Comparative response of six grapevine rootstocks to inoculation with arbuscular mycorrhizal fungi based on root traits

by

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Thesis Committee

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Comparative response of six grapevine rootstocks to inoculation with arbuscular mycorrhizal fungi based on root traits

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Abstract

Arbuscular mycorrhizal fungi are soil fungi that form a symbiotic association with plant roots. The symbiosis is largely nutritional. The fungi depend on the plants for carbon resources, and the plants benefit from increased access to soil nutrients. The magnitude of plant benefit, however, can vary significantly. Understanding the factors that influence plant growth response is important, especially in agroecosystems where high yield is desirable. It has been suggested that root architecture is a key factor that determines plant responsiveness to arbuscular mycorrhizal fungi, and some studies have been conducted and support this notion. However, a major limitation in such studies is the lack of control for phylogenetic constraints among tested plants, making it difficult to control for confounding variables that are not associated with root architecture. In this dissertation, I explored the variation in plant responsiveness among closely related species (all grapevines) and investigated the potential relationship between root architecture and responsiveness to arbuscular mycorrhizal fungi. I found that root colonization by arbuscular mycorrhizal fungi can improve grapevine growth, although the degree of growth responses differed among grapevine cultivars. The magnitude of the benefit (plant growth response to a fungi) can be partially explained by the pre-colonization root architecture, and in particular with branching intensity, which is largely associated with plant nutrient foraging ability. However, root architecture alone is a poor predictor of plant response to arbuscular mycorrhizal fungi and more factors need to be considered to better understand plant nutrient foraging ability. In addition, mycorrhizas can influence the expression of root traits, such as root branching intensity, average root diameter and the root to shoot ratio.

Preface

I was responsible for designing the study, but I received help from Taylor Holland, Dr. John Klironomos and Dr. Pat Bowen. I set up and carried out the greenhouse experiment, but I received valuable advice from Carl Bogdanoff and Rob Brownlee. I conducted the root scanning procedure, with some help from Jared Hrycan.

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Abbreviations and Glossary:

AM – arbuscular mycorrhizal

AMF – arbuscular mycorrhizal fungi

PGR – plant growth response to arbuscular mycorrhizal fungi

SRL – specific root length

Schman – Schwarzmann (grapevine rootstock)

R.G – Riparia Gloire (grapevine rootstock)

Rms – Ramsey (grapevine rootstock)

5C – 5C Teleki (grapevine rootstock)

101-14 – 101-14 Millardet (grapevine rootstock)

3309 – 3309 Coudrec (grapevine rootstock)

Cont. – control

g – grams

V. – *Vitis*

P – Phosphorous

N – Nitrogen

no. – number

Acknowledgements

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To my beloved Grandmother

1 Introduction

1.1 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal (AM) fungi (Greek: μύκητας, myco- ¨fungi¨ and ρίζα, -rrhiza "root") are members of the phylum Glomeromycota (Schüβler et al. 2001). They are obligate biotrophs associated with the majority of terrestrial plants (Gerdemann 1968) with a role in plants' trophic relations (Smith and Read 2010). They colonize roots forming an extensive mycelium which is expanding outside the colonized roots (extraradical mycelium). AM extraradical hyphae are capable on acquiring nutrients that are usually not accessible by roots and finally transfer them to host plant in exchange of photosynthate (hexoses) (Koide 1991; Hohenheim 1994).

AM fungal spores germinate after exposure to specific root exudates, largely strigolactones or other signaling compounds (Akiyama et al. 2005; Badri and Vivanco 2009; Yoneyama et al. 2009). The germ tubes from AM fungal spores extend towards the root, enter the root, and penetrate cortical cells (Varma and Schüepp 1994). Within the cortex, AM fungal hyphae (usually non-septated) ramify either intercellularly (Arum-type) or intracellularly in coils (Paris-type) (Smith and Smith 1997), forming their arbuscules intracellularly. Arbuscules are branched haustorial structures that result in a large surface of contact between the fungus and host plant, facilitating the exchange of resources (Smith and Gianinazzi-Pearson 1988). Vesicles are formed either inter- or intra-cellularly (Smith and Smith 1997). They are hyphal swellings, rich in lipids, considered to act as storage structures.

1.2 Symbiosis and plant responsiveness to AM fungi

This symbiotic relationship between plants and AM fungi spans more than 460 million years of evolution (Brundrett 2002) and has been systematically studied for the last century (Koide and Mosse 2004). The multifunctional role of AM fungi on plants has been demonstrated. For example, AM fungi can enhance plant resistance against root-infecting pathogenic fungi (Azcón-Aguilar and Barea 1996; Tchameni et al. 2012), bacteria (D'Amelio et al. 2011), and nematodes (Diedhiou et al. 2003; Liu et al. 2012); they can alleviate salt stress (Giri and Mukerji 2004) and improve water relations (Augé 2001; Ruiz-Lozano 2003). However, arguably, the most important contribution is enhanced nutrient acquisition, especially phosphorus (P). P is often a limiting resource for plants, due to its low mobility in the soil (Schachtman et al. 1998). AM fungal mycelia can access additional P by extending beyond the rhizosphere (Bolan 1991).

AM fungi, however, are obligate biotrophs, and maintaining a symbiosis has a significant carbon cost for the host plant. Plant growth responses to colonization by AM fungi (mycorrhizal responsiveness; MR; Janos, 2007) can range from positive (mutualism) to negative (parasitism). The direction and magnitude can vary depending on the plant identity (Klironomos 2003; Jones and Smith 2004), the fungal identity (Maherali and Klironomos 2007) and environmental factors such as water and nutrient availability (Koide 1991; Johnson 1993; Johnson et al. 2010).

In agricultural systems, AM inoculation might result in negative responses with parasitic effects for host plants (Johnson 1993). Under conditions of optimum fertility (application of fertilizers) the acquisition of nutrients is not a limiting factor, although the carbon cost to

maintain the symbiosis is still high (Johnson 2010). However, even in cases where AM fungi do not increase net nutrient uptake, there may be other benefits, such as higher resistance to pathogens or tolerance to abiotic stress.

1.3 AM fungal and host plant identity

It is difficult to talk about consistent patterns within the Glomeromycota with regards to plant benefit, especially when the plant benefit involves different mechanisms (protection from pathogens or resource uptake) or a combination of them. However, there is some indication that members of Gigasporaceae may be more effective at nutrient uptake, whereas members of the Glomeraceae are more effective at protecting plants from pathogens (Sikes et al. 2009).

Plants show a wide range of responses to AM fungi depending on morphological (rooting pattern) or physiological (photosynthetic pathway) traits. It has been shown that C4 grasses and forbs tend to be more dependent on AM fungi compared to C3 grasses (Wilson and Hartnett 1998; Hoeksema et al. 2010). However, for the purpose of my project I will focus more on the effects of morphological traits. Root system architecture can determine the degree and type (enhance nutrient uptake or protection from pathogenic infection) of benefit (Hetrick et al. 1992; Graham and Eissenstat 1994; Newsham et al. 1995). Plants with highly branched roots can more efficiently explore soil and scavenge for nutrients, but they are also more susceptible to soil pathogens due to the high number of meristems. Therefore, they are more likely to receive mycorrhizal benefit related with pathogen protection. In contrast, plants with coarser, and less branched, roots are more dependent on AM fungi for nutrient uptake. Hetrick (1992), grouped 23

forbs in three distinct categories depending on the level of dependency on AM fungi for acquiring phosphorus from soil: obligate mycotrophs, facultative and non-AM responsive.

1.4 Model plant system

Grapevines are highly responsive to AM colonization, responding with increased growth and tolerance to different types of soil stress (Linderman and Davis 2001; Trouvelot et al. 2015). Commercial grapevines (*Vitis vinifera*) can grow either on their own roots or grafted as scions on rootstocks.

North American grapevine species (Serra et al. 2014) are resistant to phylloxera (Forneck et al. 2000), which is caused by *Phylloxera vitifoliae*, a sap-sucking insect that perforates roots, deposits a secretion, and inhibits the root from healing. These grapevine species exude a sap, repelling nymphs or developing a protective gall, covering the wound and protecting the root from secondary infections (Forneck et al. 2001). In 1880, the worldwide grape devastation led growers to use phylloxera-resistant rootstocks (American grapevine species): *Vitis riparia, Vitis berlandieri*, and *Vitis rupestris* (Weaver 1976). Interspecific hybrids of these rootstocks were bred to be resistant to phyloxera and/or to tolerate or be resistant to other pests or stressors, such as parasitic nematodes, high pH, saline soils, drought, or poorly drained soils (Reynolds and Wardle 2001; Gu 2003; Serra et al. 2014).

I have decided to use grapevine rootstocks in this project because of their high variability in root phenology. For example, *Vitis rupestris* roots typically penetrate soils deeply with "sinker roots" compared to *Vitis riparia* roots, which grow more horizontally with "feeder roots" that have high branching angles. Hybrids of *V.riparia x V.rupestris* (3309 Coudrec, 101-14 Millardet

et de Grasset, Schwarzmann) typically grows roots with intermediate angles (Smart et al. 2006; Table 1.1).

Table 1.1 Description of American Vitis species [information taken from Pongracz (1983)]

SPECIES	DISTRIBUTION	NATURAL HABITAT	DESCRIPTION
Vitis riparia	From the center of Canada in the north, to Texas and Louisiana in the south and to the Rocky Mountains in the west	River banks and on islands in deep moist, fertile soils	Roots: Very thin, yellow in color, very tough structure Root system: Well branched and shallow-growing
Vitis rupestris	From Texas in the south, extending to New Mexico, Indiana, Tennessee and southern Pennsylvania	Gravelly banks of mountain streams In stony soils	Roots: medium to thick, reddish-brown in color, rough surface, very tough in structure Root system: Very deep growing, well branched, branching angle 30°
Vitis berlandieri	From the limestone hills of southwest Texas, to New Mexico in the south and north of Mexico	Calcareous soils	(no published information)
Vitis candicans	From the south of United States, extending from Arkansas River to the center of Mexico	(no published information)	(no published information)

Table 1.2 Description of the rootstock cultivars are used in this project [information taken from Pongracz (1983)]

ROOTSTOCK	PARENTAGE	SOIL PREFERENCE
Riparia Gloire	*Vitis riparia*	Deep / fertile
3309 Coudrec	*V. riparia x V. rupestris*	Deep / well drained
101-14 Millardet	*V. riparia x V. rupestris*	Heavy clay
Schwarzmann	*V. riparia x V. rupestris* (natural hybrid)	Fertile
5C Teleki	*V. berlandieri x V. riparia*	Clay
Ramsey	*V. champinii* (natural hybrids of *V. candicans* and *V. rupestris*	Light sand/ low fertility

1.5 Root traits of host plant

It is not well understood what plant traits are most important in determining plant response to mycorrhizas, although traits related to nutrient foraging such as root architecture and morphology seem to be linked with the observed variation in plant response (Baylis 1970; Yang et al. 2014; Eissenstat et al. 2015). Root morphology refers to the features of a single axis such as the diameter or existence of root hairs (Lynch 1995). Root architecture refers to the spatial configuration in soil and is associated with the ability of plants to scavenge and acquire nutrients and water (Fitter 1987). Comparing root traits among plants evolved under distinct soil characteristics, the architectural root traits are more variable than morphological trails (Fitter 1987). Therefore, plants might alter more the architectural traits in response to soil heterogeneity

(Lynch 1995; Bouma et al. 2001). Considering this, it is more likely that root architecture is going to be the target of belowground evolution (Fitter et al. 1988).

1.6 Rooting strategies and nutrient uptake

There is both a genetic and environmental influence on the development of root systems. There is always an interaction between these two factors and, depending on the resource strategy (nutrient-conservative vs nutrient-demanding species), some species are more plastic to the soil environment than others (Comas and Eissenstat 2009). Nutrient-conservative plants are typically slow-growing with low rates of root proliferation, high root diameter (Comas and Eissenstat 2009; Bardgett et al. 2014), low root hair density (Fitter 2004), and typically root traits that characterized by low turnover and high longevity (resource conservative traits). They are frequently related with low specific root length (SRL; defined as the root length per unit dry mass) (Eissenstat et al. 2000) due to costly, thick roots. Although high SRL index is frequently associated with high nutrient exploitation ability (Fitter 1987), it fails to take account root hierarchy (Eissenstat et al. 2000), which basically distinguishes roots of different functional groups (absorptive, storage, transfer). Specifically, root hierarchy classifies root branches into different orders depending on their position, with most distal roots identified as 1st-order (root tips). Lower order roots (1st and 2nd) are typically thinner and they function in nutrient absorption (Comas and Eissenstat 2009; McCormack et al. 2015), while higher order roots provide structural support, transportation or storage of resources, and root proliferation (Eissenstat et al. 2000; Comas and Eissenstat 2009). Absorptive roots are also classified by diameter lower than 2 mm, due to the greater ability to penetrate soil aggregates and the higher contact surface with

them. Furthermore, absorptive fine roots are typically shorter-lived as their cells are non-lignified, providing less resistance, which, in turn, allows easier diffusion of water and nutrients. Additionally, plants tend to increase the density of root hairs under high nutrient availability (Fitter 2004). Root hairs are outgrowths at the tip of a roots that increase the absorptive surface. However, the high-energy cost and the limited length allow root hair proliferation only in high nutrient patches.

Root traits are influenced by factors such as soil texture, resource availability, and interactions with soil microbes (Eissenstat et al. 2000; Meister et al. 2014). The distribution of the most limiting resources (e.g., high nutrient or water patches) usually determine root system plasticity. "Selective root placement" is a common pattern and refers to the increase in root branching in the most fertile zones of the rhizosphere (Bardgett et al. 2014). Under water stress conditions, plants develop deep, thin roots, which are effective at reaching moist zones in the soil (Bardgett et al. 2014).

1.7 The relationship between rooting pattern and plant growth response to AM fungi

The main goal of my research is to examine the potential association between plant response to mycorrhizas and root traits. There are few studies that have focused on the relationship between plant rooting pattern and growth response to AM fungi. In the next few paragraphs I provide a summary of the most relevant papers on this topic.

Newsham (1995a) introduced the hypothesis that the "continuum of AM benefit" that occurs among plant species is determined by root architecture. This hypothesis is supported with the fact that P-inflow (rate of P uptake per unit root length) is correlated with specific root tip

number (number of root tips per root mass) indicating that plant species with highly branched root systems are more efficient in P acquisition and thereby less likely to benefit from mycorrhizal P acquisition (Newsham et al. 1995). It is frequently reported, that root systems with greater nutrient foraging capacity are frequently associated with lower PGR due to the lower plant dependency on mycorrhizal acquisition (Yang et al. 2014; Liu et al. 2015).

Maheralli (2014) argued, using a meta-analysis (including 12 studies in total), that there is no overall association between root architecture and PGR. However, within this meta-analysis, three studies show that specific root length (SRL) is negatively correlated with PGR (Pope et al. 1983; Graham and Syvertsen 1985; Manjunath and Habte 1991). One study shows that root : shoot was positively correlated with PGR (Zangaro et al. 2005). However, there is no consistent relationship between root diameter and PGR; Hetrick et al., (1988) and Manjunath & Habte, (1991) showed positive correlations, whereas Declerck et al., (1995) and Zangaro et al., (2005) found negative correlations. Similar inconsistent patterns were found for root hair length [negative relationships by Manjunath & Habte, (1991) and Declerck et al., (1995), and a positive one by Siqueira & Saggin-Júnior, (2001)] and also for root hair density [(positive: Zangaro et al., (2005) and Hill et al., (2010) and negative: Manjunath & Habte, (1991); Declerck et al., (1995)].

Considering the limited number of studies that specifically examined the effect of rooting pattern on the responsiveness of AM plants, Yang (2015) tried a different approach, using the root system "type" as an approximation for root traits. In this meta-analysis, he showed that AM function differs between taprooted and fibrous rooted plants under different types of stressors. Taprooted plants showed higher PGR under no stress and abiotic stress, and no difference under biotic stress; he found an interaction between AMF and biotic stress only for fibrous plants

(Yang et al. 2014). This confirms Sikes' theory (2009) that complex root systems possess higher number of meristems, are more susceptible to infections by pathogens, and such plants may receive a limited nutritional benefit from the AM symbiosis, but a major benefit via protection from soil pathogens (Sikes et al. 2009).

It is important to mention that within a study, the outcome (referring to the association between PGR and a root trait) can shift from positive to negative and vice versa, depending on the plants used. For example, for given plant A (average root diameter =1, PGR =0.5), plant B (average root diameter =3, PGR =1), plant C (average root diameter =2, PGR =1), the relationship between average root diameter and PGR will be positive if we choose to compare plants A, B, and neutral for plants B, C. Therefore, the model plant selection is critical for this type of research. Following this, one reason studies are so variable in their findings, could be due to the fact that phylogenetic distance among tested plants is not taken into account. Thus, the differences in PGR observed among tested plants may be a result of physiological factors, rather than root traits. For example, Newshan (1995a) used bluebells (Asparagaceae) and fescues (Poaceae) as model plants. In such contrasts, it is difficult to control for confounding variables, as many traits other than root architecture will be different. An alternative approach would be using plants that are genetically and functionally similar with some variation in their root traits. Here, I decided to use a phylogenetically restricted group of plants, specifically grapevine rootstock cultivars.

1.8 Research objectives and predictions

The first objective of this study was to determine how root architecture may influence the plant growth response to AM fungi (PGR) and more specifically, to evaluate the relationship between individual root traits and PGR. To accomplish this I had to take several steps in my research. First, I compared PGR of the individual grapevine rootstocks, as the study requires that there is variation in PGR among rootstocks. Then I compared the rootstocks for different root traits. I expected that plants with a greater ability to scavenge soil nutrients would have lower PGR. Therefore, I hypothesized a negative correlation between PGR and root traits that facilitate nutrient uptake such as branching intensity, specific root length and root surface area. I also hypothesized a positive correlation between PGR and root diameter, a variable that is often associated with reduced root length or root branching.

The second objective of this study was to determine if inoculation with AM fungi influences the expression of root traits. I hypothesized that AM fungal inoculation would induce a simpler root architecture with reduced root branching due to the complementary function of AM extraradical hyphae and root traits that facilitate nutrient uptake. Alternatively, AM fungal inoculation may promote a more complex root architecture (Aðalsteinsson and Jensén 1990; Hermans et al. 2006; Fusconi 2014), due to the increased nutrient supply. Thus, the plant may invest more resources on root system development and fungal exudates (Myc-factors), which are involved in the signaling between AM fungi and host plants prior to colonization. Such exudates have been shown to stimulate the formation of lateral roots (Oláh et al. 2005).

2 Materials and methods

2.1 Experimental Design

The experiment consisted of a factorial combination of two AM treatments (+/- AM fungi; AMF) and six rootstock cultivars arranged in a completely randomized design. The rootstock cultivars were: Riparia Gloire (*Vitis riparia*), 3309 Coudrec (*Vitis riparia x Vitis rupestris*), 101-14 Millardet et de Grasset (*Vitis riparia x Vitis rupestris*), Schwarzmann (*Vitis riparia x Vitis rupestris*), Ramsey (*Vitis champinii*), 5C Teleki (*Vitis berlandieri x Vitis riparia*). Each treatment combination had eight replicates for a total of 96 plants.

2.2 Experiment setup

The greenhouse experiment was conducted at Agriculture and Agri-food Canada's Summerland Research and Development Centre (SuRDC) (May 4th to September 24st, 2015). Dormant cuttings of each rootstock cultivar were cut into two-bud segments, dipped in an auxin solution (1500 ppm Indole-3-butyric acid) for 30 seconds and placed in moist perlite filled flats in a growth chamber at 28 °C until callus formation. Sixteen cuttings from each rootstock cultivar were selected based on the callus development stage, and rooted upright in moist Turface-filled flats until root induction. Air humidity in the growth room was gradually decreased before the rooted cuttings were planted into 7.6-liter pots (May 25th) and transferred to the greenhouse. At transplanting, the AMF treatment was applied using 15g (~55 spores) of single species inoculum, *Rhizophagus irregularis* (BioSyneterra Solutions Inc.) was added per pot at 5 cm depth. The non-AMF treated plants were supplied with the same amount of AMF-

free mixture. The growth medium was expanded clay (Turface; Profile products LCC). Plants were supplied with approximately 60 mL (3 x 20 mL) of water every day, using an 8-emitter circular line within each pot, to provide water uniformly onto the medium surface. A low P-fertilization was applied initially and increased along with plant nutrient demand. Plants were hand fertilized every 2 weeks with 60 mL of: (1) 0.42 g L^{-1} solution of 12-2-14 (50 ppm N) for the first 2 months and (2) 1.5 g L^{-1} of 20-20-20 (300 ppm N) for the next two months. Plants were sprayed once with Sulphur (Kumulus DF, BASF) to prevent powdery mildew after pots were covered thoroughly with plastic sheeting film.

2.3 Parameters determined

2.3.1 Biomass

At harvest, roots and shoots were separated and weighed. Roots were carefully cleaned with water and stored in 35% aqueous ethanol solution, at 4°C for the architectural analysis. Finally, roots and shoots were dried at 65°C for 48 h and then weighed again.

2.3.2 Root colonization by AM fungi

To confirm the successful inoculation with AM fungi, I assessed root samples for colonization with AM fungi. Approximately 20 root segments were sampled from each plant. These were stored in root cartridges in 30% ethanol at 4°C. Roots were cleared in 20% KOH for 2 days at room temperature, followed by addition of 3% H_2O_2 (3:2 ratio of 20% KOH : 3% H_2O_2) and heating the roots in a water bath at 70°C for 5-10 minutes until they became transparent. Roots

were then stained in a 0.05% Trypan blue solution. Root colonization was confirmed using a compound microscope (Fig. A1; Appendix A).

2.3.3 Root system analysis

For each plant, the entire root system was washed again to remove any substrate particles and was untangled carefully in a 40 L bucket containing water. The roots were scanned while suspended in distilled water with a versatile large-format scanner (Epson expression 11000XL) using a greyscale at 400 dpi and analyzed using WinRHIZO Pro image analysis system (Regent Instruments Inc., Quebec, Canada, 2013). Multiple scans for each root system were taken. The first scan contained the entire root system. The root system was then divided into two or three parts for separate higher-resolution scans depending on the root system size. By combining the scans, the entire root sample could be used to estimate root parameters. (Table 2.1).

Table 2.1 Description of the plant traits (measurements)

	Units	**Description** (determination)
Dry weight	g	
Total root length	cm	(WinRHIZO software)
Total root surface area	cm^2	(WinRHIZO software)
Total root volume	cm^3	(WinRHIZO software)
Average root diameter	cm	(WinRHIZO software)
First-order roots		Total number of root tips (WinRHIZO software)
Root : Shoot ratio		Dry root to shoot biomass
Root tissue density	$g\ cm^{-3}$	Dry mass per unit root volume
Specific root length	$cm\ g^{-1}$	Total root length per dry root biomass
Root branching intensity	m^{-1}	Number of root tips per length (= first-order roots per length)

2.4 Statistical analysis

For statistical computing and graphing I used the R software program (R version 3.3.1, 2016, Open Source, http:// www.r-project.org/). I checked for deviations from normal distribution of the residuals for all models using the Shapiro-Wilks test and transformed using logarithmic transformation when required. Differences at $P < 0.05$ were considered significant, while differences between $P < 0.05$ and $P < 0.1$ were considered marginally significant. Data analysis

was conducted to answer the following questions that were also posed in the Introduction section.

1) Do grapevines show growth response to AM fungi and is there variation among the cultivars of grapevines?

First, I assessed the effect of AM fungal inoculation on plant growth, using using separate one-way ANOVAs with dry biomass (total, shoot and root biomass) as the dependent variable. AM fungi (+/-) was the independent variable and rootstock cultivar was the blocking factor. *Post hoc* mean comparisons were done using a Tukey test. One-tailed t-test were performed to determine increase in root and shoot biomass within each individual cultivar.

To compare the growth response among the six rootstock cultivars, I calculated the difference (Δ) in biomass of plants colonized with AM fungi relative to non-colonized controls using the equation: $PGR = 100 (Xi – Xn) / Xn$ (Hetrick et al. 1992), where Xi is plant biomass for the + AM fungal treatment and Xn is the biomass mean values for -AM fungal control. One-way ANOVA tests were run to compare the differences in PGR among rootstock cultivars, with PGR as the dependent variable and rootstock cultivar as the independent variable. *Post hoc* mean comparisons were done using a Tukey HSD test.

2) Is the variation in plant growth response to AM fungi explained by variation in root traits?

To examine this question, I used a linear mixed-effects model (multible regression; Winter 2013) with PGR as a response variable. Root parameters were fixed factors and the rootstock cultivar was a random factor. For this model, root parameters were measured from non-mycorrhizal plants (- AM). (Manjunath and Habte 1991; Zangaro et al. 2007).

3) Does colonization by AM fungi influence expression of the root traits?

I compared the root systems of mycorrhizal and non-mycorrhizal plants, to investigate the effect of AM fungi on the expression of root traits. For each root trait, cultivar and treatment differences were analyzed using separate two-way ANOVAs with AM fungi (+/-) and Rootstock cultivar as the independent variables and each root parameter as dependent variables. Also, since plant size differed among the treatments, I used an allometric approach to comparing the data. To compare the effect of AMF on root traits among the six rootstock cultivars, I calculated the difference (Δ) in each root traits of plants colonized with AM fungi relative to non-colonized controls using the equation, % change = 100 (Xi – Xn) / Xn (Hetrick et al. 1992). Changes between treatments and controls were evaluated using one-tailed t-tests.

3 Results

3.1 Do grapevines show growth response to AMF and is there variation among cultivars?

As expected, plants in the + AMF treatment were mycorrhizal (Fig. A.1), and plants in the non-AMF controls were not mycorrhizal. However, there was significant variation in the staining intensity among plants and it was not possible to compare colonization levels among the grapevine cultivars with any confidence.

Inoculation with AM fungi had a positive effect on the growth of the grapevines; over four months post-inoculation, total dry biomass of AMF-inoculated plants was significantly higher compared to control plants ($P < 0.001$, $F_{1, 89} = 16.61$; Fig. 3.1). Both, shoot and root biomass

were significantly increased, for the cultivars Ramsey ($P < 0.01$, $T = 2.7$, $df = 12.5$; $P = 0.051$, $T = 1.7$, $df = 13.8$) and 3309 C ($P < 0.05$, $T = 2.4$, $df = 13.9$; $P < 0.05$, $T = 2.4$, $df = 13.6$), while only shoot biomass for 101-14 Mgt ($P < 0.05$, $T = 3.6$, $df = 12.9$) (Fig. 3.2).

The growth increase varied with individual rootstock cultivars, from a low of less than 15 % in 5C Teleki and Schwarzmann to a high of over 50 % in 101-14 Mgt and Ramsey (Fig. 3.3). Therefore, PGR depended on the rootstock cultivar ($P < 0.05$, $F_{5,42} = 2.67$) with Ramsey showing a significantly higher PGR compared to Schwarzmann and 5C; Riparia Gloire, 3309 and 101-14 showing an intermediate PGR compared to the others (Fig. 3.3).

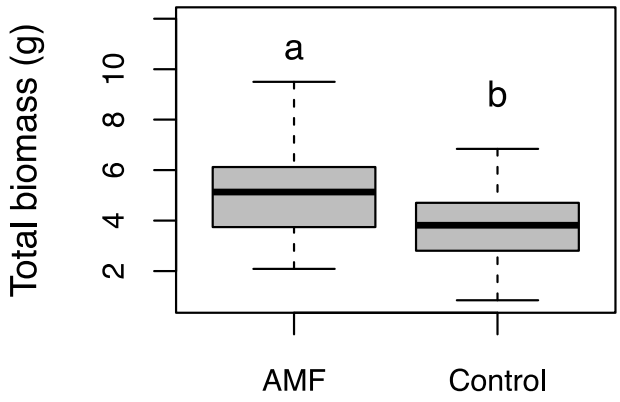

Figure 3.1 Total dry biomass including all rootstock cultivars grown in the presence (AMF) and absence (control) of AM fungi. Treatments not sharing a letter are significant different (Tukey; 95% confidence interval). The horizontal line within the box indicates the median, boundaries of the box indicate the lower and higher quartile, and the whiskers indicate the highest and lowest values.

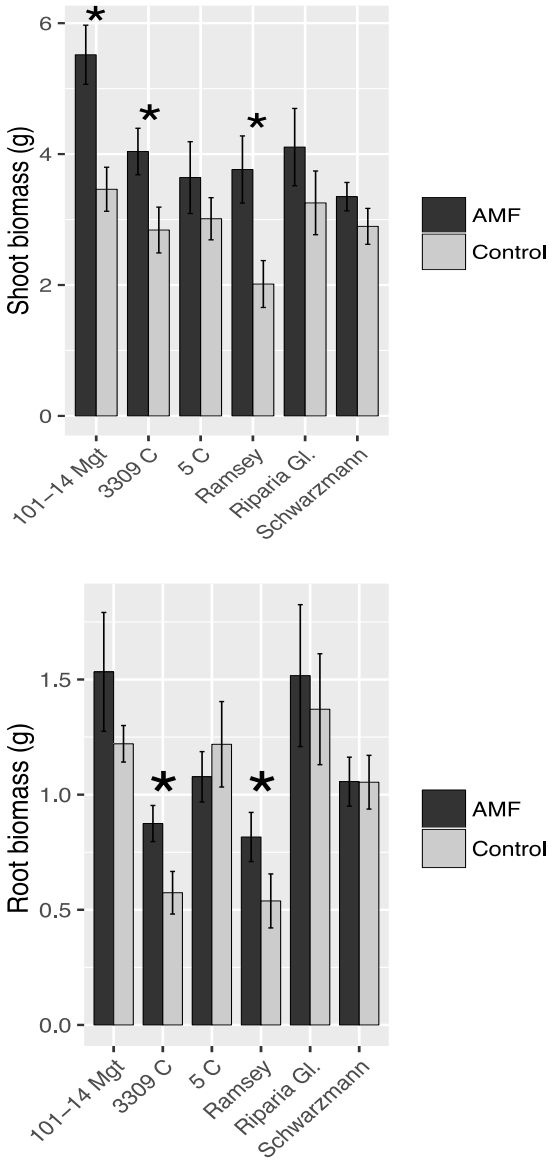

Figure 3.2 Shoot (top) and root (bottom) dry biomass for individual rootstock cultivars growing in the presence (+) and absence (-) of AM fungi. The bars represent the means of eight replicates ± 1 standard error of the mean for each rootstock cultivar. Within a rootstock cultivar, asterisk (*) indicate significant difference following a one-tailed t-test; *P* < 0.05.

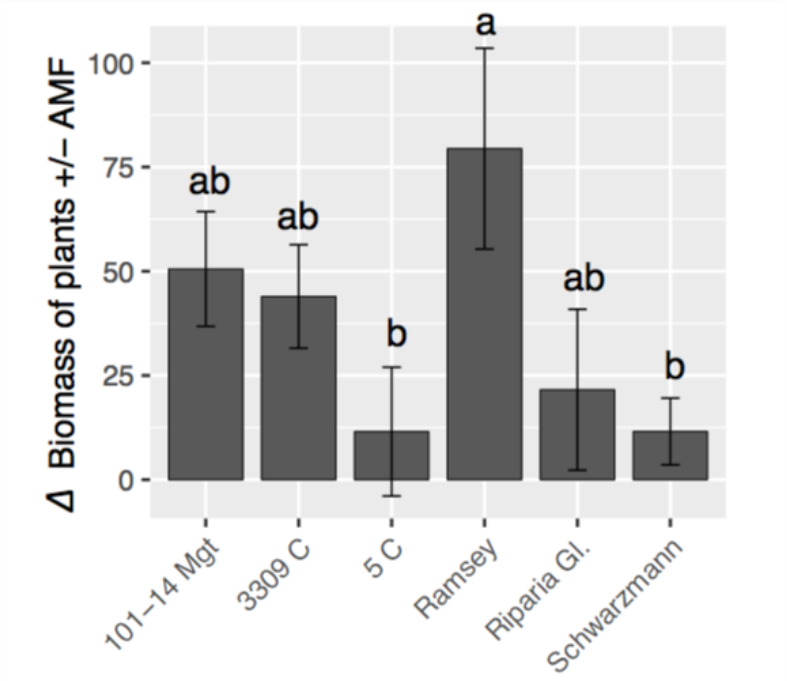

Figure 3.3 Δ biomass of plants colonized with AM fungi relative to non-colonized controls, "Plant growth response to AM fungi" = 100 (Xi – Xn) / Xn, where Xi is plant biomass for the + AM fungal treatment and Xn is the mean values for - AM fungal control. Letters indicate significant differences in PGR among rootstock cultivars (Tukey; 95% confidence interval; n=8). Bars represent the mean of eight replicates ± 1 standard error of the mean for each rootstock cultivar.

3.2 Is variation in plant growth response to AMF explained by variation in root traits?

As expected (Table 1.1), rootstock cultivars differed in their root traits (Table 3.1). A brief description of each rootstock follows, and these are ordered from high to low PGR:

- **Ramsey** had a relatively high average root diameter, low root branching intensity, low root to shoot ratio, intermediate root tissue density, low SRL, and the lowest root surface area compared to the other rootstock cultivars.
- **101-14** had a relatively low root diameter, the lowest root branching intensity, intermediate root to shoot ratio, the highest root tissue density, intermediate SRL, and high root surface area.
- **3309** had the lowest average root diameter, intermediate root branching intensity, the lowest root to shoot ratio, the lowest root tissue density, and highest SRL, and relatively low root surface area.
- **Riparia Gloire** had the highest average root diameter, high root branching intensity, the lowest root to shoot ratio, high root tissue density, the highest SRL, and relatively high root surface area.
- **Schwarzmann** had intermediate root diameter, the highest root branching intensity, intermediate root to shoot ratio, relatively high root tissue density, low SRL, and intermediate root surface area.
- **5C** had relatively low average root diameter, low root branching intensity, high root to shoot ratio, intermediate root tissue density, low SRL, and the highest root surface area.

Given that the rootstocks varied in their degree of growth response to AM fungi (previous section), I then compared the root traits among rootstock cultivars and tested whether root traits were related with the growth response to AM fungi (PGR). Specific root length, root surface area, average root diameter and tissue density were not correlated with PGR (Table 3.2).

However, I detected a significant negative correlation between PGR and (a) root branching intensity ($P < 0.01$; Table 3.2), and (b) root : shoot ratio ($P < 0.05$; Table 3.2). Nonetheless, it is interesting to note that the rootstock with the highest PGR (Ramsey) had the lowest root surface area, while the rootstock with the lowest PGR (5C) had the highest root surface area (Table 3.1).

Table 3.1 Traits associated with root morphology and architecture for six grapevine rootstocks (parameters are measured in the absence of AM inoculation)

Rootstock	Root : Shoot ratio		Average diameter (cm)		Tissue density (g/cm^3)		Surface area (cm^2)		Specific root length (cm/g)		Branching intensity (tips/m)	
5 C	0.400	a	0.359	b	0.122	ab	1102	a	84.7	b	74.5	b
101-14	0.362	ab	0.355	bc	0.135	a	1015	ab	74.4	b	64.1	b
R.G	0.412	a	0.393	a	0.133	a	1020	ab	64.4	b	98.1	a
Schman	0.363	ab	0.374	ab	0.133	a	843	ab	70.6	b	112.7	a
3309	0.194	c	0.338	c	0.101	b	653	bc	113.8	a	76.5	b
Ramsey	0.269	bc	0.391	a	0.126	ab	423	c	69.5	b	77.1	b

Within a trait, means not sharing a letter are significant different (Tukey; 95% confidence interval)

Table 3.2 Dependence of mycorrhizal plant responsiveness on the root traits. Root traits were used as fixed effect and rootstock cultivars as random effect in a linear mixed effect model.

Trait	***F*-value**	***P-value*** (n = 8)
Branching intensity	7.65	**0.008**
Specific root length	1.20	0.27
Tissue density	1.96	0.16
Average diameter	1.93	0.17
Root : Shoot ratio	6.83	**0.012**
Root surface area	0.31	0.57

P-values in bold type were significant at $P < 0.05$ (n = 8)

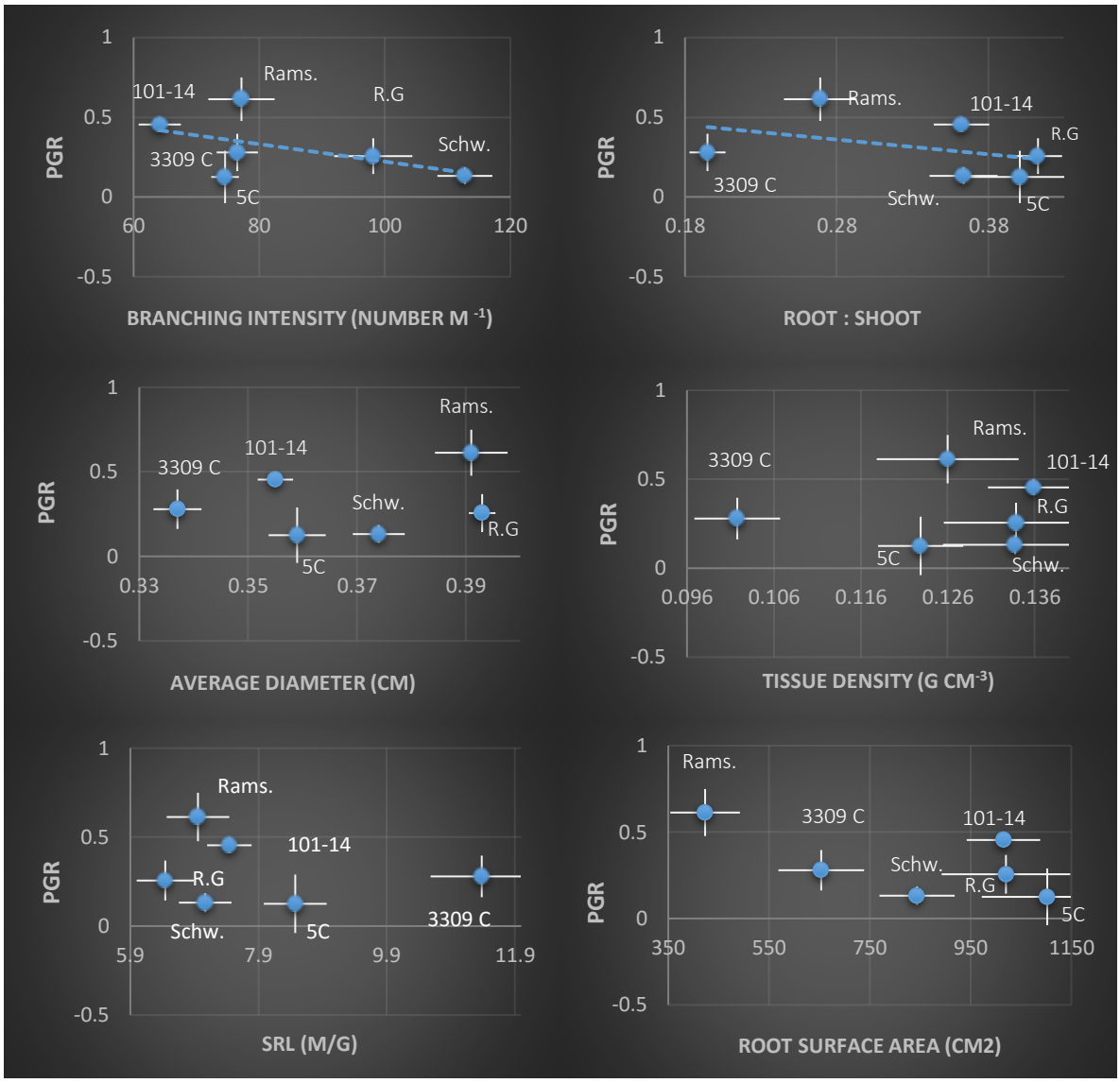

Figure 3.4 The relationship between mycorrhizal growth responsiveness and various root morphological traits: average root diameter, root to shoot ratio, branching intensity, tissue density, specific root length and root surface area. The figure represents the means of eight replicates ± 1 standard error of the mean for each rootstock cultivar. Regression lines are present only for significant relationships (branching intensity and root:shoot ratio; *P* < 0.05).

3.3 Does colonization by AM fungi influence expression of the root traits?

Due to the positive mycorrhizal growth response (PGR) that was shown in previous section, total biomass was significantly higher for the + AMF treatment (Fig. 3.1). Then, differences on root traits between +/- AMF treatments could be related to allometric growth. Therefore, I tested for root parameters that could account for the root size (e.g. branching intensity instead of absolute number of root tips). Overall, inoculation with AM fungi had a significant stimulative effect on root branching intensity ($P < 0.05$, $F_{1, 84} = 6.25$; Table 3.3). Average root diameter, surface area, and the root : shoot ratio were also affected by AM fungal inoculation, however the effect was not consistent for all rootstock cultivars (Figure 3.5). There was interaction between AMF treatment and rootstock cultivar for average diameter ($P < 0.05$, $F_{1, 84} = 3.16$; Table 3.3), root : shoot ratio ($P < 0.1$, $F_{1, 84} = 2.20$; Table 3.3), and root surface area ($P < 0.1$, $F_{1, 84} = 1.99$; Table 3.3).

and Riparia Gloire ($P < 0.01$, $T = 3.88$, $df = 13.7$). Ramsey was the only cultivar with an increase in root surface area ($P < 0.01$, $T = 2.6$, $df = 12.7$). Although Ramsey had the greatest increase in first-order roots (an average 60 % increase compared to - AMF treatment; Figure 3.5), the branching intensity (tips / length) was not increased due to the higher increase in root length. Although 3309 had a similar increase in root biomass as Ramsey (Figure 3.2), the root surface area was not increased as much as Ramsey (Figure 3.5) and conversely root diameter was increased and specific root length was decreased (Figure 3.5).

Table 3.3 The effect of AM fungal inoculation on the expression of root traits.

	AMF Treatment		**Rootstock cultivar**		**Rootstock X treatment**	
Trait	*F*-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Root : Shoot ratio	14.48	***	21.60	***	2.20	.
Average diameter	11.93	***	38.12	***	3.16	*
Specific root length	2.69		8.69	***	2.09	.
Branching intensity	6.25	*	28.66	***	1.29	
Surface area	3.79	*	10.48	***	1.99	.
Root length	1.51		9.31	***	1.38	
Root tips	4.80	*	11.50	***	1.29	

*‘***’P<0.001 / '**'P<0.01 / '*'P<0.05 / ‘.’P<0.1*

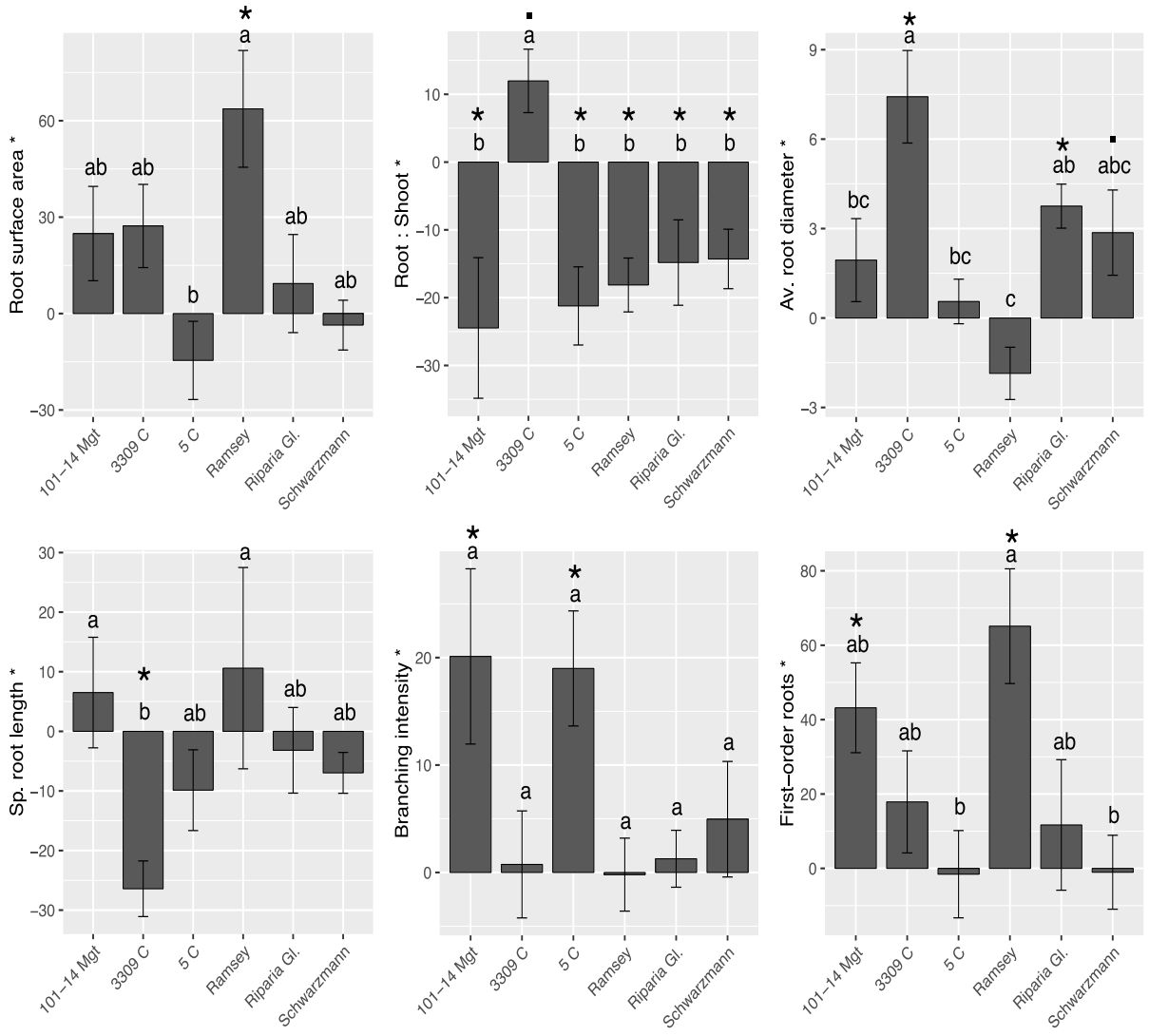

Figure 3.5 The difference (Δ) in each root trait of plants colonized with AM fungi relative to non- colonized controls using the equation, (% change in trait) * = 100 (Xi – Xn) / Xn, where Xi is the root parameter or the + AM fungal treatment and Xn is the mean values for - AM fungal control. The bars represent the mean of eight replicates ± 1 standard error of the mean for each rootstock cultivar. An asterisk (*) indicates significant difference from zero (one-tailed t-test; *P* < 0.05). A dot (.) indicates a difference from zero (one-tailed t-test; *P* < 0.1). Letters indicate significant differences among rootstock cultivars (Tukey; 95% confidence interval; n=8).

4 Discussion

4.1 Do grapevines show growth response to AMF and is there variation among cultivars?

The results of this study clearly show that there is significant variation in growth response to AM fungi among the grapevine rootstocks. Such variation has been reported previously with many other plants, albeit most other studies compare different plant species within a plant community, as opposed to different genotypes with the same species or closely related congeneric taxa (Declerck et al. 1995; Siqueira and Saggin-Júnior 2001; Klironomos 2003).

Some of this variation has been attributed to host plant functional traits. For example, C_4 grasses tend to have higher PGR than C_3 grasses, N-fixing forbs have higher PGR than non-N-fixing forbs, woody species show higher PGR compared to forbs and grasses, and non-N-fixing woody species have higher PGR than N-fixing species (Yang et al. 2016). Finally, taproot plants have higher PGR than plants with fibrous root system (Yang et al. 2014).

Grapevines are woody, non-N-fixing plants with a root system that can spread deeply and broadly in the soil. However, they have low root density (number of roots per unit area), and large-diameter fine roots (Smart et al. 2006). Considering these functional traits, we would expect that grapevines would have high PGR. Overall, I found an increase in plant growth that ranged from 11-79 %. Interestingly, Ramsey, which shows the highest growth response is the only hybrid rootstock of V. candicans (and the only one that is not hybrid of V. riparia) (Table 1.1). V. riparia has a shallow, and more highly branched root system. This may be a result of it having evolved in more fertile habitats (Shaffer et al. 2004). Indeed, in comparison to other rootstocks, Ramsey has the lowest number of first-order roots and the lowest root length (Table

3.1). Furthermore, 5C which shows the lowest growth response is the only hybrid rootstock of V. Berlandieri, also had the highest root surface area and a high root to shoot ratio compared to the other rootstocks (Table 1.1).

Although the high variation in PGR that is reported in other plants ranges from positive to negative (Johnson et al 1997, Klironomos 2003, Jones and Smith 2004), in this study I found an overall positive PGR. The positive growth responses are perhaps because grapevines are woody perennials, and may depend more on mycorrhizas compared to herbaceous plants that are typically used in other studies. Other research on grapevines has shown that they can benefit significantly from AM fungi. Trouvelot et al. (2015) in a comprehensive review, refers to the benefits of AM fungi adaptation in viticulture, such as improving nutrient uptake (P, N), reducing the need for fertilizers, improving tolerance to abiotic stresses (water stress, salinity, iron deficiency) and enhancing the plant's defensive capacity (mycorrhiza-induced resistance; Cameron et al. 2013). Nicolás et al. (2014), found that AM inoculation can increase P, K and Ca in the leaves, as well as stem water potential (indicator of grapevine water status; Choné et al. 2001) under field conditions. Similarly, Ftekhari et al. (2012), found that AM inoculation can increase root length, leaf area, as well as total chlorophyll, phenol and soluble sugars content in the leaves. This positive PGR in grapevine has been shown by others as well (Linderman and Davis 2001; Aguín et al. 2004; Bleach et al. 2008; Ftekhari et al. 2012; Nicolás et al. 2014). Although we do not have a detailed description on life strategies and evolutionary background to describe the distance among rootstocks, it seems that phylogenetic distance among plants may have a significant effect on the growth response to AM fungi. However, we have too few plant cultivars to conduct a proper phylogenetic analysis.

4.2 Is variation in plant growth response to AMF explained by variation in root traits?

One of the main objectives of this project was to examine and evaluate the relationship between PGR and various root morphological traits. Overall, I found a negative relationship between PGR and root branching intensity and a negative correlation between PGR and root : shoot ratio. There were some other notable patterns. For example, the rootstock with the highest PGR (Ramsey) had the lowest root surface area, whereas the rootstock with the lowest PGR (5C) had the highest root surface area. Also, the rootstock with the highest PGR (Ramsey) had the second highest root diameter and second lowest specific root length, whereas the rootstock with the lowest PGR (5C) had the highest specific root length. Despite these patterns, no relationships could be detected between other variables when including all rootstocks in the analyses. It is possible that there are no relationships between PGR and root traits, however, I would argue that the lack of significance is a likely a result of having too few rootstock cultivars in the analyses, and thus low power.

The observed patterns, albeit weak, are consistent with the hypothesis that less branching and coarser roots would be associated with higher PGR, a relationship that has been reported in many other studies (Pope et al. 1983; Graham and Syvertsen 1985; Manjunath and Habte 1991; Declerck et al. 1995; Newsham et al. 1995; Yang et al. 2014). Provided that extraradical AM hyphae and absorptive fine roots both contribute to nutrient foraging, a complementary association among them is possible (Liu et al. 2015). Plants with greater nutrient uptake ability are expected to benefit relatively less from mycorrhizal nutrient uptake, and therefore have a

lower PGR. This may explain the relationship between some root traits and PGR (Manjunath and Habte 1991; Newsham et al. 1995; Yang et al. 2014; Eissenstat et al. 2015).

However, it is worthwhile to mention that root traits and PGR are not directly related, and that several factors are involved. For example, root traits are dependent on plant identity and influenced by soil environment through root plasticity (López-Bucio et al. 2003). The magnitude of root plasticity (the ability to respond to nutrient-heterogeneity by altering root traits) is also dependent on plant identity (Hodge 2004; Koide 1991; Newsham et al. 1995). In addition, different root traits may facilitate the uptake of particular nutrients. For example, the uptake of poorly mobile nutrients such as phosphorus may be increased with greater root surface area (Brundrett 2002). Considering this complexity and the several factors involved, it is not surprising that there is a high variability among the studies looking at the relationship between root traits and PGR (Maherali 2014).

Nonetheless, root traits did not evolve only with regards to mycorrhizal-mediated nutrition. Roots may display different foraging strategies (Paula and Pausas 2011) among different rootstock cultivars as a function of selection to different habitats (Morano 1994). For example, *Vitis rupestris* typically grows in gravelly-stony soils, and thus it has deep roots, whereas *Vitis riparia* grows in fertile soils with roots that spread more horizontally and near the soil surface (Smart et al. 2006; Table 1.1). These different strategies do not only function for P nutrition, but also for water, other nutrient resources, and anchoring to the ground.

4.3 Does colonization by AM fungi influence expression of the root traits?

This study does provide evidence that inoculation with AM fungi can influence the expression of root traits. Specifically, I found an overall higher root branching intensity when inoculated with AM fungi. In addition, five of six cultivars also had lower root/shoot ratios when mycorrhizal. This indicates that mycorrhization alters resource allocation in plants towards shoot production compared to root production, and roots are more highly branched. There were some other changes as well (root diameter, root surface area and specific root length) but these were not consistent, but rather they changed differently on particular cultivars. In the literature, the patterns are inconsistent (Atkinson et al. 2003), and effects of AM fungi on root morphology are dependent on the identity of the plant and the fungus. Gutjahr et al. (2009) showed that *Glomus intraradices* increased the number of first-order lateral roots in rice, while Berta et al. (1990) found no effect of *Glomus sp*. strain E3 on leek. Berta et al. (1995) found an increase on the total root length and first-order lateral root length by both *Glomus intraradices* and *Glomus mosseae* on plum , while Hooker et al. (1992) found no effect of *Scutellospora calospora* on poplar. Studies more similar to mine, that focused on grapevine rootstocks showed an increased number of first-order lateral roots (Schellenbaum et al. 1991; Aguín et al. 2004) and an increased total root length (Schellenbaum et al. 1991).

The mechanisms for AM-mediated changes in root morphology are not clear. Fusconi (2014) argues that AM roots are expected to have increased branching for two reasons. First, fungal exudates (Myc-factors), which are involved in the signaling between AM fungi and host plants prior to colonization, have been shown to stimulate the formation of lateral roots (Oláh et al.

2005). Second, the improved nutrient status (and specifically phosphorus content) of AM plants can stimulate the transfer of plant carbohydrates from shoots to roots, which can help with increased root branching (Hermans et al. 2006).

Furthermore, phosphorus is related with the formation of lateral roots (Aðalsteinsson and Jensén 1990). However, the change in root branching is not correlated to internal phosphorus concentration, but it is linked to the phosphorus uptake rate (Amijee et al. 1989). Thus, improved phosphorus acquisition of AM plants, may explain the higher number of first-order roots (tips).

4.4 Conclusions and Limitations

Plant biomass was stimulated by AM fungi in all grapevine rootstocks tested, thus it is clear that grapevines benefit from the symbiosis. However, it is not known whether inoculating vines before field planting is beneficial, as young vines would typically be transferred to the field. Mycorrhizal growth response of young grapevines varies among rootstock cultivars and this response was negatively correlated with root branching intensity, suggesting that PGR is negatively correlated with nutrient foraging ability. Although interesting, and supportive of Newsham and Fitter's (1995) hypothesis, there are many traits that are involved in nutrient foraging ability of a plant. A thorough examination of plant traits and how they respond to AM fungi would be needed for a better understanding of mycorrhizas and their influence on plant life history strategies. I also found that inoculation with AM fungi can increase root branching intensity, which may help plants forage for resources more efficiently. There is no consistent effect of AM fungal inoculation on root diameter, surface area and specific root length.

A limitation of the current study is that it was conducted in the greenhouse. This was necessary, as I needed to control for certain factors, especially the +/- AM fungal treatments. This is very difficult to achieve in the field. Nonetheless, it is important to recognize that the greenhouse environment differs from the field, and thus the observed responses should be interpreted with caution. For example, the biological, chemical and physical properties of field soil are different from soil that has been sterilized, sieved and placed in pots. AM fungi behave differently in different soils (Carrenho et al. 2007), and they may interact differently with plant roots. For example, to establish +/-AM fungal treatments in pots we add prepared inoculum, which is usually spores, root fragments, and hyphal fragments. To establish a functioning mycorrhiza, the plant would need to invest significant carbon towards establishing an intraradical and extraradical hyphal network. There may be less of an investment required in the field as the extraradical network may be largely intact. Similarly, field soil contains a high diversity of rhizosphere organisms, where in pots this diversity is greatly reduced, if not absent. This may have positive and negative influences on mycorrhizal functioning, as some organisms may compete or stimulate mycorrhization (Diedhiou et al. 2003; Malusá et al. 2012; Larimer et al. 2014).

Nonetheless, in some cases, growing plants in pots is relevant, as some commercial plants are grown in containers. This is often the case for grapevines as well prior to transplant in the field, ensuring the high-quality of planting material (Waite et al. 2015).

Another bias is the identity of the AM fungus that is used in pot studies. Here, I used a common commercial inoculant, *Rhizophagus irregularis* (BioSyneterra solutions inc.), largely because of convenience. However, in the field, the plant would be exposed to a complex

community of AM fungi, and it is well known that the identity (Sikes et al. 2009; Jin et al. 2013) and diversity (Sharma et al. 2009) of AM fungi can have profound effects on plant productivity.

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Appendix A: Supporting material for root colonization by AM fungi

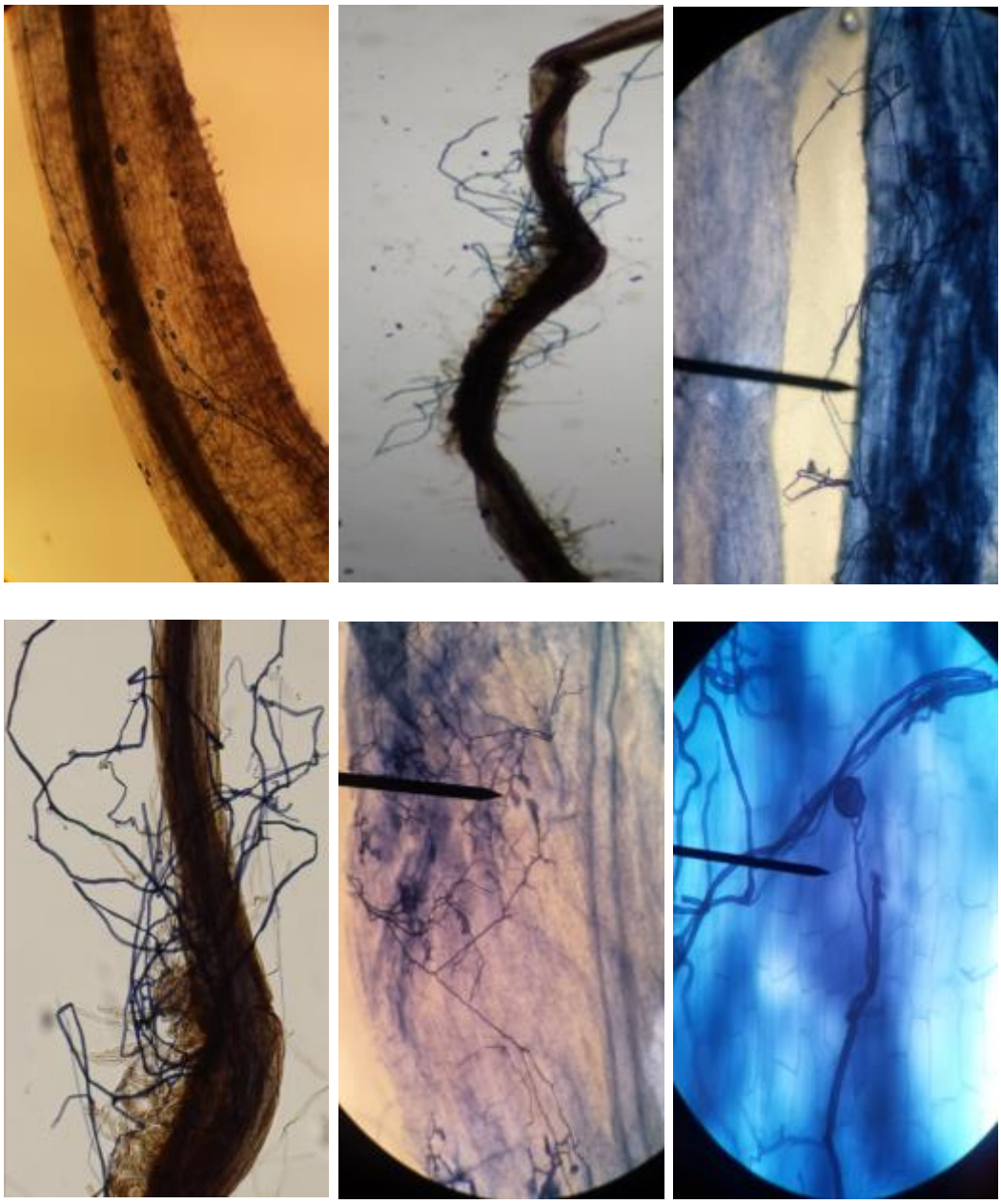

Figure A. 1 Root colonization for +AM treatment was confirmed, after the roots were cleared (KOH), stained (Trypan blue) and observed using a compound microscope.