

The Occurrence and Infectivity of Arbuscular Mycorrhizal Fungi in Inoculated and Uninoculated Rhizosphere Soils of Two-year-old Commercial Grapevines.

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Arbuscular mycorrhizal (AM) fungal populations present in the rhizosphere of vine roots in the vineyards of a commercial farm in the Stellenbosch Region were investigated using microscopic analyses. AM root colonisation levels of between 70% and 90% were found in both grapevine roots that were previously artificially inoculated with commercial AM inocula, and in uninoculated (control) grapevine roots. The AM fungal isolates in the rhizosphere soil, identified using morphological criteria, belonged to the genera *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. The majority of species found was not present in the commercial inocula and was either indigenous to the vineyard or originated from the nursery where the vines were obtained. Isolates of *Glomus* and *Acaulospora* appeared to be the most abundant. The AM fungal species occurred at a soil phosphorus (P) concentration of up to 80 mg/kg P and a soil pH (KCl) that ranged between 5.63 and 6.10. Total spore counts ranged between 1 000 and 3 779 spores/100 g dry soil. In accordance with literature, lower spore concentrations were recorded for the heavier soil types with no cover crop system, compared with the sandy soil type on which cover crops were sown annually.

Some agricultural soils, particularly those deficient in beneficial soil organisms, due in part to side effects of practices such as fumigation, require reconditioning to render them suitable for optimum crop performance. From time to time, artificial inoculation with selected arbuscular mycorrhizal (AM) fungi is used to reintroduce these fungi to such soils (Abbott & Robson, 1982; Menge, 1983).

It is generally accepted that AM colonisation of vine roots contributes to the optimum performance of grapevines (Possingham & Groot Obbink, 1971; Karagiannidis *et al.*, 1995; Karagiannidis *et al.*, 1997). It is for this reason that commercial AM fungal strains have been available to farmers abroad for nearly two decades (Menge, 1983). However, large-scale inoculation of cultivated crops in South Africa only emerged recently (M. Venter, personal communication, 2000) and there is little known about the mycorrhizal status of SA vineyard soils.

In a previous study, AM root colonisation and subsequent host plant response in young grapevines of a commercial vineyard were determined after artificial inoculation with AM fungi (Meyer *et al.*, 2004). It was, however, found that grapevine roots were adequately colonised with indigenous populations of AM fungi. Consequently, no significant improvement in the performance of most of the young grapevines could be detected, since it was believed that these fungi masked the effects of inoculation.

To obtain additional information on the mycorrhizal status of the vineyard, including the diversity and abundance of indigenous

species, AM fungal spores isolated from trap pot cultures, prepared from the inoculated and uninoculated (control) grapevine rhizosphere soils, were identified using morphological criteria. In addition, AM fungal root colonisation patterns in roots from the trap pot cultures and the field-grown vines were studied and spores obtained from the field were quantified.

MATERIALS AND METHODS

Experiment layout and experiment procedures were described previously (Meyer *et al.*, 2004). Briefly, the study was carried out in a commercial Merlot vineyard, grafted onto three different rootstocks. The first two, i.e. 101-14 Mgt and 110 Richter (110 R), were planted on a ridged Westleigh soil and the third, 99 Richter (99 R), on an unridged Fernwood soil (Soil Classification Working Group, 1991). Five treatments were applied at the time of planting. Three of the treatments (Biocult[®], *Glomus* sp. 1054 and Vaminoc[®]) each involved inoculation with different AM inoculums. The nutrient-rich Biocult[®] was compiled of spores and other infective propagules belonging to *Glomus mosseae*, *Glomus intraradices*, *Glomus fasciculatum*, *Glomus etunicatum*, *Acaulosporae* spp. and *Trichoderma*. The total number of spores in this inoculum amounted to ca 160 per 50 mL. The *Glomus* sp. 1054 inoculum, consisting of soil amended with colonised roots, containing ca 7200 spores per 50 mL. The Vaminoc inoculum contained a total of ca 920 spores in 5 g clay amended medium. These spores represented the species *G. mosseae*, *G. fasciculatum*, *G. caledonium* and *Glomus versiforme*. The fourth treatment

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involved sterilisation of soil by a combination of fungicides, Benlate® WP and Rovral Flo® SC, in order to establish a control not affected by soil fungi. The fifth treatment (control) received neither fungicides nor AM inocula. All treatments, except the one where nutrient-rich Biocult was used as AM inoculum, received 50 mL per vine of sterilised Biocult (steam sterilised in an autoclave at 121° C, 100 kPa, for 60 minutes) to ensure that all vines received the same amount of nutrients. In the present study, the procedures below were followed.

Sampling procedures and establishment of trap pot cultures

In winter (June 2000) composite soil samples, representative of each rootstock, were taken at the following soil depths: 0-150 mm, 150-300 mm, 300-600 mm and 600-900 mm. Soil analyses were carried out in accordance with methods prescribed by The Non-affiliated Soil Analysis Work Committee (1990). In addition, rhizosphere soil and roots of each of the four replicates per treatment were sampled. Roots were stored in 50% (v/v) ethanol at room temperature until analysed. Each soil sample (ca 1.5 kg) was divided into two sub-samples. The one sub-sample (ca 1 kg) was air-dried for three weeks, before it was subjected to microscopic analyses.

The other sub-sample (ca 500 g) was subjected to a cold shock treatment for approximately six weeks at 0°C to enhance germination amongst spores that were dormant at the time of field collection (Vimard *et al.*, 1999). Thereafter, each of these 500 g cold shocked, sub-samples was mixed with 500 g sterilised sand (gamma-irradiated at a minimum absorbed dose of 20 kGy per kg soil) and transferred to a free-draining pot. Surface sterilised grain sorghum seeds, treated with 5% (v/v) hypochlorite solution for 20 minutes, were sown onto these pots, covered with a thin layer of sand, and watered to field capacity. No nutrients were added to the potting mixture. Pot cultures were watered on a supplementary basis via a splash-free drip irrigation system to permit limited drainage from each pot. All possible precautions were taken to minimise the likelihood of contamination. The pH (KCl) of the potting mixture was ca 5.9. Pot plants were allowed to reach maximal growth (after eight to twelve weeks) at which time roots and soils were sampled for microscopic examination.

Microscopic analyses of roots and spores

The level of root colonisation by AM fungi was estimated in the field-collected vine roots using a compound microscope as described by Brundrett *et al.* (1994). Both the field-collected vine roots, as well as the sorghum roots from the trap pot cultures, were used to identify root colonisation patterns and diagnostic structural features of the AM fungi.

Spores were separated from the soil in the trap pot cultures using wet sieving and sucrose gradient centrifugation (Brundrett *et al.*, 1994). These spores were then selected and grouped according to morphological types using dissecting microscopy. Subsequently, semi-permanent microscope slide preparations of the spores were made. Spores were stained and mounted in Melzer's reagent and Polyvinyl-Lacto-Glycerol (PVLG). Using a compound microscope, the spores were then identified according to the methods in Brundrett *et al.* (1996) and the INVAM website (Morton, 2001). The same method was used to separate AM spores in the air-dried sub-samples. These spores were then enumerated using a stereo microscope (Nikon SMZ -10A). Counts

were expressed as the total number of spores per 100 g of dry field soil and included both dead and viable spores.

Statistical procedure

Statgraphics version 7 and SAS version 6.12 packages were used for the statistical analyses of the data:

1. The percentage data (root colonisation data) were arcsine transformed (Zar, 1981). The influence of the factors and their interactions were tested with a two-way analysis of variance (2-way ANOVA). Where the ANOVA revealed significant effects by the factors, the differences between treatments were separated using a *post hoc* least significant difference (LSD), multiple comparison test ($p \leq 0.05$). Data for each rootstock were analysed separately.
2. The rest of the data were analysed with SAS version 6.12 (SAS, 1990). The influence of the factors and their interactions were tested with a 2-way ANOVA. The analysis was done for each rootstock separately and with rootstock as a factor. For the ANOVA with rootstock as a factor, treatments were used as a sub-plot factor. Fisher's Least Significance Differences were calculated at the 5% significance level to compare treatment means. Shapiro-Wilks's test was performed to test for non-normality (Shapiro and Wilk, 1965).

RESULTS AND DISCUSSION

From the results obtained during the 1998/99-season, it was evident that AM fungal strains, other than those included in the inocula, were colonising the vine roots (Meyer *et al.*, 2004). This resulted in a relatively high level of AM fungal root colonization of between 40% and 85%. During the 1999/00-season, higher levels of root colonisation were obtained, ranging between 70% and 90% (Fig. 1). The modes by means of which the AM fungi spread through the root cortex were typical of the *Paris*- and *Arum*- types (Gallaud, 1905), which were, respectively, characterised by the formation of noticeable intracellular coils in the cortical tissue and a relatively parallel spreading of intercellular hyphae. Growth of the co-existing fungal species in the roots did not seem to have been affected by the prevailing high levels of soil P, up to 80 mg/kg P (Table 1). Furthermore, this soil P-level was much higher than the level of about 7 mg/kg P, above which inhibition of fungal development in roots can be expected (Brundrett *et al.*, 1996). This tolerance of AM fungi to relatively high levels of soil P is in agreement with results from other studies (Plenchette *et al.*, 1983; Schubert *et al.*, 1990).

In the present study, 18 AM fungal species were obtained, of which 15 were positively identified on the basis of spore morphology and other diagnostic fungal structures (Table 2). These

TABLE 1

Soil P analyses of 101-14 Mgt, 110 R and 99 R rootstock plots (1999/00 season).

Soil depth (mm)	P (mg/kg)		
	101-14 Mgt	110 R	99 R
0-150	74	68	63
150-300	80	61	67
300-600	27	31	61
600-900	15	6	50

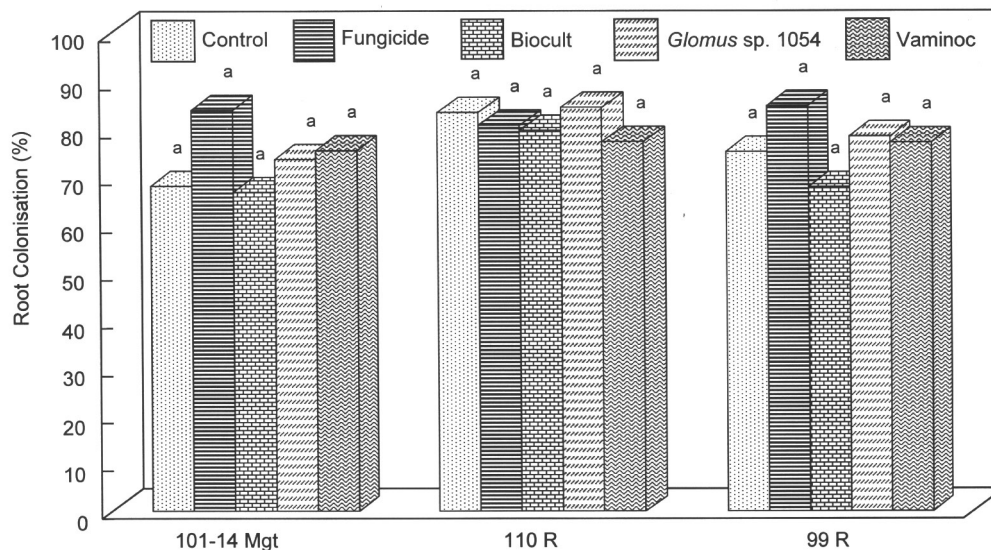


FIGURE 1

Maximum percentage of arbuscular mycorrhizal (AM) root colonisation of two-year-old Merlot grafted onto three different rootstocks (1999/00-season).
a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

species were present in the rhizosphere soils of both the inoculated and uninoculated (control) grapevines. There was considerable overlap in the species composition for the different treatments of each rootstock. Seven of the 18 species occurred at all the sites, namely: *Acaulospora spinosa*, *Gigaspora gigantea*, *Glomus mosseae*, *Glomus sinuosum*, *Scutellospora calospora*, *Scutellospora dipurpurascens* and *Scutellospora fulgida*. One species, *Gigaspora decipiens*, occurred in the soil of rootstock 101-14 Mgt, but was not detected in the other soils. This may indicate a specific association between this rootstock and *G. decipiens*, as all vines were obtained from the same nursery. The rest of the species mentioned in Table 2 were not associated with a specific treatment. Most of the AM fungal species detected belonged to the genera *Glomus*, *Acaulospora*, *Scutellospora* and *Gigaspora*, and were similar to those obtained from the soil of a vineyard located in northern Greece (Karagiannidis & Nikolaou, 1999).

In the present study, spores of *Acaulospora spinosa* and small-spored species of the genus *Glomus* listed in Table 2 accounted for the major proportion of the total volume of spores per sampling site. This is in agreement with other work, in which it was found that members of the genus *Glomus* commonly occur in field samples collected from vineyard soils (Karagiannidis *et al.*, 1997; Karagiannidis & Nikolaou, 1999). Although large spores of *Gigaspora gigantea* and *Scutellospora* species were frequently encountered at the various sampling sites, these spores accounted only for a minor proportion (*ca* 5%) of the total volume of spores per sampling site.

In a study by Franke-Snyder *et al.* (2001), where a range of agricultural soils and crops was investigated, *Gigaspora gigantea* was found to be present at higher spore concentration levels. It accounted for more than 60% of the total spore volume. The lower spore numbers of *Gigaspora gigantea* in the present study may be partially explained in terms of shifts induced in AM fun-

gal communities due to the application of nitrogen fertiliser. This was demonstrated elsewhere in fertilisation experiments, where increased nitrogen application was associated with the displacement of large-spored species of *Scutellospora* and *Gigaspora* by small-spored *Glomus* species (Egerton-Warburton & Allen, 2000). In the latter study, the nitrogen application level was about 60 kg/ha/yr, which is comparable to the 75 kg/ha/yr applied in this study.

It is important to note that only 5 out of the 18 species isolated from the vineyard soil were included in the original inocula. The rest of the species were indigenous to the vineyard and/or originated from the nursery from which the vines were obtained. Due to close similarity of morphological features between isolates of the same species (Abbott & Robson, 1982), it was impossible, using the methods employed in this study, to distinguish with certainty whether *Glomus etunicatum*, *Glomus intraradices*, *Glomus mosseae*, *Acaulospora scrobiculata* and *Acaulospora spinosa*, detected at the inoculated sites, could have originated from the Biocult and Vaminoc inocula. These species also occurred in the uninoculated soils, and similar levels of AM root colonisation were observed in the uninoculated and inoculated vine roots. Therefore, it can be assumed that the use of these species in the inocula did not result in higher levels of root colonisation. The other AM species present in the inocula, which can be positively identified using the criteria in this study, *i.e.* *Glomus caledonium*, *Glomus fasciculatum* and *Glomus versiforme*, were arguably either outperformed by the indigenous AM fungal species, or the vine roots were preferably susceptible for infection by indigenous AM fungi compared to these fungal species. A better interpretation of these results would have been possible if the infectivity potential of the individual AM strains in the inocula were measured prior to field inoculation and also if the number of spores per strain in the inocula was known.

TABLE 2

AM fungal species identified in the rhizosphere soils of 101-14 Mgt, 110 R and 99 R rootstock blocks (1999/00-season).

AM fungal species: (+) Present or (-) absent at the different treatments (T)	101-14 Mgt					110 R					99 R				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
<i>Acaulospora scrobiculata</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
<i>Acaulospora spinosa</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Gigaspora</i> sp.	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
<i>Gigaspora gigantea</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Gigaspora decipiens</i>	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>Glomus clavisporum</i>	-	-	+	-	-	+	-	+	+	+	+	+	-	+	+
<i>Glomus etunicatum</i>	+	-	+	-	-	+	-	+	-	+	+	-	+	-	-
<i>Glomus intraradices</i>	+	+	+	-	-	-	-	+	+	-	+	+	+	-	+
<i>Glomus mosseae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Glomus sinuosum</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Glomus tortuosum</i>	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
<i>Sclerocystis</i> sp.	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-
<i>Scutellospora</i> sp.	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
<i>Scutellospora calospora</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Scutellospora cerradensis</i>	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Scutellospora dipurpurascens</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Scutellospora erithropa</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Scutellospora fulgida</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

T1 = Control (uninoculated vines)

T2 = Fungicide treatment

T3 = Biocult

T4 = *Glomus* sp. 1054

T5 = Vaminoc

High spore concentrations of between 1000 and 3779 spores/100 g dry soil were observed during June 2000 (Table 3). Since spore concentrations are often higher during wet, rainy seasons than during dry seasons (Allen *et al.*, 1998; Schwob *et al.*, 1999; Picone, 2000), a decrease in the spore concentration was likely during the dry summer months normally experienced in the study area. The pH values (KCl) of soil from the spore sampling sites ranged between 5.63 and 6.10 (Table 3) and did not seem to have had a negative impact on spore abundance. It is known that most AM fungal species occur in soils around and above a neutral pH (Schubert & Cravero, 1985).

Spore numbers in soils may also indicate the ability of those soils to support infection of plant roots by AM fungi (Schubert & Cravero, 1985). The high spore numbers correlated well with the relatively high level of root colonisation, between 70% and 90% (Fig. 1). At the various sampling sites of each treatment per rootstock, there was no indication of a significant increase in spore numbers as a direct result of inoculation with commercial AM fungal species. Both the inoculated and uninoculated rhizosphere soils contained similar levels of spore concentrations (Table 3). The fungicide treatment resulted in a significant increase in the number of spores in the rhizosphere soils of 101-14 Mgt vines compared with the rhizosphere soils that received the commercial inocula, Biocult and Vaminoc (Table 3). The application of fungicides might have resulted in a reduction in hyperparasites, predators, or competing plant parasites, which could have encouraged rapid growth and dispersion of AM fungi (Menge, 1982).

TABLE 3

Spore concentrations of AM fungi and pH values measured at different soil plots of 101-14 Mgt, 110 R and 99 R rootstock cultivars (1999/00-season).

Treatment	Spore count (/100g dry soil)	pH (KCl)
101-14 Mgt		
Control	1296 ab	5.80 a
Fungicide	1458 a	5.78 a
Biocult	1000 b	5.83 a
<i>Glomus</i> sp. 1054	1234 ab	5.90 a
Vaminoc	1013 b	5.93 a
LSD (P = 0.05)	416	0.25
110R		
Control	1254 a	5.75 a
Fungicide	1317 a	5.75 a
Biocult	1075 a	5.70 a
<i>Glomus</i> sp. 1054	1925 a	5.85 a
Vaminoc	1887 a	5.63 a
LSD (P = 0.05)	973	0.26
99R		
Control	2242 a	5.78 a
Fungicide	2838 a	5.90 a
Biocult	3004 a	6.05 a
<i>Glomus</i> sp. 1054	3779 a	6.10 a
Vaminoc	2712 a	5.95 a
LSD (P = 0.05)	1594	1.27

a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level.

The high spore concentration recorded for the 99 R grapevines ranged between 2 242 and 3 779 spores/100 g dry soil, in comparison to 101-14 Mgt and 110 R grapevines where it never exceeded 2 000 spores/100 g dry soil. This may be ascribed to the presence of cover crops (grain sorghum) that were sown annually between rows of the 99 R vines. The cover crop may have facilitated a vast network of AM hyphae and spores interconnecting the roots of the cover crops and vines (Graves *et al.*, 1997). In contrast, no such network was possible in the ridged soils of the 101-14 Mgt and 110 R vines, due to the absence of cover crops and the annual removal of weeds at these sites (Meyer *et al.*, 2004).

The sandy character of the 99 R plots is another factor that might have contributed to higher spore concentrations at this sampling site. Observations regarding higher spore concentrations in sandy soils in comparison to heavier soils, such as the soils in which the 101-14 Mgt and 110 R vines were planted, were also made in other studies (Bhardwaj *et al.*, 1997; B. Bowman, personal communication, 2000).

CONCLUSIONS

This research clearly points to the importance of indigenous AM fungi in vineyard soils of South Africa. A wide range of AM species, producing an abundance of spores, might occur in a typical vineyard, depending on the soil management practices. These AM fungal taxa are similar to those recorded in vineyards of other countries by other workers.

Since it is difficult to predict the grapevine's response to inoculation in unsterile soils, this research underlines the importance of assessing the mycorrhizal status of a soil before inoculation is considered to avoid possible masking effects. It was found that inoculation is not required in soils containing adequate concentrations of efficient AM species. Adopting practices that encourage these fungal populations in soil is strongly recommended. Cover crop use may facilitate the formation of root-hyphal networks and enhance spore production.

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