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Performance and Establishment of a Commercial Mycorrhizal Inoculant in Viticulture

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Abstract: (1) Background: Arbuscular mycorrhizal (AM) fungi are symbiotic organisms that help plants acquire nutrients from the soil in exchange for photosynthetic carbon. Commercial AM fungal inoculants are widely available and are used extensively in agriculture including wine grape production. However, positive growth responses from inoculants are more consistent in the greenhouse compared to the field. (2) Methods: We grew three grapevine rootstocks with and without an AM fungal inoculant in the greenhouse for one year, then they were transplanted to the field for two years. To quantify the establishment of the inoculant, we analyzed root samples with a digital PCR assay. (3) Results: We show that AM fungal inoculation increased biomass production only in the greenhouse. After two growing seasons in the field, the commercial inoculant colonized roots but did not increase biomass production compared to uninoculated rootstocks. (4) Conclusions: This study highlights that AM fungal inoculants do not always promote growth of grapevines in the field. Future research should focus on inoculant strains designed for viticulture applications and take rootstock into consideration to maximize their efficacy.

Keywords: arbuscular mycorrhizal fungi; inoculation; rootstock; grapevine

1. Introduction

Arbuscular mycorrhizal (AM) fungi are obligate root symbionts that associate with most plants, including grapevines. They provide many benefits to their host, such as nutrient uptake and stress tolerance, in exchange for carbon [1,2]. Over the years, viticulture research has shown that grapevines are highly responsive to AM fungi [3–7] which elucidated the potential of AM fungal inoculation as a management strategy [8].

While there is evidence that inoculation of AM fungi in the greenhouse results in positive plant growth responses [3,9–14], the benefits of AM fungal inoculants in field studies are not as consistent [4,15,16]. Compared to resident AM fungi, commercial inoculants may be more successful in the greenhouse if they sporulate and grow quickly in disturbed soil [17]. AM fungal inoculation of grapevines in the field does not always result in increased shoot production [13], overall biomass [18] or yield [19]. Although inoculation may improve growth during the first few years, uninoculated plants may eventually become colonized by resident AM fungi [20] and perform as good or better than inoculated plants [21].

While commercial inoculants are beneficial in broadacre farming [22,23], there is reason to believe that perennial systems may benefit less because they have relatively intact soil ecosystems which experience low levels of disturbance after initial soil preparation [24]. Thus, they may already possess effective resident mycorrhizal populations making inoculation redundant, or even unsuccessful if priority effects are important for inoculant establishment [25].

Inoculation with commercial AM fungi may not be effective due to variation in host response to a particular fungus [26,27]. In addition, soil type and mineral profiles can heavily influence host–symbiont interactions [28]. Finally, interactions between host, symbiont, and soil source can determine the direction and intensity of the plant response to the mycorrhizal inoculant [29]. These factors make it difficult to determine if inoculation will be successful in an agroecosystem.

Despite variation in host–symbiont responses, some growers augment resident populations with commercial AM fungal inoculants to maximize the benefits of AM fungi [30]. In viticulture, grapevine rootstocks are grown in nurseries for the first season, where AM fungal inoculants are often applied aiming to increase the survival and growth rates [31]. Under controlled conditions, rootstock genotypes show differences in root colonization and shoot growth in response to AM inoculation [32]. Similarly, the composition of AM fungal communities in a vineyard could be driven primarily by host identity [33]. Variation in AM fungal response among rootstocks may affect their response to commercial inoculants and thus, the efficacy of commercial inoculants in vineyards.

This study aimed to evaluate the host response of three grapevine rootstocks to a commercial AM fungal inoculant. We hypothesized that inoculation would improve growth of grapevine rootstocks in the greenhouse but not in the field after transplantation.

2. Materials and Methods

In order to examine the effect of AM fungal inoculation on rootstock identity we conducted a three-year experiment. The first year of the experiment (nursery) took place in a greenhouse and then transitioned to the field for the next two years. The greenhouse and the experimental field were located at the Summerland Research and Development Centre (SuRDC) in Summerland, BC Canada, (49°33′49.2′′ N–119°38′19.0′′ W) and the experiment took place from September 2015 to March 2018. Additional site details including soil physiochemical properties are listed in Table A1.

The experiment consisted of a factorial combination of two AM fungi treatments (with and without AM fungal inoculation) and three rootstocks. Each treatment had eight replications (n = 48) in a complete randomized design.

2.1. Rootstock Treatments

We selected Riparia Gloire (*Vitis riparia*), Schwarzmann (*Vitis riparia* × Vitis rupestris), and Salt Creek (Vitis champinii), three rootstocks commonly used in North America [34,35]. To generate the rootstocks, dormant cuttings containing two buds each were placed in auxin solution (1500 mg L-1 of indol-3-butyric acid) for 30 s then transferred to moist perlite filled flats in a growth chamber at 28 °C until callus formation. We selected 16 cuttings from each rootstock cultivar based on callus development and rooted them in moist Turface-filled flats until appearance of roots. Finally, rooted cuttings were transplanted into 7.6-L pots containing expanded clay (Turface; Profile products LCC) and placed in a greenhouse at SuRDC. Plants were supplied with approximately 60 mL (3 × 20 mL) of water every day, using an 8-emitter circular line within each pot to provide water uniformly onto the medium surface.

2.2. AM Fungal Inoculant

We used the commercial inoculant MYKE® PRO GR (Premier Tech, Inc) that contained Rhizoglomus irregulare (DAOM 197198) Schenck & Smith (synonym Rhizophagus irregularis, Glomus intraradices) (Sieverding et al., 2015). Approximately 15 g (~2130 spores) were added to each pot, following manufacturer's instructions. We used vermiculite as a spore carrier and expanded clay as a growth medium (Turface, Profile products LCC).

During the nursery period, plants were hand watered every day with approximately 60 mL (3 × 20 mL). Plants were manually fertilized every two weeks with 60 mL of (1) solution of 0.42 g L-1 of 12-2-14 (50 ppm N) during the first two months, and (2) 1.5 g L-1 of 20-20-20 (300 ppm N) for the next two months. During foilar establishment, plants were sprayed once with sulphur (S) (Kumulus DF, BASF) to prevent mildew.

Rooted cuttings grew for four months in the greenhouse. After this growth period, each vine was trimmed with a set of pruners and the material was weighed. Leaf number was also recorded before transplantation to the field. The field trial took place over two growing seasons (2016–2017) and consisted of eight blocks spanned across one row. A block was defined as the space between two trellis posts about five metres apart. Treatments were randomly placed within each block and inoculated blocks were separated from uninoculated control blocks by a guard plant.

In 2018 after the two-year growth period, vines were removed from the soil and 10 samples of roots (~5 g each) were randomly collected from each plant for DNA extraction. Roots and shoots were separated for biomass quantification.

2.3. Biomass Production

At the end of each growing season, the shoots were collected and dried at 65 °C to constant mass and then the dry matter was determined using a precision scale. In the last growing season, when the plants were pulled out from the soil, the root dry matter was determined by following the same protocol used for shoots.

2.4. Quantification of Inoculant Establishment Via Digital PCR Assay

2.4.1. DNA Extraction

At the time of harvest each season, ten root samples of 0.25 g were randomly collected from each plant root system for the following rootstocks: Riparia Gloire (*Vitis riparia*), Salt Creek (*Vitis champinii*), and Schwarzmann (*Vitis riparia* × *Vitis rupestris*). Root samples were stored at −20 °C and surface sterilized with 5% sodium hypochlorite before DNA extraction. DNA extractions were performed using the FastDNA SPIN kit for Soil (MP Biomedicals-USA).

2.4.2. Digital PCR Assay

We used droplet digital PCR (ddPCR) to determine the establishment of the commercial mycorrhizal inoculant as well as quantify its presence in the rootstocks. Using a ddPCR probe assay, we tested rootstocks for the presence of *R. irregulare* DAOM 197198 with the Bio-Rad QX100 Droplet Digital PCR system (Bio-Rad Laboratories, Inc., Hercules, California). Droplets were generated according to manufacturer's instructions. We amplified a region of the cox3-rnl intergenic mtDNA using a primer pair and probe specific to *R. irregulare* DAOM 197198, forward 5'-AGCAAATCTAAGTTCCTCAGAG-3', reverse 5'-ACTTCTATGGCTTTGTACAGG -3', and probe 5'-FAM/CCCACCAGG/ZEN/GCAGATTAATCTTCCTT/3IABKFQ-3' [36].

Twenty µL of total reaction volume was used which contained 10 µL of ddPCR Supermix for probes (Bio-Rad Inc.), 1 µL of primer-probe mix (diluted 20×, 500 nM primers and 250 nM probe in final reaction) (Integrated DNA Technologies), 2 µL of root DNA and 7 µL of DNase free water. After droplet generation, the final reaction volume per sample was 40 µL. DNA was amplified using the Bio-Rad C1000 Thermal Cycler (Bio-Rad) using the following settings: initial heating at 95 °C for 10 min, 40 cycles with denaturation at 94 °C for 30 s and annealing at 59 °C for 30 s, and a final step at 98 °C for 10 min. A ramp rate of 2 °C/s was used for every step. Each plate contained three non-template controls (NTC) to detect contamination and three environmental negative controls (ENC) to set an accurate fluorescence threshold. ENCs consisted of a control with environmental DNA but without DNA from the inoculant used in this study. Droplets were analyzed with the BioRad QX100 Droplet Reader and amplification data was collected using Quantalife software ver. 1.7.4. (Bio-Rad).

In order to obtain abundance data per gram of root tissue, the raw Quantalife software data (copies per µL of reaction) were back-calculated with the following formula [36]:

$$Cpgr = (Quantalife\ value * (RV/SQ) * EQ)/QR$$

where *Cpgr* represents target copies per gram of root, *Quantalife value* is copies per µL of reaction, *RV* is volume of the reaction, *SQ* is sample DNA quantity used in the reaction, *EQ* is quantity of DNA extraction, and *QR* is quantity of root tissue used for the extraction.

2.5. Statistical Analyses

2.5.1. Establishment

In order to assess establishment of the AM fungi, we used a linear mixed model *lmer* [37] to compare between control and inoculated rootstocks, with "block" as a random factor and "inoculation" as a fixed factor. Data was assessed for normality by plotting a histogram of the model residuals using the *hist* function in *graphics* (3.6.2). Log transformations were applied to all analyses to satisfy model assumptions. Pairwise comparisons were made using the *emmeans* package (1.4.3.01). We asked whether the abundance of the AM fungal inoculant was due to each rootstock.

2.5.2. Software

All statistical analyses were performed using the RStudio version 1.2.5033 (R Core Team 2019).

3. Results

3.1. Growth Response

The inoculation of AM fungi in the greenhouse-grown cultivation increased the shoot dry matter production in two of the three rootstocks tested, Riparia Gl. ($p = 0.043$) and Salt Creek ($p = 0.012$) (Figure 1). AM fungal inoculation did not increase shoot dry mass production in Schwarzmann.

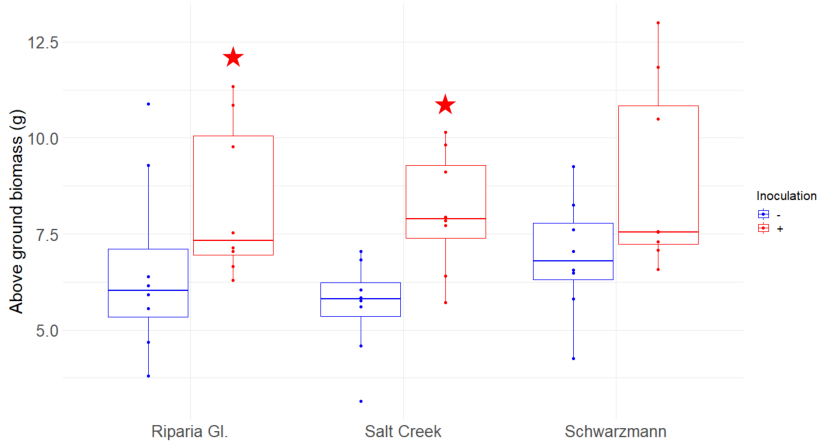

Figure 1. Above ground biomass (grams) of greenhouse-grown rootstocks during the first year. Blue plots represent uninoculated while red plots represent inoculated rootstocks. Red stars above inoculated plots depict significant positive AM fungal response compared to uninoculated control. Statistical analysis was performed on the log scale.

The number of leaves on the vines grown in the greenhouse differed among the rootstocks (Figure A1). Among inoculated rootstocks, the highest number of leaves was found on Salt Creek grapevines ($p < 0.001$), while the lowest number of leaves was found on Riparia Gl. vines.

After rootstocks grew in the field for two years, we observed that the inoculation of AM fungi did not increase shoot dry matter production, instead it decreased the dry matter production for Schwarzmann ($p = 0.06$) (Figure 2). The rootstocks, Riparia Gl. and Salt creek, neither increased or decreased shoot dry mass production by AM fungal inoculation.

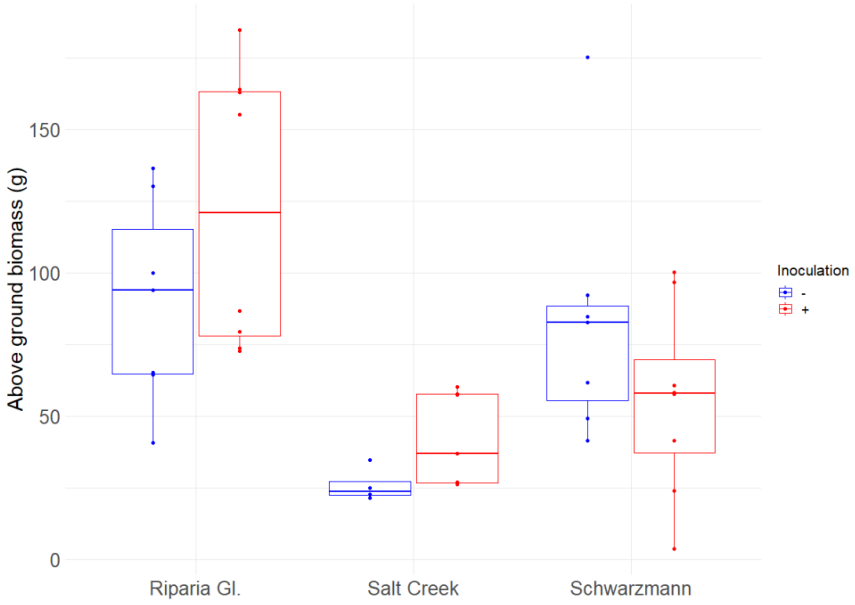

Figure 2. Above ground biomass (grams) of grapevine rootstocks after two years of field cultivation. Blue plots represent uninoculated (control) while red plots represent inoculated rootstocks. Statistical analysis was performed on the log scale.

Contrary to the growth responses in the greenhouse, the shoot dry matter obtained after one year of field cultivation was highest in Riparia Gl. vines ($p < 0.001$). Salt Creek produced the lowest amount of shoot biomass although not statistically different from Schwarzmann (Figure A2).

We also observed that the root dry matter production from rootstocks grown in the field for two years, increased by the inoculation of AM fungi for Salt Creek ($p < 0.001$) (Figure 3). Riparia Gl. and Schwarzmann neither increased nor decreased in shoot dry mass production by AM fungal inoculation.

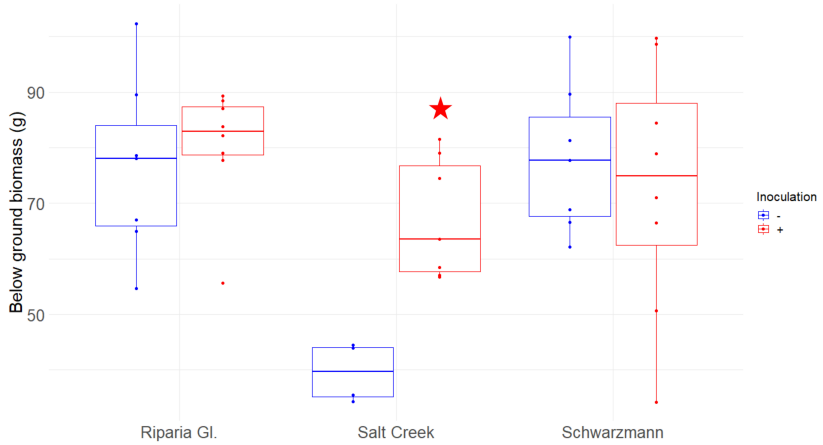

Figure 3. Below ground biomass (grams) of grapevine rootstocks after two years of field cultivation. Blue plots represent uninoculated while red plots represent inoculated rootstocks. Red stars above inoculated plots depict significant positive AM fungal response compared to uninoculated control. Statistical analysis was performed on the log scale.

Similar to the first growing season, the shoot dry matter yield obtained after two years of field cultivation was highest in Riparia Gl. vines ($p < 0.001$). Salt Creek produced the lowest amount of shoot biomass although not statistically different from Schwarzmann (Figure A3). The root dry matter of the vines obtained at two years of field cultivation showed no difference between the rootstocks ($p = 0.278$) (Figure A4).

3.2. Establishment of Commercial Inoculant

Overall, we found that the inoculant established and persisted in the field over all growing seasons (Figure 4). All of the three rootstocks, Riparia Gl., Salt Creek, and Schwarzmann, tested positive for *Rhizoglomus irregulare* (DAOM 197198) which could not be detected in high numbers in control vines.

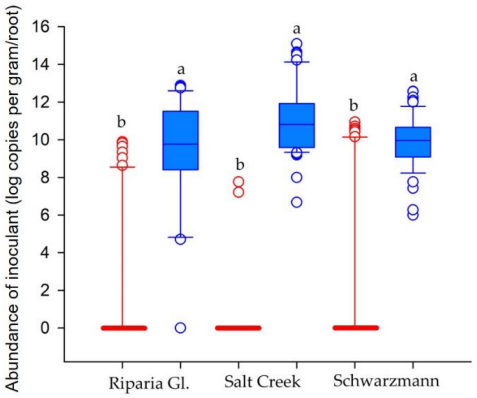

Figure 4. DNA copies per gram of roots (log(x)) of Rhizoglomus irregulare (DAOM 197198), found in the roots of grapevine rootstocks in the second year of the field study. Red boxes represent control vines and, the blue boxes represent AM fungi inoculated vines. Letters above boxes indicate significant differences within the rootstock (control and inoculated) by *t*-test ($p < 0.001$).

4. Discussion

In this study, we observed limited success of AM fungal inoculation on the performance of grapevine rootstocks. Only two rootstock cultivars in the greenhouse showed increased dry biomass, Riparia Gl. and Salt Creek, but this success was not maintained when the vines were transplanted to the field.

Several studies have found positive results on plant performance using AM fungi-containing inoculants [38–41]. However, in other cases inoculation was not effective [42–44]. Greenhouse studies that evaluate AM inoculants on plant performance do not always capture the complexities of field conditions [45]. This makes it clear that positive growth responses observed from inoculation in the greenhouses are not a great predictor of success under field conditions.

In many cases greenhouse studies are performed using autoclaved soil which means there is no comparison between the AM inoculant and resident AM fungi. [46,47]. In addition, the range of environmental conditions that the isolate has contact with in the field is much larger than under greenhouse conditions, which are tightly controlled and generally favourable to AM fungi. These conditions may compromise the inoculant performance in the field [48].

Failure to adapt to local field conditions can also impair the AM fungal inoculant. Agricultural fields typically harbour a lower rate of mycorrhizal fungi compared to natural environments [49,50]. This suggests that the unique agricultural conditions, including high disturbance via tillage and high fertilization, may select AM fungi strains that are better adapted to the local agricultural conditions than the introduced isolates contained in the inoculant. In their majority, mycorrhizal inoculants are mass produced under in-vitro conditions that represent a highly artificial environment which is extremely different from any natural environment [51] It is possible that studies conducted in greenhouses may overestimate the success of mycorrhizal fungal inoculant performance, since field studies often result

in poorer grapevine performance compared to the greenhouse. As a result, field studies often result in poorer grapevine performance compared to the greenhouse [52].

After two years in the field, the lack of performance increase in inoculated vines may be due to the fact that the foreign isolate was not adapted to the local environmental conditions and/or did not engage in an efficient mutualism with the rootstock. Here, we showed that the inoculant successfully colonised roots in the field but did not increase vine performance. Mycorrhizal associations act on a continuum from mutualism to parasitism [38,53] and previous studies show that suboptimal environmental conditions (light, minerals) can impact the interaction [54]. Likewise, AM fungi, although a generalist species, will benefit some plants more than others [55]. It should be emphasized that AM fungi-containing inoculants are intended for general use and are not designed for a specific host species [56] although some companies do advertise use with certain crops.

To increase the efficiency of inoculants, some authors suggest using a product that contains multiple AM fungi isolates, thereby increasing the chances that one or more isolates may better adapt. Another possibility is to select locally occurring isolates that are more likely to perform well in the local environment [48]. Future studies that test specific strains for specific applications are needed.

The fact that the commercial inoculant persisted in rootstocks during the two field years poses a risk of invasion [57,58] and alteration of resident AM fungal communities in roots [59]. If the commercial inoculant can expand its hyphal network from the rootstocks and/or sporulate rapidly, it may colonise vineyard weeds, cover crops, and other nearby plants [60,61]. This may change the priority effects of AM fungi [62], meaning that the commercial inoculant could rapidly colonize and occupy a large proportion of diverse neighboring roots which inevitably affects the colonization, growth ability, and survival of other fungal and bacterial species that naturally occur around roots and hyphae [63–65]. Such changes in fungal and bacterial communities could shift ecosystem functioning and further research is required to determine if this impacts crop performance.

5. Conclusions

Inoculation of the grapevines with *Rhizoglomus irregulare* (DAOM 197198) subtly favoured the performance of Riparia Gl. and Salt Creek grapevine rootstocks in the greenhouse. After transplantation to the field, inoculation with AM fungi did not provide any benefit to grapevine growth and decreased the performance of Schwarzmann. The inoculated fungal strain successfully established and maintained the symbiosis with grapevines detected after three years of inoculation, but without benefits. Future research should focus on the development of different strains for applications in viticulture and other perennial agroecosystems as well as elucidate the risk of invasion and potential microbial shifts if a commercial inoculant is to persist in soil.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Site information and soil physiochemical properties during the 2016 growing season.

Site Information	
Soil texture	Loamy sand
pH	6.68
Organic matter (%)	2.27
Nitrogen (Kjeldahl)	0.03
Phosphorous (Mehlich 3)	57.86
Irrigation type	Drip

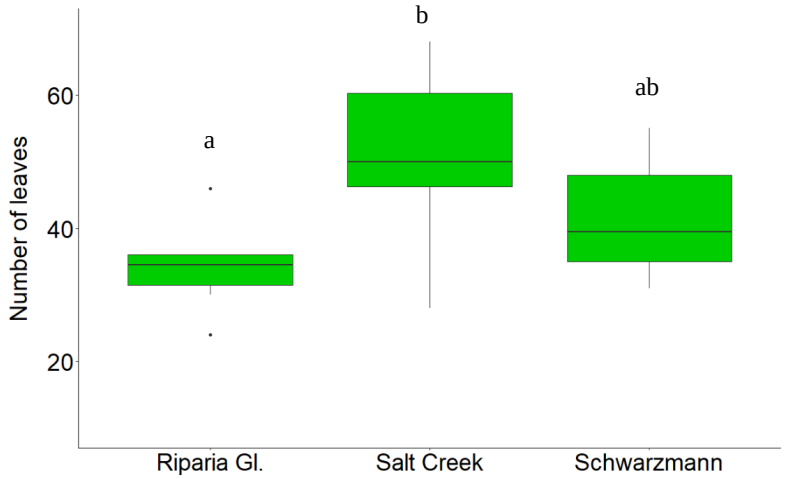

Figure A1. Total number of leaves on rootstocks inoculated with AM fungi and grown in the greenhouse. Letters above treatments represent significant differences at $p < 0.05$.

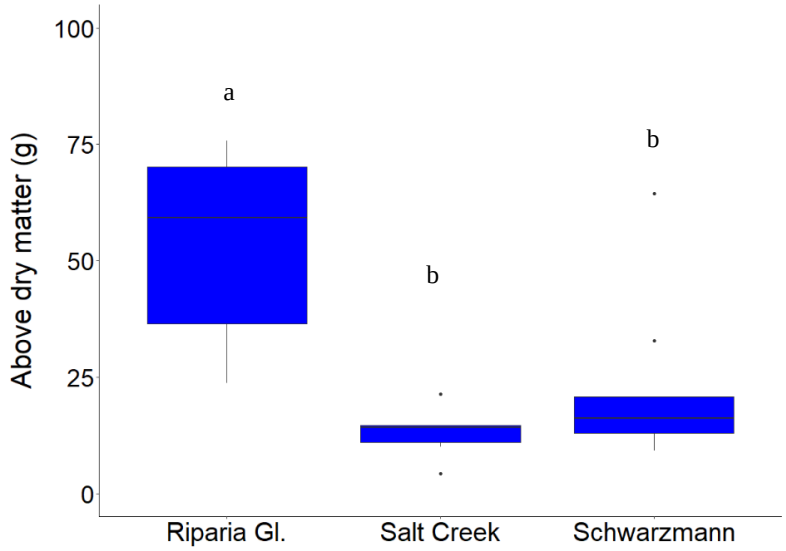

Figure A2. Shoot dry matter (g) in grapevine rootstocks inoculated with AM fungi at one year of field cultivation. Letters above treatments represent significant differences at $p < 0.05$.

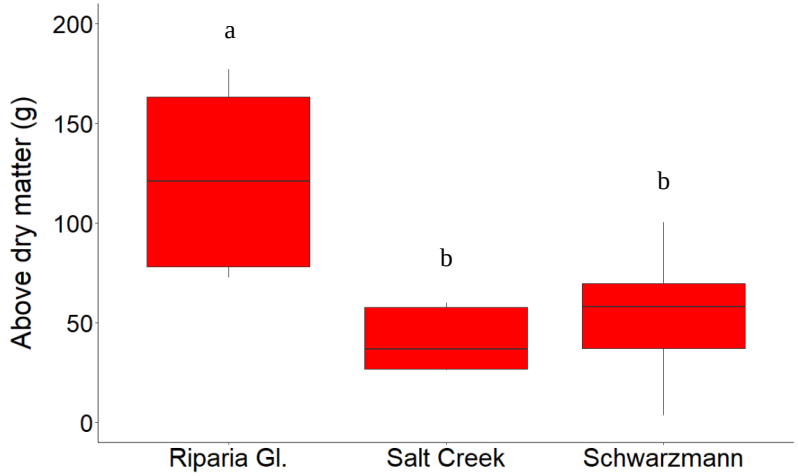

Figure A3. Shoot dry matter (g) in grapevine rootstocks inoculated with AM fungi at two years of field cultivation. Letters above boxes indicate significant differences at $p < 0.05$.

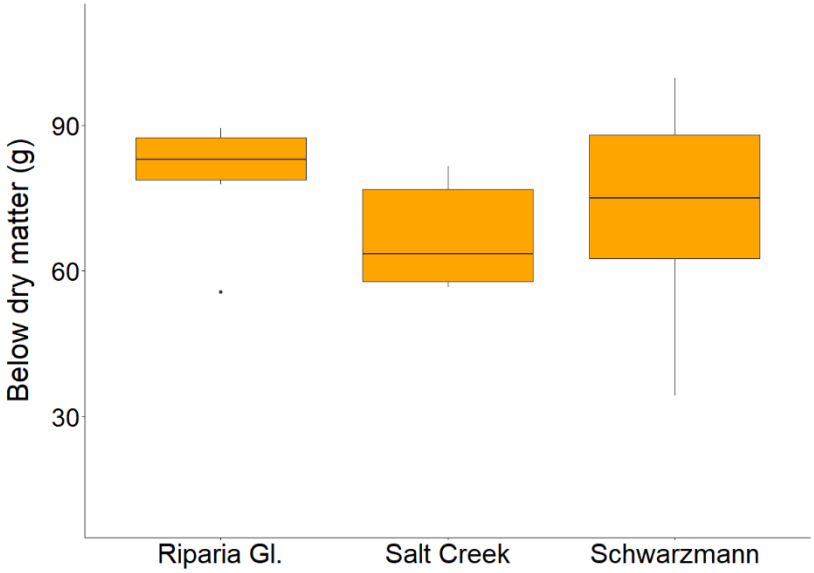

Figure A4. Root dry matter (g) in grapevine rootstocks inoculated with AM fungi after two years of field cultivation.

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