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MYCORRHIZAL *GLOMUS* SPP. VARY IN THEIR EFFECTS ON THE DYNAMICS AND TURNOVER OF FINE ALFALFA (*MEDICAGO SATIVA* L.) ROOTS

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Abstract

The distribution of fine roots in the soil profile has important implications related to water and nutrient uptake. The objective of this study was to compare the effects of different arbuscular mycorrhizal fungi (AMF) on the fine root dynamics of *Medicago sativa* L. cv. Sanditi. We used minirhizotrons to observe changes in fine root length density (FRLD, mm/cm^2) and fine root surface area density (FRSAD, mm^2/cm^2) during the growing season. Fine root P concentrations and turnover rate were also measured. The colonization rate of fine roots varied depending on the AMF species. Colonization rates were highest when roots were inoculated with *Glomus mosseae* and lowest when roots were inoculated *G. intraradices*. Inoculation with AMF significantly increased both FRLD and FRSAD. *G. versiforme* increased FRLD and FRSAD most, whereas *G. mosseae* had the least effect. Inoculation with AMF also decreased fine root turnover rates. Inoculation with a mixture of AMF species increased fine root turnover and P concentrations more than inoculation with a single AMF species. Fine root length density increased to a maximum on Aug. 6 and then decreased. In comparison, FRSAD exhibited two peaks during the growing season. Overall, the results indicated that inoculation with AMF can significantly promote fine root growth and P uptake by alfalfa growing on soil with low P availability. The AMF may preserve fine root function late in the growing season.

Key words: Arbuscular mycorrhizal fungi, Fine root, Root length density, Root surface area density, Phosphorus content.

Plant roots are important because of their involvement in (i) the absorption of water and nutrients from the soil, (ii) the synthesis of some organic compounds, and (iii) the transport and storage of carbohydrates and nutrients (Liu, 2009; Wu *et al*., 2011). Fine roots (i.e., roots that are < 2 mm in diam) make up only a small proportion of total plant biomass; however the investment of C in the development of fine roots has important implications related to water and nutrient uptake (Comas *et al*., 2002; Zangaro *et al*., 2008). Fine roots are also a major component of C and nutrient cycles in terrestrial ecosystems (Shi *et al*., 2007; Son & Hwang, 2003).

Fine root function is affected by biotic and abiotic factors. The effects of soil microbial activity are especially important (Yao *et al*., 2009; Wu *et al*., 2010). A symbiotic relationship exists between arbuscular mycorrhizal fungi (AMF) and plant roots. Arbuscular mycorrhizal fungi promote the growth of their host plants by increasing water and nutrient uptake (Wu *et al*., 2011). The host plants aid AMF by providing energy in the form of carbohydrates. Many plant species in natural ecosystems rely heavily on AMF for water and nutrient uptake (Read & Perez-Moreno, 2003; Hinsinger *et al*., 2005). The hyphae of AMF can acquire nutrients from well beyond the limits of the rhizosphere depletion zone (Li *et al*., 1991). Reports also indicate that AMF can reduce biological stresses on the host plant (Zangaro *et al*., 2008), increase plant tolerance to drought, and improve root longevity (Eissenstat *et al*., 2000) and decrease the fine root turnover rate.

Arbuscular mycorrhizal fungi can modify the root architecture and morphology of their host plants; however, reports are inconsistent about the exact modifications. Studies have shown that plants with short roots and larger diameter fine roots benefit more from symbiosis with AMF than do plants with long roots and smaller diameter fine roots (Zangaro *et al*., 2007). Mycorrhizal root colonization is reported to be negatively correlated with fine-root diameter in fertile and in infertile soil (Zangaro *et al*., 2007).

Alfalfa (*Medicago sativa* L.) has a large root system. Many lateral roots of alfalfa are colonized by AMF. These AMF improve both the persistence and the aboveground biomass production of the alfalfa. The physiological function of fine roots may be better reflected by fine root length density (FRLD, length of fine roots per unit area) and fine root surface area density (FRSAD, surface area of fine roots per unit area) than by biomass. Minirhizotrons permit the observation of roots *in situ*, with minimal disturbance to surrounding soil (López *et al*., 2001; Baslam *et al*., 2012). This makes it possible to observe temporal changes in the number, length, area, and diameter of fine roots without disturbing the soil or damaging the roots. The objectives of this experiment were (i) to compare the ability of different AMF species to colonize alfalfa roots, (ii) to measure seasonal changes in the FRLD and FRSAD of alfalfa inoculated with different AMF species and (iii) to determine the relationship between AMF and fine root P concentrations per unit surface area.

Materials and Methods

Preliminary survey: We isolated AMF fungi from the rhizosphere of alfalfa growing in 21 fields near Shihezi City, Xinjiang Province, China (44°18′N, 86°03′E, 399 m

a.s.l). Three AMF species (*Acaulospora*, *Glomus* and *Scutellaspora*) were observed most often in the samples. Among these three species, *Glomus* had the highest frequency of occurrence (Table 1). Therefore, we chose to use *Glomus* in the experiment described below.

Table 1. Frequency of occurrence of arbuscular mycorrhizal fungi in the rhizosphere of alfalfa growing in 21 fields near Shihezi City, Xinjiang Province, China.

Species	Frequency (%)	Species	Frequency (%)
A. foveata	50	*S. pellucida*	25
A. laevi	25	*S. calospora*	25
G. claroideum	25	*G. versiforme*	100
G. mosseae	75	*G. caledonium*	25
G. intraradices	75	*G. etunicatum*	50

Greenhouse experiment: Five inoculation treatments were compared in this study. In four of the treatments, the alfalfa plants were inoculated with a single species of *Glomus*: *G. mosseae* (Gm), *G. intraradices* (Gi), *G. etunicatum* (Ge), or *G. versiforme* (Gv). In the fifth inoculation treatment, the alfalfa plants were inoculated with a mixture of six *Glomus* spp: *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. cladoideum*, *G.. microagregatum* and *G. caledonium*. The AMF species were provided by the Mycorrhiza Laboratory, Qingdao Agricultural University, Qingdao, China.

Alfalfa seeds (cv. 'Sanditi') were surface sterilized in 10% H_2O_2 for 10 minutes, washed five times in distilled water (2 min each time), and then cultured in an incubator at 25ºC. After germination, the seeds were sown in plastic pots (14 cm diam × 25 cm tall) containing 1.5 kg sterilized loam soil. Ten grams of inoculum was added to each pot along with the germinated seeds and then both the seeds and inoculum were covered with an additional 60 g sterilized soil. Uninoculated plants were used as a control. The treatments were arranged in completely randomized design and replicated 28 times. The pots were kept in a greenhouse with maximum photosynthetic active radiation of 1,200 μmol m^{-2} s^{-1} and a temperature of 25±2ºC. The soil water content of the pots was kept at 65~75% (w/w) of field water capacity.

Field experiment: The field portion of this experiment was conducted near Shihezi University. The average frost free period in the area is 170 d. The mean annual temperature is 6.9°C. Annual precipitation ranges between 125 and 208 mm. The soil at the site is a heavy loam soil with the following characteristics: pH, 7.79; organic C, 18.4 $g \cdot kg^{-1}$; alkali hydrolysable N, 75.3 $mg \cdot kg^{-1}$; available P, 5.27 $mg \cdot kg^{-1}$; and available K, 195 $mg \cdot kg^{-1}$.

The experiment site was divided into 1.5 m × 3 m plots. A transparent acrylic tube (1.00 m long and 6.4 cm diam) was installed at a 45° angle to the ground surface of each plot in 2012. A 10 cm section of each tube protruded above the ground surface. This section was covered with duct tape to exclude light. A rubber stopper was put in the top of each tube.

The AMF-inoculated plants were transplanted to the plots in 4 May, 2013. Each treatment was replicated three times. The soil temperature in each plot was recorded at the 15 cm depth of plot throughout the growing season.

Images of fine roots on the upper surfaces of the tubes were taken with a digital camera system (CI-600; In-Situ Root Imager, CID Bio-Science, Camas, WA, USA) on seven dates: 25 June, 19 July, 6 Aug, 22 Aug, 6 Sep, 24 Sep and 10 Oct 2013. The images of the fine roots were traced manually using a mouse. The length, mean diameter, and surface area of the fine roots were determined using image-analysis software (WinRHIZO Tron MF; Regent Instruments, Quebec, Canada). The size of each observation window was 21.59 cm × 19.56 cm. The FRLD and FRSAD were estimated using the following formulas:

$$FRLD\ (mm \cdot cm^{-2}) = FRL\ (mm) / A\ (cm^2)$$

$$FRSAD\ (mm^2 \cdot cm^{-2}) = FRA\ (mm^2) / A(cm^2)$$

where FRL is fine root length, FRA is fine root surface area, and A is the area of the observation window (421.71 cm^2). Only live roots were measured in this study.

Bulk root samples were collected on each sample date by excavating the soil around five plants to a depth of 15 cm. The soil was washed from the roots by wrapping plastic mesh around the sample and gently massaging the sample under water until only roots and debris were left within the mesh enclosure (Julia *et al*., 2010). The fresh fine roots were picked by hand from the samples and washed with distilled water. The fine roots were cleared in 10% KOH for 1 h at 80ºC, acidified with 1% HCl for 15 min, and then stained with trypan blue in lactoglycerol (0.05%). Roots were left in clear lactoglycerol overnight, and then 1 cm pieces of the roots were placed on microscope slides. The percent colonization was measured using the gridline intersect method as described by Lutgen *et al*. (2003).

Fine root P concentrations were determined using samples collected on 10 Oct. After washing the fine roots, they were dried in an oven at 60°C for about 12 h until they reached a constant weight. The P content of the fine root samples was measured with the molybdate blue method (Murphy & Riley, 1962). The dry weight of the alfalfa shoots was also determined on 10 Oct.

Data analysis: One way analysis of variance was performed using SPSS 18.0 software (SPSS, Chicago, IL, USA). Multiple comparisons of means were performed using least significant difference tests at the 5% level. Results are presented as means ± SE.

Results

AMF colonization: No AMF were observed in the uninoculated treatment (Fig. 1a). Colonization rates in the inoculated treatments varied significantly across time. The colonization rates were lowest (14.2 to 29.8%) on 19 July. Colonization rates then increased to between 49.1 and 86.2% on 24 Sep. Colonization rates decreased between 24 Sep and 10 Oct.

Colonization rates averaged across the entire growing season varied significantly among the AMF species (Fig. 1b). The Gm-inoculated treatments had the highest average colonization rate (42.3%). The differences among the Gv, Ge, and mixed inoculum treatments were not significant. The Gi treatment had the lowest average colonization rate (31.3%).

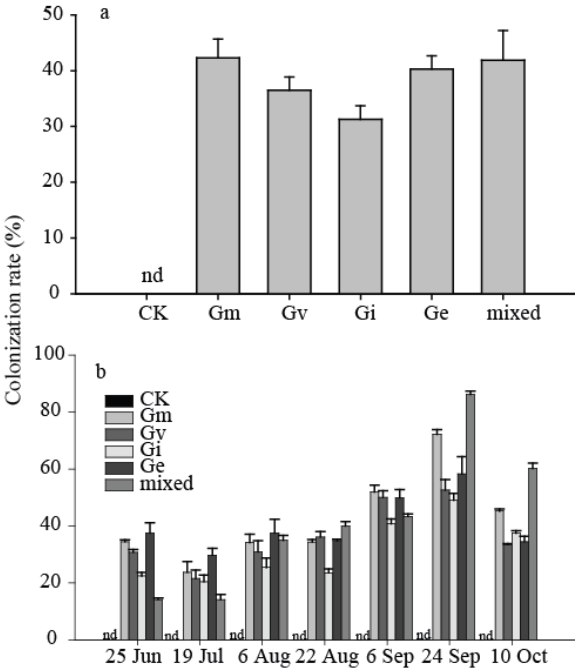

Fig. 1. Colonization rates of fine alfalfa roots after inoculation with mycorrhizal *Glomus.* (a) Average colonization rate for the entire growing season. (b) Colonization rate on each sample date. Abbreviations: CK, uninoculated; Gm, *G. mosseae;* Gi, *G. intraradices*; Ge, *G. etunicatum*; Gv, *G. versiforme.* Plants in the mixed treatment were inoculated with six *Glomus* spp: *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. cladoideum*, *G.. microagregatum* and *G. caledonium*. Error bars represent SE.

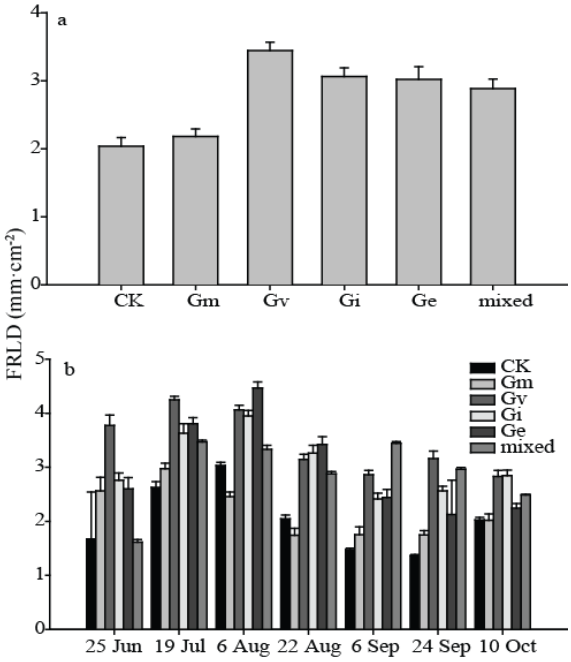

Fig. 2. Fine root length density (FRLD) of alfalfa roots after inoculation with mycorrhizal *Glomus.* (a) Average FRLD for the entire growing season. (b) FRLD on each sample date. Abbreviations: CK, uninoculated; Gm, *G. mosseae;* Gi, *G. intraradices*; Ge, *G. etunicatum*; Gv, *G. versiforme.* Plants in the mixed treatment were inoculated with a mixture of six *Glomus* spp: *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. cladoideum*, *G.. microagregatum* and *G. caledonium*. Error bars represent SE.

Fine root length density: Inoculation with AMF generally increased FRLD (Fig. 2a). Among the inoculated treatments, FRLD was highest in Gv-inoculated plants treatment (3.441 mm·cm^{-2}) and lowest in Gm-inoculated plants (2.181 mm·cm^{-2}). There were no significant differences among the Ge, Gi and mixed inoculum treatments. The FRLD was lowest in uninoculated plants (2.035 mm·cm^{-2}).

Fine root length density changed significantly during the growing season (Fig. 2b). The FRLD of Gm- and Gv-inoculated plants were highest on 19 Jul (2.976 mm·cm^{-2} and 4.251 mm·cm^{-2}). In comparison, the fine root length densities of Ge- and Gi- and uninoculated plants were highest on 6 Aug (4.470, 3.952, and 3.036 mm·cm^{-2}, respectively). The FRLD of plants in the mixed inoculum treatment had two peaks (3.469 mm·cm^{-2} on 19 July and 3.444 mm·cm^{-2} on 6 Sep). The FRLD decreased in all treatments between 6 Aug and 24 Sep. The FRLD of Gi-, and Gm-inoculated plants increased between 24 Sep and 10 Oct, whereas the FRLD of the other species remained the same or decreased.

Fine root surface area density: The fine root surface area density was significantly affected by the AMF species (Fig 3a). Averaged across the growing season, Gv-inoculated plants had the highest FRSAD (5.9381 mm^2·cm^{-2}), whereas Gm-inoculated plants had the lowest FRSAD (3.7069 mm^2·cm^{-2}). There was no significant difference among the Ge-, Gi- and 6G-inoculated plants.

The FRSAD was generally highest on 6 Aug (Fig. 3b). The exception was Gm-inoculated plants, which reached their maximum on 19 July. The FRSAD decreased in all treatments between 6 Aug and early 6 Sep and then generally increased during the remainder of the growing season (except for Gv- and Gm-inoculated plants).

Fine root turnover rate and P concentrations: Total fine root turnover rate was highest for in uninoculated plants (1.19 year^{-1}), followed by the mixed inoculum treatments (0.99 year^{-1}), Ge-(0.75 year^{-1}), Gi-(0.56 year^{-1}), Gm-(0.39 year^{-1}) and Gv-inoculated plants (0.18 year^{-1}) (Fig. 4). Fine root P concentrations per unit surface area were significantly highest in the mixed inoculum treatment (7.58±0.37 mg P/cm^2) and lowest in the uninoculated treatment (3.77±0.02 mg P/cm^2). The differences among the Gm, Gi, and Ge treatments were not significant (Fig. 5).

Shoot biomass production: Shoot dry weight was less in the uninoculated plants than in the AMF inoculated plants (Fig. 6). There were significant differences among the AMF treatments, with 6G-inoculated plants having the greatest biomass and Gi-inoculated plants having the least biomass.

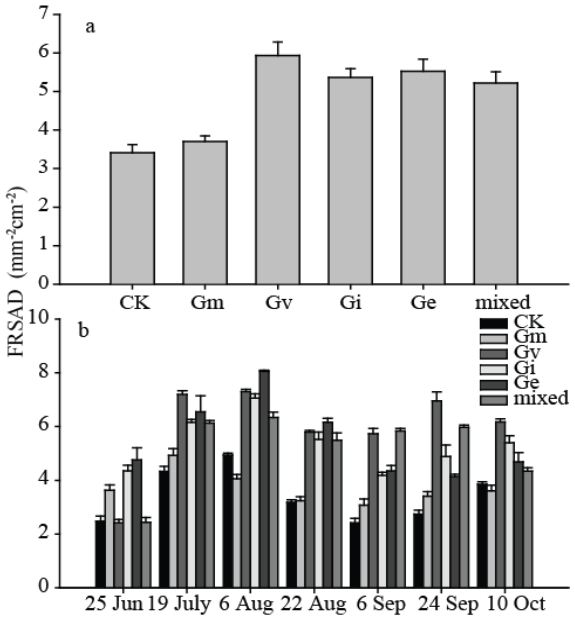

Fig. 3. Fine root surface area density (FRSAD) of alfalfa roots after inoculation with mycorrhizal *Glomus.* (a) Average FRSAD for the entire growing season. (b) FRSAD on each sample date. Abbreviations: CK, uninoculated; Gm, *G. mosseae;* Gi, *G. intraradices*; Ge, *G. etunicatum*; Gv, *G. versiforme.* Plants in the mixed treatment were inoculated with a mixture of six *Glomus* spp: *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. cladoideum*, *G.. microagregatum* and *G. caledonium*. Error bars represent SE.

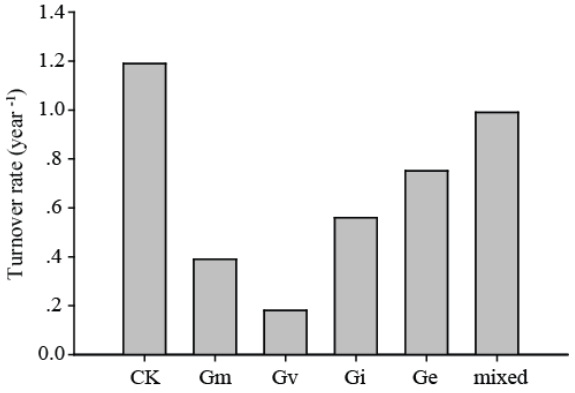

Fig. 4. Effect of mycorrhizal *Glomus* spp. on the fine root turnover rate of alfalfa. Abbreviations: CK, uninoculated; Gm, *G. mosseae;* Gi, *G. intraradices*; Ge, *G. etunicatum*; Gv, *G. versiforme.* Plants in the mixed treatment were inoculated with a mixture of six *Glomus* spp: *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. cladoideum*, *G.. microagregatum* and *G. caledonium*. Error bars represent SE.

Discussion

The colonization rates of fine roots differed significantly among the AMF species, with plants in the Gm and mixed inoculum treatments having the highest colonization rates in this study. The Gi treatment had the lowest colonization rate. A previous study indicated that colonization of bermudagrass were higher when plants were inoculated with a single AMF species rather than a mixture of AMF species (Ye *et al*., 2013). However, our results suggest that the symbiotic effects were best when alfalfa was treated with a mixed inoculum of AMF.

The colonization rates of fine alfalfa roots by AMF declined between 25 June and 19 July, and then rose steadily until 24 September. A previous report indicated that AMF colonization of fine grape roots increased after budbreak and generally remained high until the end of the growing season (Schreiner *et al*., 2007). In contrast, Bohrer *et al*. (2004) observed that AMF colonization rates in a wetland ecosystem were highest in March and April and lowest in August and September. The different seasonal patterns may be related to either plant species or environmental factors. The colonization rates in our study declined during the last weeks of the growing season. This is probably related to soil temperature, which declined from 24ºC on 30 Aug to 11ºC on10 Oct.

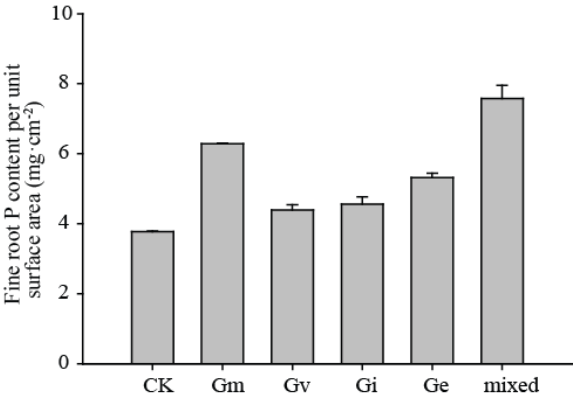

Fig. 5. Fine root P concentration of alfalfa roots on Oct 10. Abbreviations: CK, uninoculated; Gm, *G. mosseae;* Gi, *G. intraradices*; Ge, *G. etunicatum*; Gv, *G. versiforme.* Plants in the mixed treatment were inoculated with a mixture of six *Glomus* spp: *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. cladoideum*, *G.. microagregatum* and *G. caledonium*. Error bars represent SE.

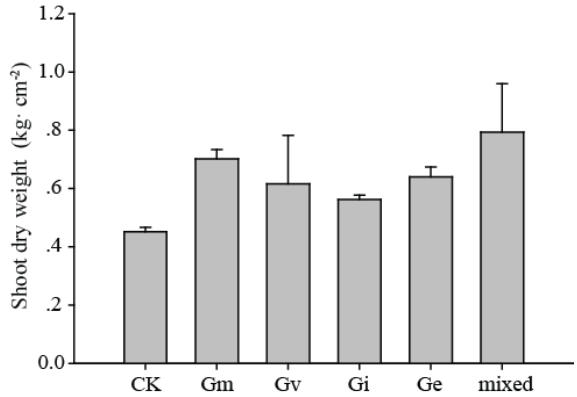

Fig. 6. Effect of mycorrhizal *Glomus* spp. on the above ground dry weight of alfalfa on Oct 10. Abbreviations: CK, uninoculated; Gm, *G. mosseae;* Gi, *G. intraradices*; Ge, *G. etunicatum*; Gv, *G. versiforme.* Plants in the mixed treatment were inoculated with six *Glomus* spp: *G. mosseae*, *G. intraradices*, *G. etunicatum*, *G. cladoideum*, *G.. microagregatum* and *G. caledonium*. Error bars represent SE.

Both FRLD and FRSAD were significantly higher in AMF-inoculated plants than in uninoculated plants. This meant that AMF promoted root extension and increased fine root surface area. Similarly, Zangaro *et al*. (2008) reported that AMF root colonization was correlated with root morphology. In our study, FRLD and FRSAD were highest in plants inoculated with either Gi, Ge, or Gv. In contrast, Gm-inoculated plants generally had the lowest FRLD and FRSAD. The ability of AMF to promote root growth depends on many factors, including the ability of the AMF to colonize the roots, the AMF metabolic activity, and the length and spatial distribution of the AMF hyphae.

Our study indicated that FRLD and FRSAD varied significantly during the growing season. Both variables increased from late June until early August. This was expected because it was a time of vigorous plant growth. The two variables exhibited different patterns after 6 August. The FRLD decreased steadily from 6 August until the end of the growing season. In contrast, FRSAD generally increased on the two last two sample dates.

The decline in FRLD may be attributed to a decrease in the amount of photosynthate available to the root system at the end of the growing season and the shedding of small fine roots (Pregitzer *et al*., 2000). We observed that many leaves began to drop from the alfalfa plants in mid- to late September. Soil temperatures also declined rapidly after 30 August and this would also contribute to a decline in FRLD (Pregitzer *et al*., 2000). It is important to note that FRLD during the last part of the growing season was still significantly higher in inoculated plants than in uninoculated plants. This suggested that the AMF either increased the longevity of the fine roots or promoted new fine root growth.

The increase in FRSAD on the last two sample dates may be attributed to an overall shortening and thickening of roots that occurs in fall (Kaspar & Bland, 1992). These changes may help the roots adapt to lower temperatures by reducing root respiration (Pregitzer *et al*., 2000; Hendrick & Pergitzer, 1993). Another explanation for thickening is that carbohydrates are being stored in the roos to meet plant needs in early spring before the leaves fully expand (Pregitzer, 2003).

In our study, AMF colonization significantly decreased the turnover rate of fine alfalfa roots. Furthermore, the turnover rates of fine roots differed significantly among the AMF treatments. In contrast, Hodge *et al*. (2000) found that AMF colonization did not affect the longevity of *P. lanceolata* roots. This suggests that the effect of AMF colonization on root turnover depends upon the species. The rapid turnover of fine alfalfa roots in the uninoculated treatment suggests that plants must continually produce new fine roots to replace the old ones in order to maintain vigorous growth. One hypothesis is that fine root turnover rate increases with nutrient availability (Pregitzer *et al*., 1995). Our results showed that the turnover rate of AMF inoculated roots increased as fine root P concentrations per unit surface area increased. A previous study by Son & Hwang, (2003) indicated that longevity of fine *L. leptolepis* roots decreased as soil nutrient availability increased.

Fine root P concentrations per unit surface area and shoot biomass were both significantly higher in AMF-inoculated plants than in uninoculated plants. Plants inoculated with mixed inoculum or Gm had the highest fine root P concentrations and shoot biomass. These plants also had the highest AMF colonization rates in our study. This suggests that the AMF promoted shoot growth by taking up P from the soil and transporting it to the alfalfa roots. Interesting, plants in the mixed inoculum and Gm treatments did not have the highest FRLD and the FRSAD in this study. Previous reports have emphasized the importance of fine roots, and especially fine root surface area, on nutrient uptake (Marschner, 1998). This may be true, but our results suggest that that the AMF were more important to P uptake than fine roots.

Conclusion

Our results indicate that mycorrhizal *Glomus* spp differ significantly both in their ability to colonize alfalfa roots and in their ability to promote alfalfa root growth and P uptake. The beneficial effects were greater when plants were inoculated with a mixture of *Glomus* spp rather than a single species. The AMF decreased root turnover rates; however, the highest root P concentrations were observed in alfalfa plants with slightly lower FRSAD. This suggests that the AMF are more important than fine root surface area to P uptake. The FRLD at the end of the season was higher in AMF inoculated plants than in uninoculated plants, suggesting that AMF preserved the function of fine roots late in the growing season.

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