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Effect of Two *Glomus* Species Inoculations on Survival, Photosynthetic Capacity, Growth, Morphology and Root Ginsenoside Content of *Panax quinquefolius* L.

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Abstract : Vesicular-arbuscular mycorrhizae (VAM) fungi naturally colonise American ginseng roots and this relationship is highly beneficial to enhance plant productivity. Our goal was to determine the effect of adding two *Glomus* species (*Glomus etuticatum, G. intraradices*) on survival, photosynthetic capacity, growth, morphology and root ginsenoside content of one-year-old American ginseng plants grown in a broadleaf forest. While our study revealed that VAM inoculations significantly affected root morphology and Re ginsenoside content, the survival, photosynthetic capacity and root growth of American ginseng plants were not significantly influenced by VAM inoculations. Surface area and volume of rootlets were 16-25% higher for ginseng grown in VAM-inoculated soil compared to those grown in the control plots. Also, Re ginsenoside content was 18 % higher in VAM-inoculated roots compared to controls.

Key words : *Glomus etuticatum, G. intraradices*, American ginseng, VAM mycorrhizae.

INTRODUCTION

American ginseng (*Panax quinquefolius* L.) is an esteemed medicinal plant indigenous to North American broadleaf forests.[1] Unfortunately, natural populations of American ginseng are dangerously close to extinction because of overharvest[2] and destruction of their natural habitat.[3] Presently, American ginseng is commercially grown under artificial shade and also increasingly in forests.[3,4] Forest-grown ginseng roots are more valuable on markets[3,5] and contain higher ginsenoside contents[6,7] than those cultivated under artificial shade. However, root yield of forest-grown American ginseng is greatly stunted,[3] and the time required to obtain marketable roots is two to eight years longer compared to those cultivated under artificial shade.[8]

Both in field and forest cultures, American ginseng roots are naturally colonised by vesicular-arbuscular mycorrhizae (VAM) fungi,[9,10,11] and a higher VAM fungi soil content generally equates with a faster colonisation rate.[12] American ginseng plants colonised by VAM fungi show higher survivorship and increased growth,[13] higher photosynthetic rate[14] and enhanced nutrient uptake.[10] Ginseng plants colonised by mycorrhizae also exhibit resistance to root rot caused by *Fusarium* spp.[15]

Although few studies have looked at the influence of adding various *Glomus* species to the soil in natural settings, previous studies have found that one-year-old American ginseng plants grown in greenhouses are successfully inoculated with *Glomus* species (*Glomus etuticatum, G. intraradices*), and plants contain increased amounts of phosphorus (P), potassium (K) and magnesium (Mg) in their roots compared to non-inoculated plants.[10] Also, greenhouse-cultivated American ginseng grown in VAM-inoculated soil has 15%[10] to 43%[13] higher root weight than control plants. The objective of this study was to elucidate the effect of adding a mixture of *Glomus etuticatum* and *G. intraradices* mycorrhizae to forest soil during seeding on survival, photosynthetic capacity, growth, and ginsenoside content of one-year-old American ginseng grown in a broadleaf forest.

MATERIALS AND METHODS

1. Experimental design

The experiment was conducted in a broadleaf forest located at Île d'Orléans, Québec, Canada (lat. 46.58, long.

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70.58) with one-year-old American ginseng (*Panax quinquefolius* L.) plants collected in August 2000. In September 1999, stratified American ginseng seeds were sown at a density of 30 kg ha^{-1} in either VAM-inoculated or non-inoculated soil (control) in sixteen experimental units measuring 1 m^2 arranged in a randomised block design (total of 8 blocks). The VAM inoculate was a mixture of *Glomus etuticatum* and *G. intraradices* (Premier Tech, QC, CAN).

Daily light levels were measured at the end of the growing season in each experimental unit and averaged 8.7 mol m^{-2} d^{-1} in control plots and 8.6 mol m^{-2} d^{-1} in VAM-inoculated plots, equivalent to less than 5 % light penetration rates. Light variables were included in the statistical analyses as co-variables. ANOVA and LSMEANS tests were performed with SAS, version 6.12 (SAS Institute Inc., NC, USA). Since only four plants survived in one of the experimental units, samples were collected in 15 units (15 samples per unit), except for roots collected for ginsenoside analyses. In this case, five samples were collected in 8 experimental units (4 blocs).

2. Survival, photosynthetic capacity, growth and morphology measurements

Plant survival was determined as the percentage of individuals that survived to August 11 2000 compared to those that had successfully emerged by June 19 2000. Chlorophyll *a* fluorescence induction kinetics were measured in 15 experimental units on third leaflets on August 13 2000 using a Plant Efficiency Analyzer (PEA, Hansatech Ltd., Norfolk, UK) during a 1-sec flash of bright red light (3250 μmol m^{-2} s^{-1} centred at 650 nm). The minimal (F_o) and the maximal (F_m) unquenched yields of Chlorophyll *a* fluorescence were measured, and the maximum quantum yield of photosystem II (PSII) photochemistry was estimated using the ratio F_v/F_m, where $F_v=F_m$ F_o.[16,17] The measurements were recorded from 8:30 to 10:00 on 25-minute dark-adapted.

To evaluate growth and morphology, five ginseng plants were collected in 15 experimental units on September 6 2000. Growth parameters were determined for each sample, and then an average was calculated for each experimental unit. The fresh and dry weights of the shoots and roots were determined prior to and after a 48-hour drying period at 40°C.[18] The third leaflet surface area, width and length were measured using a LI-3000A planimeter (LI-COR, inc., Lincoln, Nebraska, USA) and stem length was also determined. Root morphological analyses were performed on fuchsin (0.005% solution) stained roots using Win Rhizo™, version 3.8a (Régent Instrument Inc., Québec, Canada).[7]

3. Root ginsenoside analyses

On September 6 2000, five one-year-old American ginseng roots were collected from eight experimental units in VAM-inoculated and control plots, and then dried in an oven at 45°C for 72 hours prior to ginsenoside analyses.[7] Content of six major ginsenosides (Rb_1, Rb_2, Rc, Rd, Re and Rg_1) were determined in American ginseng roots[7] for each sample, and then an average was calculated for each experimental unit.

RESULTS

Our results showed that the effect of adding two *Glomus* species to the soil did not significantly affect survival of American ginseng plants during the growing season, although we noted that there was an overall higher survival of ginseng plants growing in VAM-inoculated plots (76%) compared to control units (66%) (data not shown). Our results demonstrated that the photosynthetic capacity,

Table 1. Effect of VAM inoculations on root morphology of one-year-old American ginseng plants collected in September 2000 in a broadleaf forest

	Average diameter (mm)	Total volume (m^3)	# tips	# forks	Surface area (cm^2)		Volume (m^3)	
					Diam.≤1 mm	Diam.>1 mm	Diam.≤1 mm	Diam.>1 mm
Treatments								
Control	0.64±0.02[z]	0.09±0.01	63.2±7.3	78.7±8.8	4.3±0.5	1.2±0.2	0.04±0.00	0.04±0.01
VAM	0.62±0.02	0.10±0.01	69.1±6.5	81.6±5.0	5.0±0.4	1.3±0.1	0.05±0.00	0.05±0.00
GLM procedure								
Total light	NS[y]	NS	NS	NS	NS	NS	NS	NS
Bloc	NS	*	NS	NS	NS	NS	NS	NS
VAM	NS	*	NS	NS	*	NS	*	NS

[z]Each value represents the mean (±standard error) for 15 experimental units (5 samples per unit).
[y]NS, *: non-significant and significant at P≤0.05.

estimated using F_v/F_m, was not significantly influenced by VAM inoculations where average F_v/F_m values were 0.82 for plants grown in VAM-inoculated and controls plots (data not shown). Our results further showed that effect of blocs significantly influenced photosynthetic capacity where the average F_v/F_m for one bloc was significantly lower (0.79) than for the others, which had F_v/F_m above 0.82 (data not shown).

Shoot morphology (Leaflet area, length, width and stem length), and shoot and root weights of one-year-old American ginseng plants were not significantly influenced by VAM inoculations (data not shown). On the other hand, surface area and volume of rootlets (Diameter≤1 mm) grown in VAM-inoculated soil were 16 % and 25 % higher than of those cultivated in the control plots (Table 1). While surface area and volume of taproots (Diameter >1 mm) remained uninfluenced by VAM inoculations, the total root volume was significantly higher when plants were grown in VAM-inoculated soil compared to controls (Table 1).

VAM inoculations significantly influenced Re ginsenoside content in one-year-old American ginseng plants, while Rg_1, Rb_1, Rc, Rb_2, Rd, and total ginsenoside contents were not significantly influenced (Table 2). However, ginsenoside contents were consistently higher in VAM-inoculated roots than in control roots (Table 2). While Rb_2 ginsenoside content was not significantly affected by VAM inoculations, both total light and blocs significantly influenced its content (Table 2).

DISCUSSION

In contrast to previous studies performed in greenhouses,[10] our results showed that there was no significant difference between survivorship of American ginseng plants grown in VAM-inoculated and control plots. The survival of ginseng plants varied greatly in the experimental site, ranging from 41% to 100% (data not shown). Nonetheless, the average survivorship (70%) of one-year-old ginseng plants was similar to that obtained by previous studies,[5,18] and it was within the optimal range for stratified American ginseng seeds.[5]

In the present study, VAM inoculations did not significantly influence the photosynthetic capacity of American ginseng plants, but values were high compared to previous studies with Asian ginseng (*Panax ginseng* C.A. Meyer) and American ginseng.[19,20,21] To our knowledge, only one study, tested on two-year-old American ginseng plants cultivated in a forest, showed average F_v/F_m values of 0.82 at the end of the growing period.[7] Thus, in our experimental site, VAM inoculations were not necessary to enhance the photosynthetic capacity of one-year-old American ginseng, which was already at optimal levels.

The shoot morphology and growth of American ginseng plants were not significantly influenced by VAM inoculations. Growing American ginseng plants in VAM-inoculated soil containing *G. intraradices* could have contributed to decreasing root growth since Asian ginseng plants grown in *G. intraradices*-inoculated soil have significantly lower protein contents compared to non-inoculated plants, causing lower root weight.[22] Compared to previous studies, the fresh weight of one-year-old American ginseng roots was 59% and 78% lower than that obtained when plants are grown in a forest[7] and under artificial shade,[8] respectively. A closer examination of our results showed that ginseng plants were exposed to low light levels: less than 10% of the solar radiation. Root growth for Asian and American ginseng is maximised when plants are cultivated under 20% of the solar radiation but not when they are exposed to less than 10% of the solar radiation.[7,20,23] Similarly, Charest *et al.*[18] showed that the root fresh weight of one-year-old ginseng plants

Table 2. Effect of VAM inoculations on root ginsenoside contents in one-year-old American ginseng plants collected in September 2000 in a broadleaf forest

	Rg_1(mg·g^{-1})	Re(mg·g^{-1})	Rb_1(mg·g^{-1})	Rc(mg·g^{-1})	Rb_2(mg·g^{-1})	Rd(mg·g^{-1})	Total(mg·g^{-1})
Treatments							
Control	1.8±0.1[z]	7.0±0.4	4.3±0.4	4.1±0.8	0.8±0.2	2.8±0.2	20.9±1.0
VAM	1.9±0.2	8.3±0.6	4.5±0.8	6.0±0.7	0.9±0.3	3.1±0.2	24.7±0.5
GLM procedure							
Total light	NS[y]	NS	NS	NS	*	NS	NS
Bloc	NS	NS	NS	NS	**	NS	NS
VAM	NS	*	NS	NS	NS	NS	NS

[z]Each value represents the mean (±standard error) for 8 experimental units (5 samples per unit).
[y]NS, *, **: non-significant and significant at p≤0.05 and p≤0.01.

grown under 11 μmol m^{-2} s^{-1}, equivalent to less than 6% of the solar radiation, was 0.06 g, which is 74% lower than that recorded in our study.

The root morphology was significantly influenced by VAM inoculations, as was previously shown for American ginseng[10] and other plants.[24] Our results showed that one-year-old ginseng grown in VAM-inoculated soil had higher rootlet surface area and volume than plants grown in control plots, which has been shown to maximise the absorption of P, Mg and K by the roots.[10,25] Ginseng plants grown in VAM-inoculated soil could also have absorbed higher levels of Zn and Mn, as was demonstrated for cucumber plants.[26]

Our results provided the first evidence that root ginsenoside contents increase when American ginseng plants are grown in VAM-inoculated soil compared to non-inoculated soil. Although Han *et al.*[15] showed that the penetration of VAM fungi in ginseng rootlets stimulates plant resistance to *Fusarium* spp., a pathogen that causes substantial profit loses in commercial exploitation,[27] the effect of VAM on secondary metabolite contents had not been reported. Our study showed that the Re content was significantly influenced by VAM inoculations, and both Re and total ginsenoside contents in ginseng roots were 18 % higher in plants grown in VAM-inoculated plots compared to controls. The Re ginsenoside is generally one of the most abundant ginsenoside in American ginseng roots,[3,8,28] accounting for between 33% and 34% of total ginsenoside content in our study. We also noted that the content of individual ginsenosides (Rg_1, Re, Rb_1, Rc, Rb_2 and Rd) was higher in VAM-inoculated roots than in control roots, suggesting that *Glomus* species inoculations stimulated the overall ginsenoside production and not only the accumulation of one or several ginsenosides. Other factors also significantly influenced the ginsenoside content in one-year-old ginseng roots since both the bloc formation and light conditions affected the Rc content. Our results are concordant with previous results which demonstrated that the understory light conditions affected more significantly Rc than the other five ginsenosides.[7]

In summary, our results are concordant with previous studies performed within controlled conditions and also provide great insight on the effect of VAM inoculations on American ginseng growing in natural conditions. Our results suggest that *Glomus* species added to forest soil during seed sowing (autumn) survived winter freezing even if they were not associated with plants. Our study further suggests that VAM fungi remained viable and successfully associated with ginseng seedlings in spring, which is concordant with results obtained by Addy et al.[29] with external mycelium of VAM fungus. Furthermore, our study showed that VAM inoculations significantly increased root surface area and root ginsenoside contents of American ginseng plants, which might be beneficial not only for enhanced phytopathological protection of plants, but also for ginseng growers that can produce high quality roots.

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