0.72	0.33	0.63	0.63	0.03	0.75
Product 2[ii]	0.78	0.99***[iii]	0.83**	−0.82**	0.79	0.72	0.81**
Product 3[ii]	0.67	0.46	0.64	−0.52	−0.47	−0.51	0.75
Product 4[ii]	0.68	0.76	0.79	−0.82**	0.87**	0.78	0.82**
Riesling/3309C	N vs RLC	RD vs RLC	RL vs RLC	RLD vs RLC	SRL vs RLC	YAN vs RLC	N vs H
Control	−0.27	0.61	−0.84**	−0.89**	−0.25	−0.37	0.10
Product 2	0.72	0.88**	0.95**	0.55	0.75	0.85**	0.88*
Product 3	0.71	0.71	−0.67	−0.59	−0.66	0.66	0.67
Product 4	0.77	0.82*	0.85*	0.57	0.79	0.95**	0.92**

[i] H = hyphae (%); RLC = root length colonization (%); N = total nitrogen content (%); C = total carbon content (%); RL = root length (cm); RD = root diameter (mm); RLD = root length density ($cm \cdot cm^3$); SRL = specific root length ($m \cdot g^{-1}$); YAN = yeast assimilable nitrogen ($mg \cdot L^{-1}$).
[ii] Treatment details are available in Table 1.
[iii] Significant correlations for Riesling/SO4 and Riesling/3309C (n = 3): $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

nutrient concentrations through the AMF hyphae network (Smith and Smith 2012), thus allowing exploration beyond the nutrient depletion zone that develops around roots. Furthermore, different AMF species may have distinct roles in plant health and growth under different environmental conditions than single AMF species (Mensah et al. 2015).

Interestingly, the most effective commercial inoculants in this study, products 4 and 2, contained nine AMF species, and product 1 contained only four AMF species (Table 1). Although product 3 included nine AMF species, its low performance could be attributable to unviable and/or an insufficient amount of AMF propagules in the bioinoculants. This product may also contain incompatible AMF species that are not adapted to the soil and climate conditions of this research location (Salomon et al. 2022). Inhibitory effects may also progressively occur among the AMF species, the growth promoters, and the native AMF species present in the soil (Owen et al. 2015). However, the multiple benefits obtained with the different bioinoculants could be attributed to several factors, such as the source of AMF isolates, the diversity and efficacy of AMF species, the AMF propagule density within the inoculant at the time of production, and/or the substrate carrier of the inoculant (Salomon et al. 2022). Some of the bioinoculants (products 1, 3, and 4) contained abiotic amendments such as worm castings, humic acids, kelp, and other microorganisms, including ectomycorrhizae and bacteria (Table 1), that may optimize the efficiency of the product. For example, product 4 contained slow-release organic fertilizer (3N–0.4P–0.8K), five beneficial bacteria species, and ectomycorrhizae fungi that help to improve the plant nutrient status and growth. The positive benefits observed with product 4 may not be directly from AMF species, as was the case for product 2 (Table 1), but rather from a positive interaction between the microorganisms and abiotic amendments.

Root colonization by AMF also affects root characteristics of host plants (Chen et al. 2021); however, there is limited information about how AMF affects diverse traits of grapevine root morphology (Aguín et al. 2004). These changes may alter the morphology of the root system in a structural, quantitative, spatial, and temporal manner (Kapoor et al. 2008), but impacts seem to vary according to specific plant–fungal combinations and environment (Holland et al. 2018). In this study, the greatest increase in root diameter for Riesling/3309C, higher RLD and SRL for Riesling/3309C and Riesling/SO4, and the higher RL only for Riesling/3309C were observed with products 4, 2, and 3, respectively (Table 5). The prevalence of significant positive correlations between RD, RL, SRL, specifically with products 4 and 2 (Table 7), corroborate these effects and reveal that AMF colonization alone (for product 2, which contained only an inorganic carrier of clay) affected grapevine plasticity by inducing morphological changes in the root system. Our results are consistent with those of previous studies that focused on AMF effects on grapevines and other crops (Aguín et al. 2004; Chen et al. 2021), indicating that roots of AMF-inoculated plants can improve their ability to absorb and use nutrients and water in the soil under different environmental stresses (Comas et al. 2013; McCormack and Iversen 2019).

Mycorrhizal inoculation did not impact most berry traits determined during this study (data not shown), as in a previous study of 'Tempranillo' (*V. vinifera*) (Torres et al. 2019). The exceptions were products 4 and 2, which increased berry YAN for Riesling/3309C. The improvement of the N concentration in petioles observed for inoculated plants (Table 4) may have contributed to the increased YAN; furthermore, the positive and significant correlation found between RLC and YAN support this premise, particularly for Riesling/3309C plants inoculated with products 4 and 2 (Table 7). An adequate YAN concentration is necessary for successful wine fermentation; however, wine grapes in the northeastern United States tend to have a low YAN (Karl et al. 2016). Even though an improvement in the YAN concentration was observed during this study, grapes from all treatment groups and the control group had lower YAN concentrations than those recommended for healthy fermentation (Boulton et al. 2013)

During this study, statistically significant small differences were observed for several parameters evaluated; however, we cannot suggest whether they were biologically relevant because the biological effects of 1 year of treatment were not visually observed. To confirm whether mature vines in this study were biologically affected, more data, such as those of different phenological stages of vine growth, yield production, nutrient concentrations in leaves and roots, starch concentrations of different vine tissues, root morphology, and berry primary and secondary analyses, are necessary.

Conclusions

This study evaluated the ability of commercial bioinoculants with diverse AMF species—and some with additional amendments—to increase AMF colonization in grapevine roots and improve plant mineral nutrition and berry composition and alter root traits of field-grown grapevines. The results of this study indicate that bioinoculants can alter grapevine root morphology, resulting in positive impacts on nutrient accumulation. These results could be altered by soil characteristics, soil microbiomes, indigenous AMF, and interactions between scion cultivars and rootstocks. These factors should be further investigated. Future research should focus on identifying key mycorrhizae from bioinoculants that colonize and affect vine growth under varying conditions. Similarly, more research is needed to understand the role and interactive effects of abiotic amendments and AMF on rootstocks in a changing environment.

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