RESEARCH NOTE

Young Grapevine Response and Root Colonisation following Inoculation with Arbuscular Mycorrhizal Fungi

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The host plant response following inoculation with commercially available arbuscular mycorrhizal (AM) fungi was determined in young grapevines under field conditions which would prevail on a typical farm. Measurements regarding growth improvement, nutrition and water relations were made in a field trial carried out on a commercial farm in the Stellenbosch region. Merlot noir grafted onto 101-14 Mgt and 110 Richter (110 R) in a Westleigh soil form and 99 Richter (99 R) in a Fernwood soil form was planted in December 1998. Vine roots were inoculated during planting with Biocult, Vaminoc and *Glomus* sp. 1054. Inoculation generally had little effect on xylem sap and leaf nutrient concentrations, water relations or growth responses. This was mainly ascribed to indigenous AM fungi, which seemed to have masked the effects of inoculation. A high soil P concentration was also implicated as a possible contributing factor to the general lack of grapevine response to AM inoculation.

The inoculation of vine roots with arbuscular mycorrhizal (AM) fungi may possibly promote healthy vine growth and survival, especially during the first year of vineyard establishment. Various species of AM fungi positively affected growth and development of young, pot-grown vines under greenhouse conditions with sterilised soil (Menge *et al.*, 1983). Other pot trial studies have shown that AM fungi allow for greater uptake of nutrients, thereby stimulating vine growth (Deal *et al.*, 1972; Karagiannidis *et al.*, 1995; Biricolti *et al.*, 1997). This increase in nutrient uptake by AM fungi is restricted to nutrients that are present at low concentrations and/or nutrients with low diffusion rates in soil, such as phosphorous (P) (Bolan *et al.*, 1987). Increased uptake of P was found to be the primary reason for increased growth in plants showing AM colonised roots (Gianinazzi-Pearson & Gianinazzi, 1983).

Under conditions of drought AM fungi may also play an important role in the acquisition of readily mobile nutrients such as nitrate (NO_3^-) (Smith *et al.*, 1985). It was found that the external mycelium of AM fungi is actively involved in NO_3^- uptake and transport (Tobar *et al.*, 1994). AM-facilitated uptake and transport of the readily mobile potassium ion, K^+, has also been demonstrated (Marschner & Dell, 1994), and it was found that AM fungal species might differ in K^+ uptake (Sieverding & Toro, 1988). Furthermore, it is known that AM fungi play an important role in plant water relations, but there is a distinct absence in AM-water relations reports in grapevines. However, since AM colonisation has been shown to improve the water relations in non-grapevine host plants (Auge *et al.*, 1986; Ruiz-Lozano & Azcon, 1995; Ruiz-Lozano *et al.*, 1995; Taylor, 1996), it is possible that AM inoculation may also enhance grapevine water relations.

The above-mentioned beneficial effects of AM fungi were demonstrated mainly during pot trials under controlled conditions, during which at least some of the plants were grown under sub-optimal conditions in sterilised soil. However, on a well-managed commercial farm indigenous AM fungi may commonly occur in the vineyards (Schubert & Cravero, 1985) and sub-optimal conditions regarding plant nutrition and water relations rarely exist. Adequate soil P concentrations usually occur and AM root colonisation may be inhibited by high soil P (Menge *et al.*, 1978; Brundrett *et al.*, 1996). AM-facilitated enhancement of plant growth may therefore be unlikely (Abbott & Robson, 1982). However, vines were shown to benefit from root colonisation, even when up to 40 mg/kg P was present in the soil (Schubert *et al.*, 1990).

The aim of the present study was to test commercially available AM inocula on vines under field conditions which would prevail on a typical farm. This entailed measuring vine growth, nutrition, drought stress and percentage root colonisation over the first season.

MATERIALS AND METHODS

Experimental layout

The study was carried out in a commercial vineyard planted on

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the farm Groenland in the Stellenbosch Region. Merlot noir grafted on three rootstocks, i.e. 101-14 Mgt, 110 Richter (110 R) and 99 Richter (99 R), was used. These rootstocks were selected to accommodate different soil forms: 101-14 Mgt and 110 R on a Westleigh soil form, which was ridged, and 99 R on an unridged Fernwood soil form (Soil Classification Working Group, 1991). Vines were planted in December 1998. Three similar randomised complete block designs were used to accommodate the different rootstocks. Five treatments (control, fungicide, Biocult®, *Glomus* sp. 1054 and Vaminoc®), replicated four times, were randomly allocated. An experimental unit (plot) consisted of 2 vine rows with 5 vines in each row and two buffer vines at each end of the experimental plot. Vines were planted 2.5 x 1.2 m apart. The dimensions of the planting holes were *ca* 300 x 300 x 300 mm in depth, width and length, respectively.

Treatments

The AM inocula applied during planting are listed below. All inocula contained healthy viable AM spores as determined microscopically (i.e. spores without visible damage and containing lipid droplets). The inocula were prepared according to the instructions of the manufacturers.

1. Biocult

Biocult contained, amongst other things, phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn) and boron (B), as well as *Glomus mosseae*, *Glomus intraradices, Glomus fasciculatum, Glomus etunicatum, Acaulosporae* spp. and *Trichoderma*. Each vine was treated with 50 mL nutrient-rich Biocult containing *ca* 160 AM spores, as well as other AM infective propagules. The inoculum was mixed with the soil in the planting hole.

***2. Glomus sp.* 1054**

A dose of 50 mL of bulked inoculum, in the form of soil containing *ca* 7200 AM spores and amended with colonised roots, was mixed with the soil in each planting hole. To ensure that all vines received the same amount of nutrients, a further 50 mL sterilised Biocult (steam sterilised in an autoclave at 121°C, 100 kPa for 60 min) was added to the soil-inoculum mixture.

3. Vaminoc

The inoculum contained spores of *G. mosseae*, *G. fasciculatum*, *Glomus caledonium* and *Glomus versiforme* in a clay-amended medium. Each planting hole received 5 g, containing *ca* 920 AM spores in total, of the Vaminoc inoculum that was mixed with the soil. A further 50 mL sterilised Biocult was added to the soil-inoculum mixture.

4. Fungicides

A suspension containing the systemic fungicide Benlate® WP (100 g/100 L H_2O; active ingredient: benomyl) and the contact fungicide Rovral Flo® SC (200 mL/100 L H_2O; active ingredient: iprodione) was used as secondary control to inhibit AM fungal growth (Kjøller & Rosendahl, 2000) in and around the vine roots. A 50 mL volume of sterilised Biocult was mixed with the soil in each planting hole and soil was added to fill the hole completely. The soil was subsequently drenched with a combination of the above-mentioned fungicides. Each vine received 2 L of mixed fungicides that covered an area of *ca* 300 mm radius around the vine.

5. Control

A 50 mL volume of sterilised Biocult was mixed with the soil in each planting hole. Neither fungicides nor AM inocula were added.

Cultivation practices

Standard soil-management practices were maintained, which can be summarised as follows:

Previous crop

Wine grapes were established in 1976 and were removed during winter in 1998, prior to the establishment of the current vineyard.

Tillage

Before planting, the soils were deeply ploughed to a depth of 800 mm. At this stage P was added to meet the prescribed nutritional requirements. Lime was added to adjust the pH (KCl) to *ca* 5.5.

Irrigation

Irrigation was applied on a supplementary basis. The 99 R grapevines were irrigated with an overhead system (12 mm/h for 3h at a time), whereas 101-14 Mgt and 110 R grapevines received micro-irrigation (3 mm/h for 12h at a time).

Vine nutrition

During April 1999 limestone ammonium nitrate (LAN) was applied at 75 kg/ha on all three rootstock plots.

Pest and disease control

Pests and diseases were managed according to a standard programme, spraying approximately every 2 weeks, throughout the season. Powdery mildew was controlled with the commercially available fungicides, Olymp® 100 EW (active ingredient: flusilazole) and Sabithane® 400 EC (active ingredients: dinocap and myclobutanil). Sulphur dust was also applied. Downy mildew was controlled with Sancozeb® 80 DP (active ingredient: mancozeb) and Curzate Pro® WP (active ingredients: cymoxanil and mancozeb).

Weed control

During the course of this trial weeds in the vineyards were hand hoed.

Microscopic analyses

Root samples (*ca* 3 g sample for each of 2 randomly chosen vines per experimental unit), representative of each of the 4 replicates per treatment, were taken in April 1999 and stored in 50% ethanol at room temperature until analysed. Roots were first subjected to clearing and staining procedures as descibed by Brundrett *et al.* (1994). For microscopic analysis only the finer stained roots (0.3 – 0.5 mm in diameter) were mounted in Polyvinyl-Lacto-Glycerol (PVLG) on slides by arranging 20 mm length root segments using a fine forceps to accommodate 25 segments per slide. Mounted root segments were covered with cover slips. Four slides per sample were prepared. Percentage root colonisation by AM fungi was calculated as described by Brundrett *et al.* (1994).

Field and laboratory measurements

In December 1998 soil analyses were performed on composite soil samples (twelve in total), obtained from eight sub-sampling sites from each rootstock plot (two per replication) that represented soil variation in the vineyard. The sub-samples (*ca* 500 g each) were taken over 0-150 mm, 150-300 mm, 300-600 mm and

600-900 mm depth increments, using a standard soil auger. Soil analyses were subsequently carried out on the composite samples in accordance with methods prescribed by The Non-affiliated Soil Analysis Work Committee (1990). By using a pressure bomb, the leaf water potential was regularly measured between 10 am and 3 pm, according to standard procedures (Scholander *et al.*, 1965). For each treatment this measurement was performed on one randomly selected leaf per replicated treatment. These analyses were carried out at two-week intervals from February 1999 until April 1999.

In late April 1999 vigour was determined by measuring the total shoot length per vine. Five selected vines per treatment plot were used to calculate mean shoot length. Xylem sap samples were taken during April 1999. Sap collection was carried out in the field in the morning between 08:00 and 11:00. The sap was extracted by modifying the standard procedure for measuring the leaf water potential (Scholander *et al.*, 1965). Similar to the leaf water potential measurements, minimal pressure was exerted to extract sap from the tip of the petiole. The pressure differed slightly when different leaves were used, but on average a pressure of approximately -1300 kPa was sufficient for sap excretion. The pressure was then regulated to allow for a continuous flow of sap, which was subsequently collected using a Gilson pipette and 1.5 mL eppendorf tubes. *Circa* 90 µL sap was collected from one randomly selected leaf per replicated treatment. Each selected leaf was a fully expended, mature leaf on the same position of each plant. The time designated for each excretion varied in accordance with the different leaves. While excretion was in progress, the collected samples were kept on ice in a cooler bag. Subsequently, the sap was frozen at -80°C until the chemical analyses were conducted. The phosphate, nitrate and amino acid concentrations in the xylem sap samples were determined according to the methods of Murphy & Riley (1962), Rosen (1957) and Nydahl (1976), respectively. The leaf mineral concentrations were also determined. Leaves were sampled during March 1999, slowly dried to constant mass in a fan oven at 70°C, milled and dry ashed in a microwave furnace. The residues were taken up in acidified, distilled water, diluted to 100 mL and analysed for P and K using a Varian Liberty 200, inductively coupled plasma atomic emission spectrometer. Nitrogen was determined on the milled plant material using a Leco Nitrogen Determinator.

Statistical procedure

Statgraphics version 7 and SAS version 6.12 packages were used for the statistical analyses of the data. The analyses were done for each rootstock separately since the rootstocks were planted on different soil forms.

1. The percentage data (root colonisation data) were arcsine transformed (Zar, 1981). The influence of the factors and their interactions were tested with a one-way analysis of variance (1-way ANOVA) (Statgraphics version 7, 1993, Statgraphics Corporation, USA). 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 (xylem sap nutrient concentration, leaf nutrient concentration, vine growth and leaf water potential) were analysed using SAS version 6.12 (SAS, 1990). The analyses were performed on observations made for one season and for three different rootstocks with five different treatments. The influence of these factors and their interactions were tested with an ANOVA.

Fisher's Least Significance Differences were calculated at the 5% significance level to compare treatment means (Ott, 1993). Shapiro-Wilks's test was performed to test for non-normality (Shapiro and Wilk, 1965).

RESULTS AND DISCUSSION

Results obtained showed that the vines of all three rootstocks, i.e. 101-14 Mgt, 110 R and 99 R, were colonised by AM fungi, including the fungicide treatments and controls, ranging from 40% to 85% (Fig. 1). This is in agreement with previous observations that AM fungi naturally colonise grapevine roots (Possingham & Groot Obbink, 1971; Deal *et al.*, 1972; Gebbing *et al.*, 1977; Menge *et al.*, 1983; Nappi *et al.*, 1985; Schubert & Cravero, 1985; Nikolaou *et al.*, 1994). The high level of root colonisation occurred despite high soil P concentrations of up to 89 mg/kg P, which would usually inhibit AM fungal development in roots (Brundrett *et al.*, 1996).

Although the level of root colonisation for the treatments was generally similar, 99 R vines that were treated with Biocult exhibited significantly higher levels compared to the controls and Vaminoc-treated vines (Fig.1). In vines treated with fungicides, root colonisation may have been the result of indigenous AM fungi present in the vineyard, since it is known that the fungicidal effect eventually fades, allowing for the invasion and colonisation of vine roots by indigenous AM fungi present in soil surrounding the vine roots (Menge, 1982). The fungicides, Rovral Flo (contact fungicide) and Benlate (systemic fungicide), were presumed to have inhibited the external and internal phases of AM fungal development, respectively. These fungicides are commonly used in agriculture and are known to inhibit AM fungal growth (Kjøller & Rosendahl, 2000). However, since the immediate impact of the fungicides on fungal growth in and on the vine roots was not determined in this experiment, any discussion on possible re-invasion by AM fungi originating from the vineyard remains speculative.

Therefore, it can be agreed that a large percentage of the observed colonisation in all vine roots, including AM-treated vines, of which the morphology of the indigenous taxa was distinguishably different from those in the inocula, was due to fungi that probably originated from the vineyard soil itself and/or nursery soils (Fig. 2). Since all vines were colonised irrespective of the treatment applied, the implication was that no AM-facilitated improvements regarding growth, water stress and nutrition would be expected unless the inoculant AM fungi were superior to the indigenous AM fungi.

Compared to the controls, few AM-induced increases regarding xylem sap and leaf nutrient concentrations, as well as growth responses, were observed (Fig. 3, Table 1). This also applied for Biocult-treated 99 R vines, where a significantly higher level of root colonisation compared to the controls was observed, but no concurrent nutritional or growth benefit. The lack of AM-induced growth differences may also be attributed to the high levels of soil P (Abbott & Robson, 1982) recorded during this study, which were notably higher than the recommended phosphate fertilisation level for this vineyard [20 to 25 mg/kg P (Van Schoor *et al.*, 2000)]. The

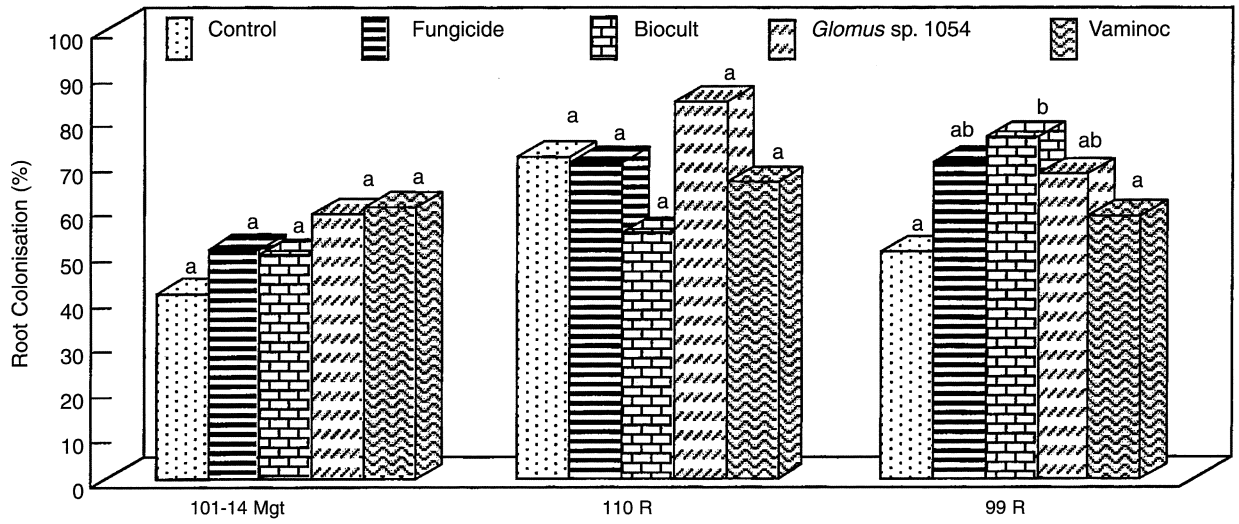

FIGURE 1

Maximum percentage arbuscular mycorrhizal (AM) root colonisation of one-year-old Merlot noir grafted onto three different rootstocks (1998/99-season). a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level. Statistical package Statgraphics version 7 was used for the statistical analyses of the data. The analyses were done separately for each rootstock.

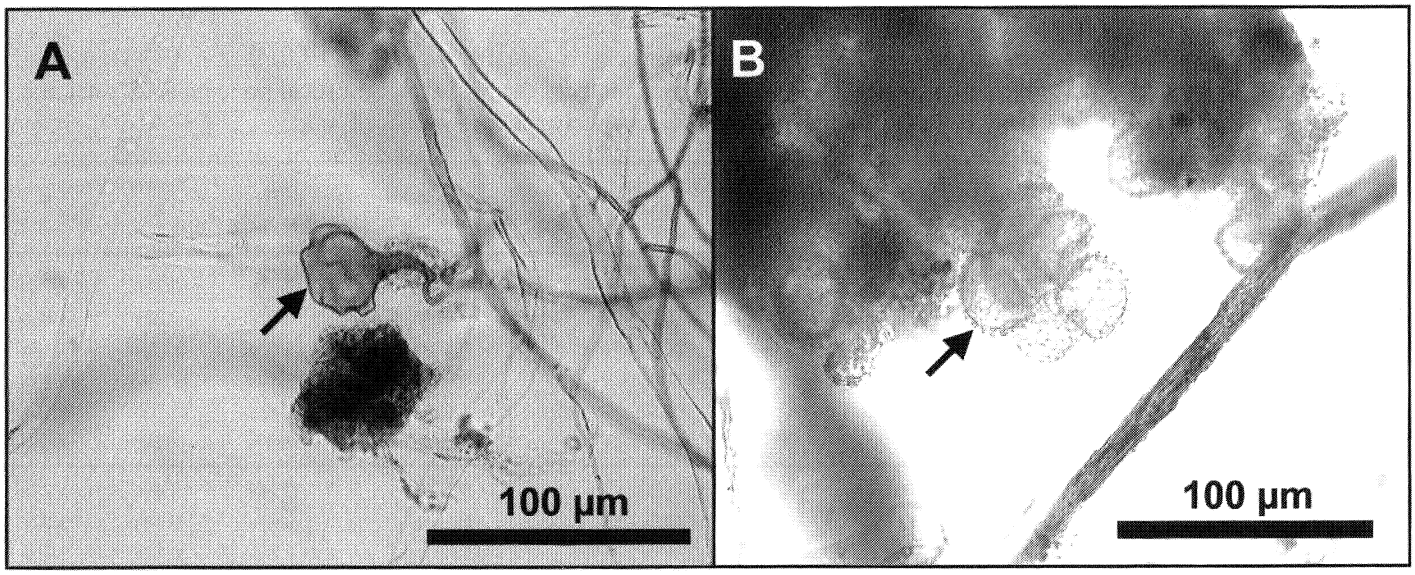

FIGURE 2

Structures typical of arbuscular mycorrhizal (AM) genera (Morton, 2001) not included in the commercial AM inocula, but which were found during microscopic analyses of the vine roots, indicating the presence of indigenous fungi originating from the vineyard and/or nursery soils. The arrows indicate auxiliary cells of *Scutellospora* (**A**) and *Gigaspora* (**B**).

only positive growth response was observed in 110 R vines inoculated with *Glomus* sp. 1054. Given the observation that these vines also tended to be the most colonised of all the vines, including the controls (Fig. 1), this may partially explain the increased growth. However, no concurrent nutritional benefit was observed (Table 1).

Vine nutrition appeared to be relatively unaffected by inoculation, since only a few measurements showed significant differences and only for 101-14 Mgt vines, i.e. differences in xylem sap nitrate concentrations between Biocult and fungicide-treated vines, as well as differences in leaf P concentrations between Vaminoc-treated vines, on the one hand, and the controls and fungicide-treated vines, on the other (Table 1). In the latter case the concentration of nutrients in the Vaminoc-treated vines, was lower than in the controls and fungicide-treated vines, thereby contradicting the observation made for root colonisation in the Vaminoc-treated vines (Fig.1). The observed root colonisation in these vines was higher (but not significant) than in the controls and in the fungicide-treated vines.

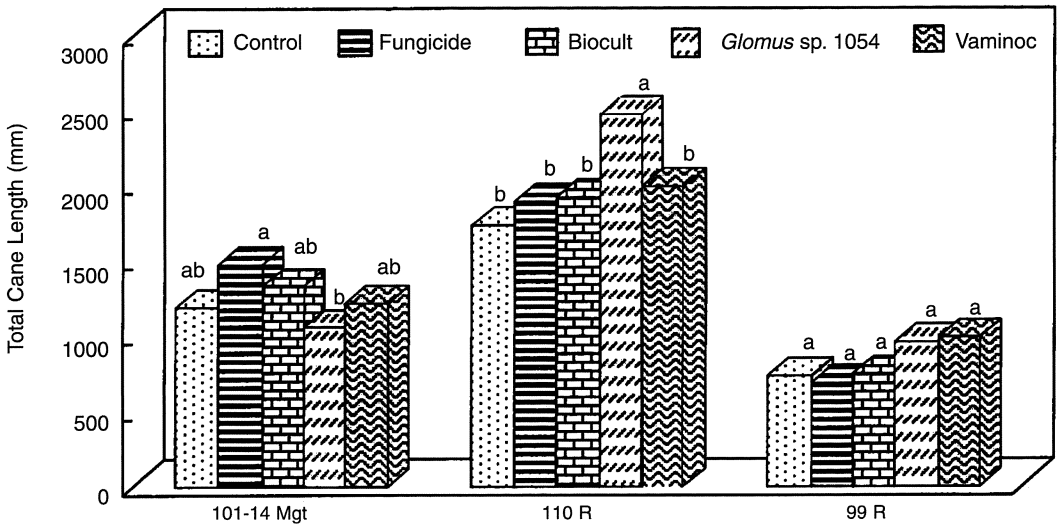

FIGURE 3

Total cane length (mm) per vine of one-year-old Merlot noir grafted onto three different rootstocks (1998/99-season). a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level. Statistical package SAS version 6.12 was used for the statistical analyses of the data. The analyses were done separately for each rootstock.

TABLE 1

*Xylem sap and leaf nutrient concentrations of one-year-old Merlot noir grafted onto 101-14 Mgt and 110 R (1998/99-season). a, b: Values accompanied by a common letter per rootstock do not differ significantly at the 5% level. Statistical package SAS version 6.12 was used for the statistical analyses of the data. The analyses were done separately for each rootstock.

Root-stock*	Treatment	Nitrate µmol/L	Xylem sap Phosphate µmol/L	Amino acids mmol/L	N % w/w	Leaves P % w/w	K % w/w
101-14 Mgt	Control	227.2ab	305.6a	0.688a	2.91a	0.25a	1.22a
	Fungicide	111.2a	231.2a	0.424a	2.83a	0.25a	1.06a
	Biocult	311.2b	371.2a	0.432a	2.83a	0.22ab	1.14a
	Glomus sp. 1054	225.6ab	372.0a	0.488a	2.79a	0.23ab	1.09a
	Vaminoc	160.8ab	311.2a	0.632a	2.85a	0.20b	1.19a
	LSD (P = 0.05)	182.4	165.6	0.400	0.13	0.04	0.18
110 R	Control	105.6a	375.2a	0.144a	2.74a	0.41a	1.27a
	Fungicide	85.6a	263.2a	0.184a	2.85a	0.34a	1.22a
	Biocult	168.8a	273.6a	0.304a	2.81a	0.32a	1.16a
	Glomus sp. 1054	80.0a	264.0a	0.112a	2.75a	0.35a	1.29a
	Vaminoc	184.0a	336.8a	0.160a	3.14a	0.32a	1.20a
	LSD (P = 0.05)	221.6	232.0	0.232	0.61	0.13	0.23

* No tests were carried out on 99 R due to insufficient sap.

Vines, planted in mid-December, were at times exposed to high summer temperatures and even dry soil conditions. The leaf water potential fluctuated variably and, with the exception of a few measurement dates, the vines generally exhibited severe water stress, above the norm of -1200 kPa (Fig. 4). In three out of seven measurement dates 101-14 Mgt vines treated with Vaminoc appeared to be more tolerant to water stress than the controls. Although not significant, the Vaminoc-treated vines exhibited water potential 0.2 MPa higher than the controls. These vines also exhibited a tendency to be more colonised with AM fungi than the controls (Fig. 1). This particular positive response should be further investigated in future.

Irrespective of a general lack of positive responses observed during the trial, no setbacks in vine growth performance during periods of fluctuating or insufficient moisture were visible and less than 1% dieback was recorded for the vines (data not shown). Vine survival occurred irrespective of the late planting or the soil type in which the vines were grown.

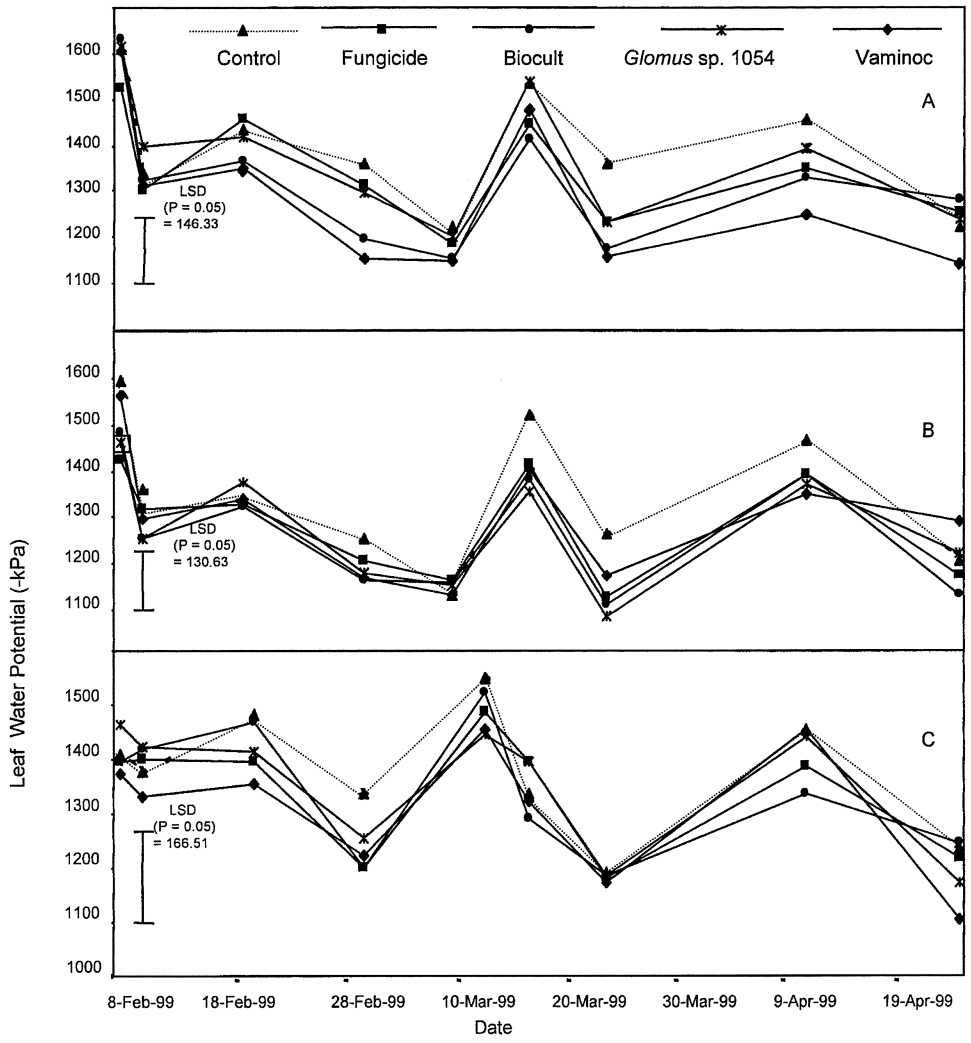

FIGURE 4

Leaf water potential of one-year-old Merlot noir grafted onto rootstocks 101-14 Mgt (**A**), 110 R (**B**) and 99 R (**C**) (1998/99-season). Statistical package SAS version 6.12 was used for the statistical analyses of the data. The analyses were done separately for each rootstock.

CONCLUSIONS

Since the study was preliminary in nature and of limited scope, the results must be interpreted in full recognition of a number of constraints. Principal among these was the fact that the commercially available inocula had not been subjected to infectivity tests prior to planting. Such tests should be included in future trials of this nature, because this may allow for better interpretation of results. Also, no prior tests had been conducted on the plant material for the presence of AM infection to conclude whether AM root colonisation had already occurred in the nursery. Furthermore, there was no indication whether the observed effects of inoculation would have persisted, had the trial been repeated over a longer time period.

Nevertheless, this study highlighted the general lack of positive responses that may occur in a typical commercial vineyard upon AM inoculation. The presence of indigenous AM fungi seemed to have masked the effects of inoculation. In addition, an adequate supply of soil P to the vines could also have contributed to the general lack of AM-mediated responses. Therefore, it is strongly recommended that the mycorrhizal status of a soil should be assessed before field inoculation is considered.

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