



## Effects of season and soil conditions on the mycorrhizal status and colonization of seven grass species

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### Abstract

In this study seven plant species were collected from the forest of Arasbaran located in the northwest of Iran. Sampling was conducted in May and August and roots were used for calculating vesicular arbuscular mycorrhizal colonization percentage during the same period. Fine roots were separated, washed and put in FAA solution as a fixative. Through the time, root colonization of all plant species increased significantly. Soil collected from rhizosphere of each plant species was physico-chemically analyzed and spore number was determined. Similar to VAM colonization of roots, spore population per 1 gram rhizosphere soil of all plants except *Melilotus officinalis* increased through the time. Rhizosphere soils were used for analysis of EC, pH and soil available N, P and K. Soil texture also was analyzed. Soil EC had very high correlation ( $r = 0.923$ ,  $p < 0.05$ ) with spore number and pH had high but negative correlation with both spore number and vesicular arbuscular mycorrhizal root colonization. Among the soil available nutrients, N showed high correlation with root colonization and specially spore populations.

**Keywords:** vesicular arbuscular mycorrhizae; root colonization; spore number; soil texture; extractable nutrients

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### Introduction

Arbuscular mycorrhiza (AM) is one of the most common symbioses worldwide and about 80% of the known plant species form AM (Mandyam and Jumpponen, 2008). Arbuscular mycorrhizal (AM) fungi biotrophically colonizes the root cortex and develops an extra-matrical mycelium that helps the plant to acquire mineral nutrients from soil (Fuzy et al., 2008). AM fungi

improve plant growth mainly through improvement of phosphorus (P) nutrition. Furthermore, they can establish mutual symbiosis with arbuscular mycorrhizal fungi (AMF), which may result in reciprocal transfer of P from the fungus to the plant in exchange for carbon from the plant to the fungus (Ezawa et al., 2002). Mycorrhizal symbiosis plays a key role in nutrient cycling in the ecosystem and also protects plants against environmental and cultivation stress (Carvalho et al., 2004). Plants, in their natural environment are colonized both by external and internal microorganisms. Some soil microbes, particularly beneficial bacteria and fungi can improve plant performance under stress environments and consequently, enhance yield

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(Jahromi et al., 2008; Kaligaric et al., 2008; Creus et al., 1998). Such information is of prime importance in identifying and utilizing the most suitable mycorrhizal conditions for large scale inoculation programs. The distribution and function of VAM in natural ecosystems are poorly understood. However, information on their prevalence and importance in natural ecosystems is limited and often contradictory. The development and seasonal fluctuations in VAM colonization has been studied in several mycorrhizal-dependent plant species or communities (Postma et al., 2007; Sharifi et al., 2007; Brundrett and Kendrick, 1990; Brundrett, 1991; Douhan et al., 2005; Merryweather and Fitter, 1998), although most of these studies have failed to find consistent seasonal patterns of VAM development. The patterns and timing of VAM development may depend on edaphic factors (Sanders, 1990) or variations in plant nutrient levels (Mullen and Schmidt, 1993; Juniper and Abbott, 2006). In this study we were interested in finding the influences of soil extractable nutrients and seasonality on root colonization quantity, the correlation between them and how these kinds of symbiosis affect spore population in the rhizosphere.

## Materials and Methods

### Sampling site

Arasbaran or Qaradag is a UNESCO registered biosphere in East Azerbaijan, Iran. This region has a varying altitude from 256 m in the vicinity of Aras River to 2896 m and covers an area of 78560 hectares (38°40' to 39°08' N; 46°39' to 47°02' E). Sampling site (38°50' N, 47°00' E) is approximately 1620 m above sea level and receives an annual rainfall of 400-600 mm.

### Soil analyses and isolation of AM fungi spores

Three soil samples (1000 g) were collected from the rhizosphere. Soil samples were bagged in polyethylene bags, sealed, brought to the laboratory and stored at 5° C until analysis for mycorrhizal spores. These samples were also analyzed to determine pH, electrical conductivity (EC) and available N, P, K using AOAC (Robertson

et al., 1960) and Olsen P protocols (Olsen et al., 1954). Mycorrhizal spore count of each soil sample was conducted by a modified wet-sieving and decanting technique of Gerdemann and Nicolson (1963). Spore number was expressed as the total number of spores per 1 g of soil. Identification of spore number was done with the help of synoptic keys adopted by Hall and Fish (1979), Trappe (1982), Schenck and Perez (1987) and Raman and Mohankumar (1988).

### Mycorrhization levels

Roots were carefully washed, fixed in FAA (6.0% formaldehyde, 2.3% glacial acetic acid, 45.8% ethanol and 45.9% H<sub>2</sub>O (v/v)) and stained with lactophenol blue (Phillips and Hayman, 1970). Fixed roots were heated at 90 °C for about 30 to 60 min (depending on the thickness and color of the roots) in 10 % KOH. Afterwards roots were rinsed in tap water and acidified with 3.7 % HCl for 10 min. They were stained for 90 min in lactophenol blue solution for staining fungi and the excess stain was removed in 50 % lactic acid for at least 12 h. Total mycorrhization levels (percentage of the examined root segments with mycorrhizal structures) were determined with a light microscope (Zeiss Axioplan) using the gridline intersect method (Ambler and Young, 1977 modified after Schmitz et al., 1991) at 100× magnification. A minimum of 300 root segments per plant were counted.

### Statistical analysis

Statistical tests were performed with SPSS version 18 (PASW statistic 18). The data were analyzed by T-test to examine the effect of the factors. In this study P-value at  $P \leq 0.05$  was used to compare means. Also Pearson correlation was calculated to know correlation coefficient between factors.

### Results

Figure (I) shows that all seven plant species were colonized by arbuscular mycorrhizal fungi at the two time points, May and August. In May, percentage of arbuscular mycorrhizal colonization ranged from 15 % in *Melilotus*

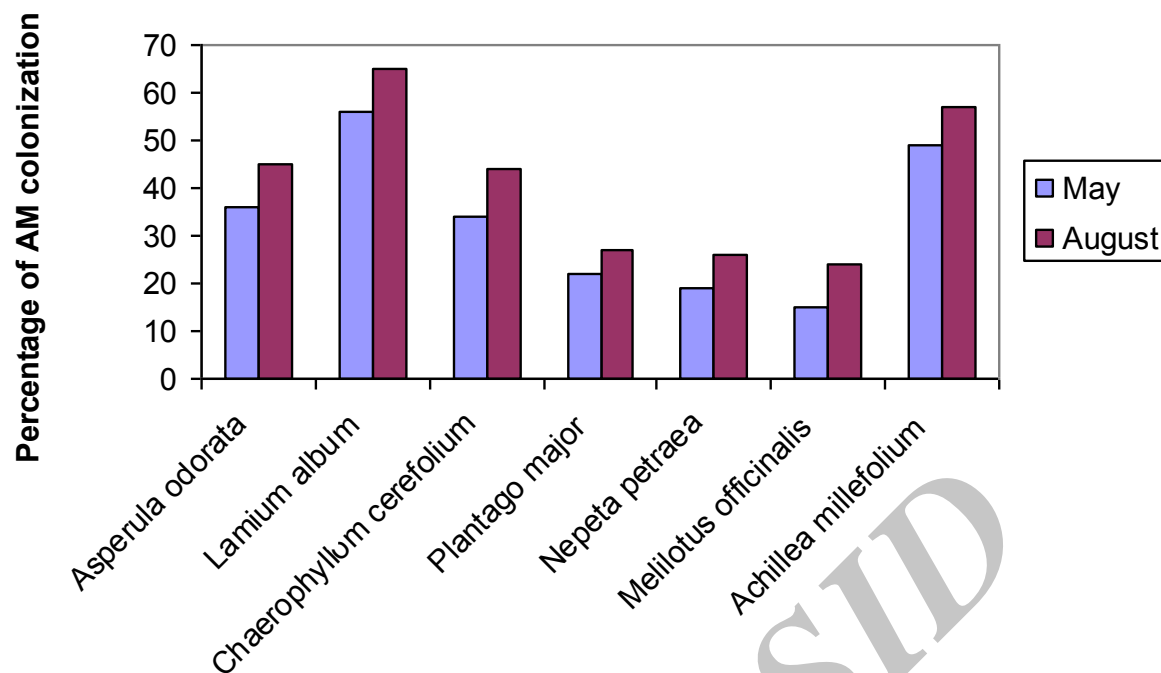


Fig. I. Comparison of percentage of root colonization in May and August

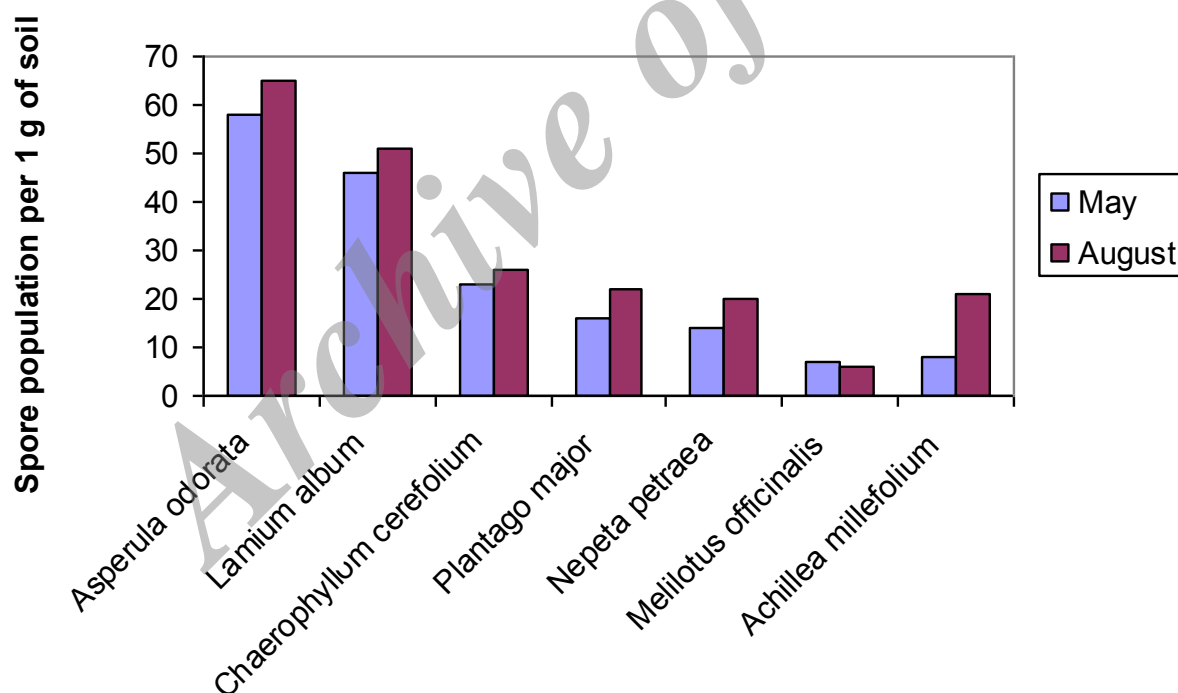


Fig. II. Comparison of spore number in rhizosphere of different species in May and August

*officinalis* to 56 % in *Lamium album*. Besides, in August it ranged from 24 % in *Melilotus officinalis* to 65 % in *Lamium album* (Table 1). Figure (I) also indicates that percentage of root colonization in all seven plant species has increased from May to August. *Chaerophyllum cerefolium* and *Plantago*

*major* showed the highest and lowest increases, respectively. Spore population per one gram soil was different in each plant rhizosphere ranging from 7 in *Melilotus officinalis* to 58 in *Asperula odorata* in May and from 6 in *Melilotus officinalis* to 65 in *Asperula odorata* in August. Except for

Table 1  
Characteristics of rhizosphere soil of different species

Plant species	Spore population per 1 g of soil		Percentage of AM colonization		EC*10 <sup>3</sup> (ds/m)	pH	Available N, P, K (mg/kg)			Soil texture			
	May	August	May	August			N	P	K	%Clay	%Silt	%Sand	Text.
<i>Asperula odorata</i>	58	65	36	45	1550	6.98	63	51.10	464	13	41	46	L.
<i>Lamium album</i>	46	51	56	65	1190	7.31	45.5	31.7	364	14	24	62	Sa.L.
<i>Chaerophyllum cerefolium</i>	23	26	34	44	990	6.91	52.5	36.4	444	12	13	75	Sa.L.
<i>Plantago major</i>	16	22	22	27	1020	7.77	38.5	18.9	327	38	38	24	C.L.
<i>Nepeta petraea</i>	14	20	19	26	1120	7.53	31.5	13.8	238	16	18	66	Sa.L.
<i>Melilotus officinalis</i>	7	6	15	24	840	7.86	59.5	13	257	32	36	32	C.L.
<i>Achillea millefolium</i>	8	21	49	57	940	7.55	52.5	18.5	322	38	29	33	C.L.

L: Loam; C.L: Clay Loam; Sa.L: Sandy Loam

*Melilotus officinalis*, spore population increased in all plants' rhizosphere from May to August particularly in *Achillea millefolium* (Fig. II). The rhizosphere soils of the plants were loam, sandy loam and clay loam which exhibited variations in pH, EC and available N, P, K contents (Table 1). Soil spore number increased with the highest soil N in August but it did not significantly correlate ( $r = 0.274$ ,  $p < 0.05$ ) with soil N. Root colonization had also low correlation with soil N ( $r = 0.247$ ,  $p < 0.05$ ) (Table 2). Root colonization and specially spore number significantly correlated with soil P and soil P significantly correlated ( $r = 0.743$ ,  $p < 0.05$ ) with soil K. As compared to P and N, little is known about the role of K in root colonization and spore number of AM fungi; however, in this study spore number ( $r = 0.493$ ,  $p < 0.05$ ) and root colonization ( $r = 0.566$ ,  $p < 0.05$ ) showed a higher correlation with soil K. Furthermore, soil pH had a negative correlation with N ( $r = -0.326$ ,  $p < 0.05$ ), P ( $r = -0.883$ ,  $p < 0.05$ ), K ( $r = -0.788$ ,  $p < 0.05$ ), spore number ( $r = -0.695$ ,  $p < 0.05$ ) and root colonization ( $r = -0.502$ ,  $p < 0.05$ ). EC significantly correlated with extractable soil P ( $r = 0.794$ ,  $p < 0.05$ ) and spore number ( $r = 0.923$ ,  $p < 0.05$ ) but negatively correlated with pH ( $r = -0.612$ ,  $p < 0.05$ ).

## Discussion

It has been shown that soil nutrient availability varies with space and time (Ritsem

and Dekker, 1994). The results of this investigation showed a significant effect of edaphic factors on VAM formation and function in natural ecosystems. The influence of soil factors on mycorrhizal colonization and spore numbers demonstrated under controlled conditions may be substantially modified in natural ecosystems. Supporting VAM fungi is costly for the host as it must allocate carbon to the VAM symbionts, the cost being proportional to the extent of colonization (Graham et al., 1997). The extent of root length colonized by VAM fungi is an interaction between root growth and spread of colonization. In general, plants tend to allocate more resources for root production irrespective of soil nutrient levels (Aerts et al., 1992; Konings et al., 1992). This is consistent with our results that root colonization and spore population increased through passing time and with root growth from May to August. Another discrepancy between present results and earlier studies is the differential influence of soil P and K on mycorrhizal colonization. Plants in nutrient-limited soils are highly adapted to low nutrient levels and may be more sensitive to small changes in nutrient inputs (Kitt et al., 1988). Increasing soil P is known to reduce or suppress VAM formation, which may either be due to the direct effect of P on the external hyphal growth or be an indirect effect associated with the P status of the plant. The positive correlation between soil P and VAM colonization, contrary to

Table 2  
Correlation coefficient (r) and significance at  $p < 0.05$  (p) between factors

		Percentage of AM colonization (May)	Spore population (August)	Soil EC	Soil pH	Available N	Available P	Available K	Soil texture		
									%Clay	%Silt	%Sand
Percentage of AM colonization (August)	r	---	---	0.281	-0.502	0.247	0.476	0.566	-0.261	-0.194	0.266
	p	0.000	---	---	---	---	---	---	---	---	---
Spore population (August)	r	---	1.000	0.923	-0.695	0.274	0.876	0.493	-0.603	0.192	0.269
	p	---	---	---	---	---	---	---	---	---	---
Spore population (May)	r	---	---	---	---	---	---	---	---	---	---
	p	---	0.000	---	---	---	---	---	---	---	---

these observations, could be due to the fact that an increase in P levels in P deficient soils can enhance mycorrhization before an expected decrease occurs (Bolan, 1991). However, suppression of mycorrhizal colonization occurs at much higher nutrient levels than those recorded in the present study. Likewise, soil K is often reported to have a stimulatory effect on mycorrhization (Furlan and Bernier-Cardou, 1989), and a minimum soil K is often a prerequisite for mycorrhization in some plant species (Ouimet et al., 1996). As reported elsewhere, the effect of soil K on mycorrhizal fungi depends, to a certain level, not only on its own availability, but also on concentrations of other exchangeable ions like Ca and Mg in the soil (Proctor and Woodell, 1975; Mengel and Kirkby, 1980). Though less documented than P, soil N affects root colonization and spore numbers (Muthukumar and Udaiyan, 2000). There are several reports that soil N could suppress root colonization by VAM fungi (Chambers et al., 1980; Buwalda and Goh, 1982; Johnson et al., 1984). The suppressive effect of soil N on VAM fungi has been attributed primarily to pH changes associated with variations in soil N (Thompson, 1986). Fluctuations in the number of VAM fungal spores would be expected to occur if they were lost during periods of mycorrhizal formation, or as a result of predation by soil organisms, as well as due to the impact of adverse soil conditions during periods of inactivity (Brundrett, 1991). However, these potential seasonal patterns in

spore numbers may also be created by the formation of new spores in association with root growth at these times. This study also highlights that assessing the influence of soil factors individually on VAM colonization or spore numbers can often lead to misleading conclusions in this type of study. The literature documents many complex interactions between soil factors on mycorrhizal colonization and spore numbers (Sylvia and Neal, 1990; Michelini et al., 1993).

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