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Indigenous populations of arbuscular mycorrhizal fungi and soil aggregate stability are major determinants of leek (*Allium porrum* L.) response to inoculation with *Glomus intraradices* Schenck & Smith or *Glomus versiforme* (Karsten) Berch

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Abstract Knowledge of physical, chemical and biological soil characteristics influencing plant response to inoculation with arbuscular mycorrhizal (AM) fungi would help to distinguish soils where inoculation could be profitable. The relationship between leek (*Allium porrum* L.) response to mycorrhizal inoculation with *Glomus intraradices* Schenck & Smith or *G. versiforme* (Karsten) Berch and soil texture, bulk density, particle density, porosity, pH, organic matter content, available P, K, Ca, Mg, Fe, Zn, Cu, and Mn, soil structure, soil mycorrhizal potential (SM), preceding crop mycorrhizal potential, composition of indigenous mycorrhizal fungal communities, and the abundance of spores of different species, was studied in 81 agricultural soils using Principal Component Analysis and regression analysis. The nature of the indigenous AM fungi population was an important determinant of leek response to inoculation (RTI). In soils with more than 200 μg available P g^{-1}, SM potential accounted for over 27% of RTI with *G. intraradices* and *G. versiforme*, RTI being high in soils with low SM potential. In low P soils, however, a positive relation between the abundance of water stable soil aggregates in the 0.5–2 mm diameter range and RTI was most important. Low soil Zn and high porosity, abundant total mycorrhizal spore as well as scarcity of spores of *G. aggregatum* and of the group *G. etunicatum-rubiforme* were also associated to high RTI. The influence of water stable aggregation of soil on RTI was modulated by soil P levels. Abundance of soil aggregates was positively related to RTI at low soil P levels, but negatively related to RTI at high P levels. Different relationships were found between soil variables and spore abundance of different AM fungi species. Some AM species appear to have as yet undefined similarities or complementarities at the biological or ecological levels.

Key words Soil properties · Soil structure · Inoculation · Glomalean fungi · Agricultural soils

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Introduction

Application of mycorrhiza biotechnology to crop production has the potential to reduce inputs such as pesticides or fertilizers and insure the sustainability of agroecosystems (Hamel 1996; Azcón Aguilar and Barea 1997). However, conditions for utilization of mycorrhizal inoculants must be defined. Soil conditions are believed to influence arbuscular mycorrhizal (AM) development. For example, Nicolson (1960) found a relationship between AM root colonization and the level of organic carbon in sand dunes, while Koske and Halvorson (1981) found that *Gigaspora gigantea* (Nicolson & Gerdemann) Gerdemann & Trappe spore number in soil was related to sand grain size. In natural environments, high AM colonization levels are observed in soils where pH (Read et al. 1976), P level (Jeffries et al. 1988), or salinity level (Gerdemann 1968; Azcón and Elatrash 1997) limit plant growth. AM fungi populations most likely adapt to their edaphic environment.

The influence of soil conditions is especially a concern for introduced AM fungi which might not be adapted to the new environment. Hence, inoculation of field-grown crops has yielded variable results. While

Baltruchat (1987) and Furlan (1993) obtained considerable yield increase after inoculation of field-grown maize (*Zea mays* L.) and leek (*Allium porrum* L.), Hamel et al. (1991) found no significant effect of inoculation on field grown maize and soybean, unless the soil was fumigated prior to seeding.

Plant response to mycorrhizal inoculation is most likely modulated by biological, chemical and physical soil characteristics and thus knowledge of these characteristics and how they influence plant response to inoculation would help to distinguish the soils where inoculation would be profitable. However, little is known of the influence of soil factors on the development and efficiency of AM fungi, other than that it is difficult to predict (Abbott and Robson 1991).

The objectives of this present study were to define the relationships between selected soil variables and leek response to inoculation with *Glomus intraradices* Schenck & Smith or *G. versiforme* (Karsten) Berch. In addition to the variables measured in the standard soil test used in field management (soil-available P and K, pH and organic matter level), Ca, Mg, Fe, Zn, Cu, Mn levels were also measured. As data on particle size distribution are easily obtained by producers, we also considered soil texture. Although more difficult to obtain on a routine basis, variables related to gas exchange and compaction (bulk density, particle density, porosity and water stable aggregation) or to indigenous mycorrhizal populations (soil mycorrhizal potential (SM), preceding crop mycorrhizal potential (PCM), composition of the communities and spore abundance) may strongly influence plant response to mycorrhizal inoculation (RTI).

Materials and methods

Experimental design

Data were collected over 2 years on a total of 81 different fields. For the first year, 30 field soils with a horticultural history (i.e. more intensively managed, often with low plant populations and high fertilization rates, sometimes with perrenial plants) and 30 soils with an agronomic history (lower input productions, often with high plant density) were studied. In the second year, experimental plots were established on 6 sites in which sweet corn (*Zea mays* L.; a good host), pea (*Pisum sativum* L.; a good host but a short season plant) and beet (*Beta vulgaris* L. ; a non-host plant) had been grown the preceding year. Experimental plots were also set in three different fields on a vegetable-production farm during the second year. Soils with a horticultural history were located at the McGill University Horticultural Research Centre (Ste-Anne-de-Bellevue, Quebec), while soils with an agronomic history and those previously cultured with corn, peas and beets were on the Quebec Ministry of Agriculture Research Station at St-Bruno-de-Montarville, Quebec. All sites were <100 km from each other.

Three contiguous plots, each receiving one of three mycorrhizal treatments (leek pre-inoculated with *Glomus intraradices*, with *G. versiforme* or pre-inoculated with sterilized inoculum) were located randomly on each soil for a total of 243 plots. Each of these plots was composed of 1 row of 5 leek plants to which one inoculation treatment had been previously applied in the greenhouse. Rows were 0.7 m apart with 0.15 m between plants. Plots were separated from each other by 1 row of non-inoculated plants, while 1 row of non-inoculated border plants was placed around each set of 3 plots.

Plant production

Leek (*Allium porrum* L., cv. Argenta) transplants were produced in plastic trays (53 cm × 28 cm × 6 cm). Three substrate-inoculum combinations were used: Mycori-Mix, SB-Mix plus 30 g of leek roots colonized by *G. versiforme* per tray, and SB-Mix plus 30 g of uncolonized leek roots per tray. Mycori-Mix, a commercial peat-based substrate containing *G. intraradices* propagules and SB-Mix, a peat-based substrate similar to Mycori-Mix but without mycorrhizal propagules, were provided by Premier Peat Ltd (C.P. 2600, G5R 4C9, Canada). Leek seeds were disinfected for 5 min in a solution of sodium hypochlorite (1%) containing 2 drops of Tween 80, and then rinsed with distilled water. In each tray, 550 seeds were broadcast, covered with 5 mm of SB-Mix and watered to saturation with distilled water (pH 6.2).

Transplants were maintained in a growth chamber with a 16-h day at 325 μmole $m^{-2}s^{-1}$, 65% relative humidity and day:night temperature of 23 °C:18 °C. From week 10 to week 13, plantlets were acclimated to cool spring temperatures in a non-heated greenhouse. Transplants received distilled water only from week 1 to week 4. From week 5 to week 8, each tray received 1 l of half-strength modified Long Ashton solution (Hamel et al. 1991) containing 1.5 mM P, adjusted to pH 6.2, and from week 9 to week 12, 1 l of full-strength solution.

After 13 weeks, leek plantlets were harvested and selected for uniformity (about 2.5 mm diameter) prior to transplantation in the fields. Ammonium nitrate was applied twice: 100 kg ha^{-1} broadcast during field preparation and 100 kg ha^{-1} banded when plants were 15 cm high.

Weeds were controlled with Prowl (3.5 l ha^{-1} in mineral soils and 6.25 l ha^{-1} in organic soils) and Goal (0.63 l ha^{-1}), while wire worms were destroyed with Ambush (1 ml l^{-1}) applied at dusk on organic soils. Plants were grown for 13 weeks in 1993 and for 14 weeks in 1994.

Plant measurements and soil analysis

The RTI of leek in the different soils was defined as the difference between the average stalk diameter at harvest of the 5 leek plants inoculated with *G. intraradices* or *G. versiforme*, and the diameter of the 5 non-inoculated plants. Roots were harvested, washed and cut into 1-cm pieces. The roots of the 5 plants comprising a plot were pooled. Root pieces were dispersed in water and a subsample was collected and used to determine the percentage of mycorrhizal root colonization. Roots were cleared at 126 °C for 10 min in 10% KOH, stained for 24 h at room temperature with acid fuchsin (Kormanik and McGraw 1982) and destained overnight in lactoglycerol at room temperature. Mycorrhizal colonization of roots was rated according to both frequency and intensity. A rating of zero was given when colonization was absent. Ratings of 1, 2, 3 and 4 were given when fungal structures were found in less than 10%, in approximately 25%, 50% and in over 75% of the roots, respectively. A second rating of 0, 3, 6, or 9 was attributed when intensity of colonization was very low, low, medium or high, respectively.

The soil texture was determined by sieve analysis, according to Sheldrick and Wang (1993). Soil bulk density (Culley 1993), particle density (1993), porosity (Carter and Ball 1993) and the relative abundance of water stable aggregates of three size classes (1–2 mm, 0.5–1 mm and 0.25–0.5 mm diameter) (Angers and Mehuys 1993) were all determined at mid-season.

Organic matter content was determined by combustion (Tiessen and Moir 1993) and soil pH (water) with a pH meter. Available P, K, Ca, Mg, Fe, Zn, Cu and Mn in the soils in spring, were measured by colorimetry (P) or atomic absorbtion (all other nutrients) after extraction with the Mehlich III solution (Sen Tran and Simard 1993).

Soil mycorrhizal potential (SM) was defined as the percentage of root colonization of one non-inoculated trap plant (leek) per plot 4 weeks after transplantation. The mycorrhizal potential of the preceding crops (PCM) was defined as the percentage of root

colonization of the crop species previously grown, 8 weeks after inoculation with *G. intraradices* in a bioassay carried out under greenhouse conditions. These preceding crops were: sorghum (*Sorghum bicolor* [L.] *Moench)*, rye *(Secale cereale* L.), barley (*Hordeum vulgare* L.), soybean (*Glycine max* [L.] Merr.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.; sweet corn and grain corn), carrot (*Daucus carota* L.), cabbage (*Brassica oleracea L.*), apple (*Malus domestica* Borkh), pumpkin (*Cucurbita maxima* Duch), buckwheat (*Fagopyrum esculentum* Moench), asparagus (*Asparagus officinalis* L.), canola (*Brassica napus* L)., zucchini squash(*Cucurbita pepos* L.), raspberry (*Rubus idaeus* L.), strawberry (*Fragaria* × *ananassa*, Duch), melon *(Cucumis melo* L.), parsley (*Petroselinum crispum* L.), beet (*Beta vulgaris* L.), pea (*Pisum sativum* L.) and radish (*Raphanus sativus* L.). Plants were grown in the commercial mycorrhizal fungi-containing peat-based potting mix, Mycori-mix, for 8 weeks after plant emergence. Percentages of root colonization levels were determined by the gridline intersect method (Giovanetti and Mosse 1980) after staining as described above. There were 3 repetitions per species in this bioassay. The PCM values used in the statistical analysis were the means of 3 values of percentage root colonization. Soils following a fallow (clean weeded mechanically) period were attributed a PCM value of zero.

The abundance of spores of indigenous species in the 0–15 cm layer was determined at the time of leek harvest by taking a total of 6 soil cores at random from the non-inoculated plot and from the border rows. These cores were pooled and homogenized, and spores present within 50 ml of soil were extracted (Dalpé 1993), counted and identified.

Statistical analysis

Principal Component Analysis (PCA) (Lebart et al. 1984) was used as an exploratory method to study the relationship between leek RTI (a continuous variable), the different indigenous mycorrhizal species and the other measured soil characteristics. Simple correlations between variables were made and Pearson's correlation coefficients calculated using the Proc corr procedure of the SAS (1987) software. Threshold P values were calculated according to a Bonferroni correction. The Stepwise regression procedure (*sle* = 0.05 *sls* = 0.05) of SAS (1987) was used to determine which of the soil variables were related to leek response to inoculation with *G. intraradices* and *G. versiforme*, and the nature of the relationships encountered.

The relationship found between mycorrhizal fungal species by PCA revealed the common occurrence of groups of species. (1) *G. etunicatum* and *G. rubiforme*, (2) *G. caledonium* and *G. macrocarpum*, and (3) *G. mosseae, G. constrictum* and *G. fasciculatum*. The sums of the spore counts of each of these groups were used as variables in the regression analysis as it improved in some cases the R^2 of the model. Counts of *G. geosporum, G. microcarpum, G. aggregatum* and two unidentified *Acalospora* species were used separately.

The data collected from soils with available P levels <200 μg g^{-1} were analysed separately from soil with P levels >200 μg g^{-1}. This division of the data set was suggested by the shape of curves obtained when RTI was plotted against other soil variables, showing a different pattern at lower and higher soil P values. Large improvement of models R^2 further justified separating the soils in two groups. The effect of treatments on leek diameter was analysed using ANOVA (SAS 1987) and difference between treatment means was assessed using a Duncan multiple range test.

Results

Leek mycorrhizal development and growth

Four weeks after transplantation, greenhouse-inoculated leeks had a larger proportion of their root system colonized and their mycorrhizal colonization was more dense than non-inoculated plants (Fig. 1). Mycorrhizal development rate seemed unrelated to soil P levels. *G. versiforme* colonized leek roots more aggressively than *G. intraradices*. Colonization levels were lowest in non-

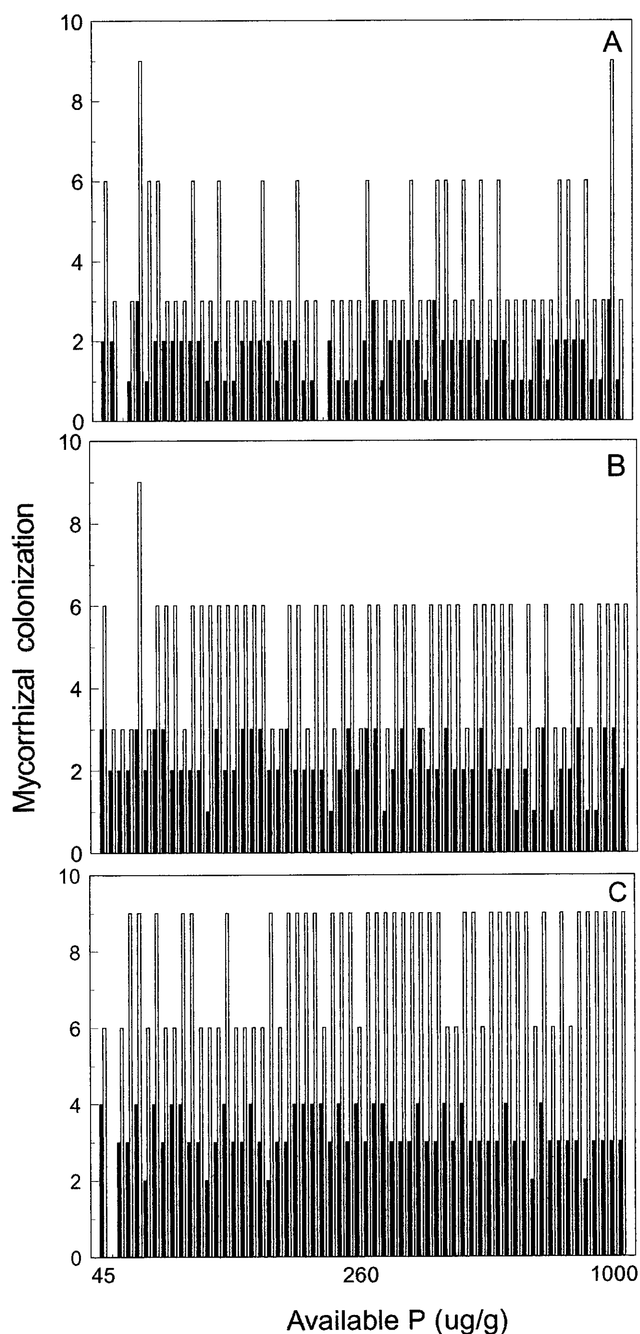

Fig. 1 Mycorrhizal colonization levels of **A** non-inoculated leeks, **B** leeks pre-inoculated in the greenhouse with *Glomus intraradices* and **C** leeks pre-inoculated with *G. versiforme*, 4 weeks after transplantation in the fields. Values are sorted according to soil available P level. *Solid bars* represent the abundance of colonized roots (0 = no colonization, 1 = <10%, 2 = approximately 25%, 3 = approximately 50%, 4 = >75% of roots colonized). *Empty bars* represent the intensity of colonization (0 = trace, 3 = low, 6 = medium, 9 = high fungal density)

inoculated plants that had been in contact with mycorrhizal fungi for only 4 weeks, compared with 17 weeks for the greenhouse-inoculated plants. At harvest, however, non-inoculated leeks still had the lowest level of mycorrhizal colonization, followed by leeks inoculated with *G. intraradices* (Fig. 2). Plants inoculated with *G. versiforme* maintained the highest level of root colonization. High soil P levels seemed to reduce root colonization at this later date.

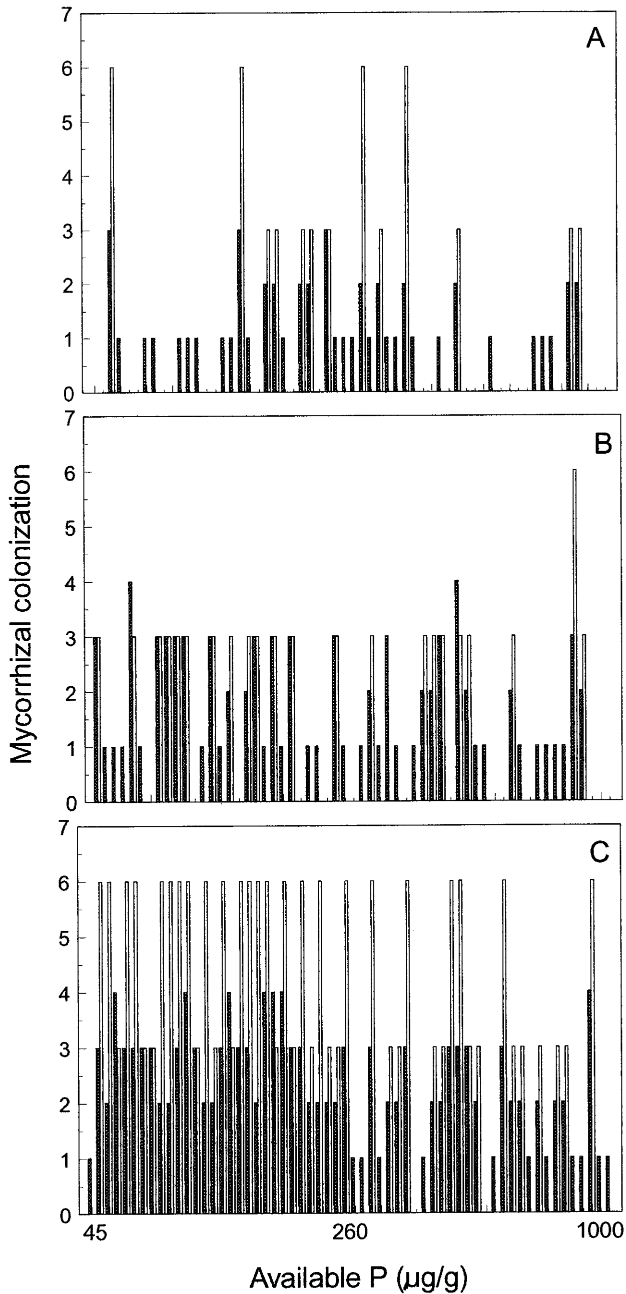

Fig. 2 Mycorrhizal colonization levels of **A** non-inoculated leeks, **B** leeks pre-inoculated in the greenhouse with *Glomus intraradices* and **C** leeks pre-inoculated with *G. versiforme*, 14 weeks after transplantation in the fields. Values are sorted according to soil available P level. *Solid bars* represent the abundance of colonized roots (0 = no colonization, 1 = <10%, 2 = approximately 25%, 3 = approximately 50%, 4 = >75% of roots are colonized). *Empty bars* represent the intensity of colonization (0 = trace low, 3 = low, 6 = medium, 9 = high fungal density)

Leek diameter at harvest was sometimes larger but sometimes smaller when previously inoculated with *G. intraradices* or *G. versiforme* in the greenhouse (Fig. 3). Inoculated plants with larger diameter were more often found in high P soils than in low P soils. Overall, benefits of inoculation with *G. intraradices* were low. The average RTI with *G. intraradices* was slight, 1.1 mm (data not shown) but significant ($P < 0.05$), while *G. versiforme* performed numerically but not statistically better, producing an average RTI of 1.4 mm.

Relationship between indigenous mycorrhizal fungal species and measured soil variables

It is important to recognize that spores of some species were very scarce (Table 1). For example, one single spore of *G. caledonium* was found, on average, in the 50 ml soil samples taken from the sites where this species was encountered. We found this species in 11% of the soils studied; however, as only 1 spore per plot was

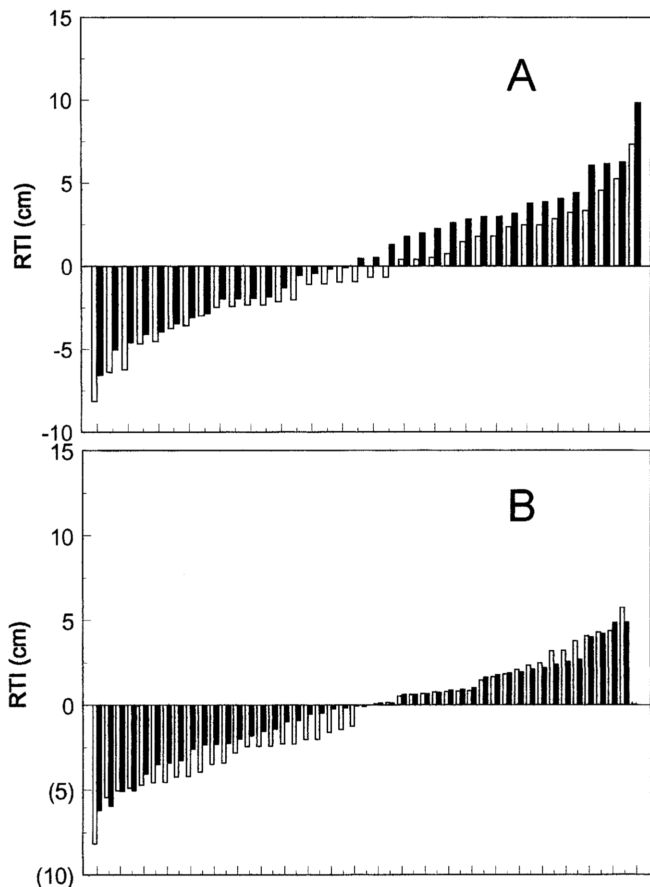

Fig. 3 Response to inoculation (*RTI*) with *G. intraradices* (*empty bars*) and *G. versiforme* (*filled bars*). Leeks were grown in soil containing **A** more than 200 µg available P g–1 and **B** less than 200 µg available P g–1

Table 1 Frequency and mean number of spores AM fungal species recovered from 81 agricultural soils. The relationship between species is shown using Pearson's correlation coefficients. *P values are italicized*. Significance (*) is given after a Bonferroni correction (*ACA* two *Acaulauspora* species, *AGG G. aggregatum, FAS Glomus fasciculatum, MAC G. macrocarpum, CAL G. caledonium, CON G. constrictum, ETU G. etunicatum, MIC G. Microaggragatum, MOS G. mossea, RUB G. rubiforme*)

Correlation coefficients (*P* values)											Frequency (% of plots)	Mean number of spores (spores/50 ml soil)
ACA	MOS	RUB	FAS	MAC	CAL	CON	ETU	MIC	AGG			
−0.14 _0.23_	0.22 _0.05_	0.42 _<0.01_ *	0.33 _<0.01_	−0.19 _0.08_	−0.17 _0.13_	−0.11 _0.32_	0.43 _<0.01_ *	0.40 _<0.01_ *	0.45 _<0.01_ *	GEO	37	30
	0.12 _0.28_	0.09 _0.39_	0.07 _0.55_	0.27 _0.01_	0.05 _0.63_	−0.03 _0.80_	−0.01 _0.91_	0.20 _0.08_	0.13 _0.22_	ACA	19	5
		0.63 _<0.01_ *	0.07 _0.54_	0.34 _<0.01_	0.23 _0.04_	0.76 _<0.01_ *	0.40 _<0.01_ *	0.52 _<0.01_ *	0.49 _<0.01_ *	MOS	51	91
			0.60 _<0.01_ *	−0.16 _0.16_	−0.14 _0.20_	0.63 _<0.01_ *	0.72 _<0.01_ *	0.69 _<0.01_ *	0.71 _<0.01_	RUB	48	101
				0.17 _0.12_	0.06 _0.57_	0.38 _<0.01_ *	0.56 _<0.01_ *	0.75 _<0.01_ *	0.80 _<0.01_ *	FAS	57	40
					0.69 _<0.01_ *	0.17 _0.14_	−0.13 _0.24_	0.28 _0.01_	−0.02 _0.84_	MAC	23	12
						0.17 _0.14_	−0.12 _0.30_	0.17 _0.14_	−0.05 _0.67_	CAL	11	1
							0.38 _<0.01_ *	0.43 _<0.01_ *	0.37 _<0.01_ *	CON	59	65
								0.62 _<0.01_ *	0.70 _<0.01_ *	ETU	23	37
									0.81 _<0.01_	MIC	11	19
										AGG	46	31

found in these soils, it is likely that this species was present though undetected in several other soils. Similarly, *G. mosseae* often produce intraradical spores which are not considered in evaluation of spore populations. In order to reduce the impact of sampling error and to improve the fit of the model in further statistical analysis, species related according to the PCA were grouped.

Seven axes were significant in the PCA. The first two axes (Fig. 4) explained 13.8% of the variability encountered. Spore counts of *G. caledonium* and *G. macrocarpum* were always close to each other on the axes retained by the PCA run on the measured variables. This spatial proximity suggests a direct or indirect relationship between these species, whic could be related directly to each other or to the same edaphic variables. We found a positive relationship between these two species (Table 1) and between these species and the proportion of water stable soil aggregates in the 0.5–2 mm diameter range (Table 2). A negative relationship between these species and available Fe in soil was also found.

The PCA suggested a relationship between *G. mosseae, G. constrictum* and *G. fasciculatum* and, therefore, they were grouped for the correlation analysis. Spore abundance of these species was positively related to available Ca and Mg (Table 2). Similarly, spore counts of *G. etunicatum* and *G. rubiforme* were associated on the PCA axes. Spores of these species also seemed associated with high levels of available Ca and Mg (Table 2). An inverse relationship between the abundance of spores of these species and leek response to inoculation with *G. intraradices* explained 17% of encountered variability, according to a Stepwise regression analysis (Table 3).

Glomus geosporum spores were inversely related to water stable soil aggregation (Table 2). However, this relationship was not significant after a Bonferroni correction. PCA clearly showed the dissociation of *G. geosporum* from other indigenous mycorrhizal species (data not shown). This relationship was not revealed by the Pearson's correlation coefficients calculated from the data set made exclusively from the spore counts (Table 1). This may indicate that *G. mosseae, G. fasci-*

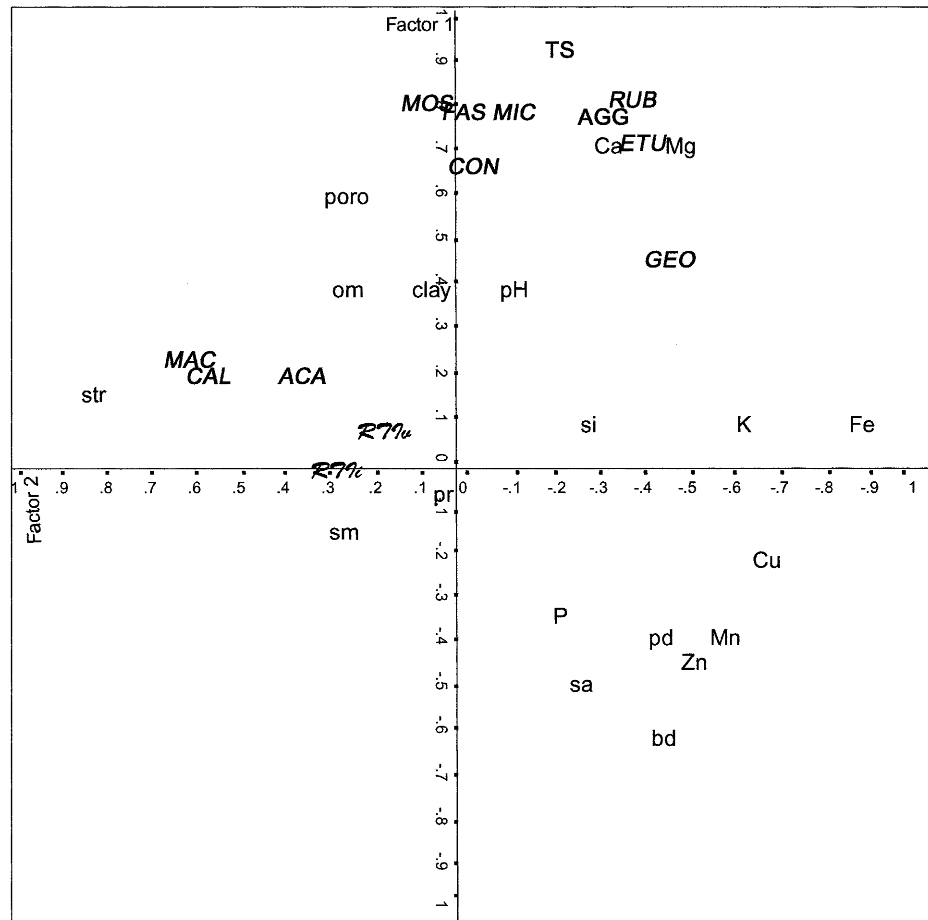

Fig. 4 Plot of the occurrence of the variables analysed against the two first axes of the Principal Component Analysis (*ACA Acaulospora* spp., *AGG G. aggregatum, bd* bulk density, *CAL G. caledonium, clay* proportion of clay, *CON G. constrictum, ETU G. etunicatum, ETU G. etunicatum, GEO G. geosporum, MAC G. macrocarpum, MIC G. microaggregatum, MOS G. mosseae* spore number, *om* organic matter content, *P, K, Ca, Mg, Fe, Zn, Cu, pd* particle density, *pH* pH, *poro* porosity, *pr* preceding crop mycorrhizal potential, RTIi response of leek to inoculation with *G. intraradices,* RTIv response to inoculation with *G. versiforme, RUB G. rubiforme, sa* proportion of sand, *si* proportion of silt, *sm* soil mycorrhizal potential, *str* total soil water stable aggregates, *TS* total spore number, *p*, *K*, *Ca*, *Mg*, *Fe*, *Zn*, *Cu* and *Mn* soil available P, K, Ca, Mg, Fe, Zn, Cu, and Mn, respectively)

culatum, G. constrictum and *G. caledonium* are found in soils with characteristics different to those where *G. geosporum* is found, rather than *G. geosporum* being antagonistic to the former species. Spores of *G. aggregatum* and *G. microaggregatum* were inversely related to soil available Ca and Mg (Table 2).

Relationship between measured soil variables and leek RTI

The regression analysis produced unsatisfactory results when run on the entire data set. Measured soil variables could explain only 34% of the variability encountered in leek response to inoculation with *G. intraradices* and only 20% of that encountered in *G. versiforme*–inoculated leeks.

The graphical relationship between the various soil variables and soil available P levels suggested two groups: those obtained from the soils with <200 µg P g^{-1} (45 soils) and those with >200 µg P g^{-1} (36 soils). This data separation greatly improved the fit. The significant variables, as determined by a Stepwise regression analysis, explained 51% of the variation observed in leek RTI with *G. intraradices* and 27% of that with *G. versiforme*, at low P levels. At high P levels, 59% of the variation observed in leek RTI with *G. intraradices* and 54% of that observed with *G. versiforme* was explained.

According to the regression analysis, SM and water stable aggregates accounted for as much as 42% and 36% of the variability encountered in leek RTI with *G. intraradices* and *G. versiforme* in high P soils (Table 3). In this case, RTI increased with decreasing SM or stable aggregates, as shown by the negative regression coefficients. In soils with P levels below 200 µg g^{-1}, soil stable aggregates (class size 1–2 mm for RTI with *G. intraradices* and 0.5–1 mm for RTI with *G. versiforme*) also accounted for much of the variability encountered in RTI with both fungi. However, in this case, RTI increased with increasing amount of soil aggregates. Leek RTI with *G. versiforme* in P-poor soils was higher when *G. aggregatum* spore number was low and total spore number was high (Table 3). In P-rich soils, leek RTI with *G. intraradices* was higher when spore number of the *G. rubiforme-etunicatum* group was low.

Discussion

The regression analyses indicate the importance of indigenous endomycorrhizal microflora as a determinant of leek RTI and the low contribution of soil inorganic variables. SM appears to be a major determinant of

Table 2 Relationship between soil variables and spore populations ion the different soils studied. The relationship between species is shown using Pearson's correlation coefficients. *P values are italicized*. Significance (*) is given after a Bonferroni correction. Species abbreviations as in Table 1

Soil variables	ACA	GEO	ETU	CAL+ MAC	MOS+ CON+ FAS	AGG	MIC	Total
Particle size analysis								
Sand (%)	−0.21 *0.06*	−0.01 *0.91*	−0.13 *0.26*	−0.30 *<0.01*	−0.23 *0.04*	0.08 *0.49*	−0.05 *0.65*	−0.16 *0.16*
Silt (%)	−0.08 *0.47*	0.06 *0.65*	− <0.01 *0.99*	0.03 *0.76*	−0.02 *0.88*	−0.03 *0.79*	0.03 *0.75*	− <0.01 *0.96*
Clay (%)	−0.04 *0.70*	0.10 *0.37*	0.25 *0.03*	0.20 *0.07*	0.29 *<0.01*	−0.03 *0.78*	0.11 *0.33*	0.25 *0.02*
Stable aggregates (%)								
1–2 mm diameter	−0.21 *0.06*	−0.16 *0.15*	−0.07 *0.53*	0.65 *<0.01* *	0.29 *<0.01*	−0.04 *0.79*	0.08 *0.48*	0.09 *0.40*
0.5–1 mm diameter	0.18 *0.13*	−0.26 *0.02*	−0.17 *0.12*	0.71 *<0.01* *	0.15 *0.19*	−0.10 *0.36*	0.06 *0.56*	−0.03 *0.80*
0.25–0.5 mm diameter	0.06 *0.65*	−0.29 *0.01*	−0.25 *0.02*	0.51 *<0.01* *	−0.03 *0.80*	−0.12 *0.30*	− <0.01 *0.99*	−0.15 *0.18*
Total	0.18 *0.14*	−0.26 *0.02*	−0.18 *0.12*	0.70 *<0.01* *	0.17 *0.14*	−0.09 *0.40*	0.06 *0.61*	−0.02 *0.85*
Organic matter (%)	0.32 *<0.01*	−0.05 *0.63*	0.05 *0.69*	0.23 *0.03*	0.15 *0.19*	0.18 *0.11*	0.11 *0.31*	0.12 *0.29*
Porosity (%)	0.19 *0.09*	0.03 *0.76*	0.21 *0.06*	0.20 *0.07*	0.32 *<0.01*	0.25 *0.02*	0.20 *0.07*	0.29 *<0.01*
Bulk density (g/cm^3)	−0.28 *0.01*	0.03 *0.81*	−0.11 *0.32*	−0.28 *0.01*	−0.29 *<0.01*	−0.22 *0.05*	−0.19 *0.90*	−0.22 *0.04*
Particle density (g/cm^3)	−0.33 *<0.01*	0.08 *0.47*	0.05 *0.60*	−0.28 *0.01*	−0.14 *0.22*	−0.12 *0.30*	−0.12 *0.28*	−0.06 *0.60*
Mycorrhizal potential	<0.01 *0.98*	−0.18 *0.13*	−0.13 *0.25*	0.04 *0.72*	−0.09 *0.45*	−0.06 *0.59*	−0.16 *0.15*	−0.13 *0.26*
pH	0.09 *0.44*	0.23 *0.04*	0.33 *<0.01*	0.19 *0.09*	0.31 *<0.01*	0.08 *0.49*	0.27 *0.02*	0.33 *<0.01*
PO_4-P (μg/g)	−0.18 *0.11*	−0.03 *0.80*	−0.23 *<0.01*	−0.22 *0.04*	−0.31 *<0.01*	−0.15 *0.19*	−0.16 *0.16*	−0.28 *0.01*
K (μg/g)	−0.17 *0.13*	0.22 *0.05*	0.15 *0.17*	−0.40 *<0.01*	−0.05 *0.67*	0.04 *0.71*	−0.02 *0.85*	0.06 *0.59*
Ca (μg/g)	− <0.01 *0.99*	0.36 *<0.01*	0.54 *<0.01* *	−0.14 *0.22*	0.41 *<0.01* *	0.56 *<0.01* *	0.45 *<0.01* *	0.55 *<0.01* *
Mg (μg/g)	−0.04 *0.71*	0.39 *<0.01*	0.63 *<0.01* *	−0.20 *0.07*	0.44 *<0.01* *	0.52 *<0.01* *	0.42 *<0.01* *	0.60 *<0.01* *
Fe (μg/g)	−0.13 *0.26*	0.30 *<0.01*	0.39 *<0.01*	−0.61 *<0.01* *	0.04 *0.73*	0.20 *0.07*	0.10 *0.37*	0.24 *0.03*
Zn (μg/g)	−0.21 *0.06*	0.03 *0.76*	−0.23 *0.04*	−0.34 *<0.01*	−0.40 *<0.01*	−0.10 *0.38*	−0.18 *0.12*	−0.31 *<0.01*
Cu (μg/g)	−0.15 *0.19*	0.03 *0.75*	0.02 *0.85*	−0.40 *<0.01*	−0.21 *0.06*	−0.08 *0.46*	−0.11 *0.31*	−0.10 *0.35*
Mn (μg/g)	−0.23 *0.04*	0.11 *0.33*	−0.04 *0.71*	−0.37 *<0.01*	−0.27 *0.01*	−0.08 *0.46*	−0.13 *0.25*	−0.16 *0.15*
Response to inoculation								
G. intraradices	−0.15 *0.19*	−0.07 *0.53*	−0.20 *0.07*	0.29 *<0.01*	− <0.01 *0.94*	− <0.01 *0.78*	0.04 *0.73*	−0.10 *0.36*
G. versiforme	−0.23 *0.04*	−0.03 *0.76*	0.03 *0.81*	0.20 *0.07*	0.13 *0.24*	0.01 *0.90*	0.18 *0.10*	0.07 *0.51*

Table 3 List ob the variables associated to leek response to inoculation (RTI) with *G. intraradices* and *G. versiforme*, in soils with available phospohorus levels above or below 200 μg g^{-1}, according to a 'Stepwise' regression analysis, with their *P* value, partial R^2 and coefficient

	P above 200 μg g^{-1} of soil					P below 200 μg g^{-1} of soil			
	P value	Partial R^2	Model R^2	Coefficient		*P* value	Partial R^2	Model R^2	Coefficient
G. intraradices									
Soil mycorrhizal potential	0.0002	0.33	0.33	−2.5989666	1–2 mm diameter aggregates	0.0003	0.27	0.27	0.73835505
G. etunicatum-rubiforme group spore no.	0.0022	0.17	0.50	−0.67436892	Intersect				−4.54252125
0.25–0.5 mm diameter aggregates	0.0124	0.09	0.59	−0.09434860					
Intersect				7.85359666					
G. versiforme									
Soil mycorrhizal potential	0.0012	0.27	0.27	−1.97536717	0.5–1 mm diameter aggregates	0.0001	0.35	0.35	0.72162586
1–2 mm diameter aggregates	0.0436	0.08	0.48	−1.40858154	Total spore number	0.0077	0.10	0.45	0.00323711
Soil Zn	0.0087	0.13	0.35	−0.10608191	*G. aggregatum* spore no.	0.0325	0.06	0.51	−0.44924330
Soil porosity	0.0447	0.06	0.545	0.17810467	Intersect				−3.81637851
Intersect				1.98104645					

leek RTI in high P soils and spore abundance of particular species or total spore abundance were sometimes retained by the analysis. Soil structure seems particularly important in the determination of RTI in both high and low P soils, with a proportion of water stable aggregates of given size classes always retained by the analysis. The importance of mycorrhizal mycelium in the formation of soil aggregates is well recognized (Tisdale and Oades 1982; Miller and Jastrow 1992) and soil structure is considered to be a physical soil characteristic with an important biological component to it. According to the hierarchical model of aggregate organization (Tisdale and Oades 1982), microorganisms and their debris are important binding agents at several different levels. The tying by AM hyphae of microaggregates into aggregates greater than 250 μm diameter is important to the stabilization of agricultural soils. AM hyphae also contribute to bacterial activity as a source of carbon and energy for the soil flora. Microbial products and debris stabilize microaggregates of lower-order aggregates, down to the level at which individual humic substance-coated clay platelets interact.

In rich soils, SM alone explained as much as 33% and 27% of RTI with *G. intraradices* and *G. versiforme.* In low P soils, aggregates of 0.5–1 mm and 1–2 mm had more weight and explained 35% and 27% of RTI with these two species. While it appears logical that leek responds to inoculation better in low SM soils, as in P-rich soils, it is more difficult to explain the positive influence of good soil aggregation on leek RTI in low P soils. If the abundance of aggregates in the 0.5–2 mm diameter range is related to the abundance of mycorrhizal mycelium, as shown in other studies (Miller and Jastrow 1992), the positive relation observed here between RTI and soil aggregation suggests that the fungal inoculation was symbiotically more efficient than strains already present. In agricultural soils, the pressure exerted by crop management practices may select for robust, fast-growing species (Giovannetti and Gianninazi-Pearson 1994) which may be less mutualistic (Johnson et al. 1992; Johnson 1993). Fungal strains with vigorous extraradical growth may be large sinks to plant carbon and thus improve soil quality more than crop growth. The larger RTI of leek in well-aggregated soil may also reflect a preference by *G. intraradices* and *G. versiforme* for well-aerated soils.

The high mycorrhizal colonization in *G. versiforme*-inoculated leeks measured both 4 and 14 weeks after transplantation suggests that this species is competitive for infection sites. However, this fungus was not more efficient than *G. intraradices*. Considering that large differences in mycorrhizal colonization were on average associated with only small differences in leek growth, the status of internal root colonization does not reflect symbiotic efficiency.

The importance of soil P levels for RTI can not be ignored. Soil P had profound effects on several variables and, hence, was the basis for dividing the data prior to regression analysis. However, its influence was not revealed in the resulting subsets. The higher RTI of leek in P-rich soil may be explained by the presence of a reduced or inefficient indigenous mycorrhizal flora in these soils. This suggests, contrary to common belief, that mycorrhizae-dependent crops achieve high RTI in P-rich agricultural soils. Our results underline the relevance of approaches such as that of Plenchette et al.

(1989), who developed a test for soil infectivity to evaluate the need to inoculate a specific crop.

The relationship between RTI and soil water stable aggregation changed with soil-available P level. RTI with either fungus was high in P-poor soils with good structure, but in P-rich soils RTI was low when soil structure was good. This interaction is probably responsible for the low R^2s obtained when the entire data set was analysed, and further justifies the split into high and low P groups.

The abundance of spores of the *G. etunicatum-G. rubiforme* group was negatively correlated with the RTI measured with *G. intraradices* in high-P soils. Similarly, *G. aggregatum* was negatively correlated with RTI and *G. versiforme* in low-P soils. This may indicate that inoculation is inefficient in soils rich in these species, presumably because they are very efficient or highly antagonistic to the inoculant fungi. However, spore abundance is not always an indicator of the size or health of a mycorrhizal fungal population in soil (Jasper et al. 1993). The negative correlation between the high spore number and RTI may thus reflect a stressed population triggered to sporulate. The introduced species may also be more susceptible to stress than the native species. The induction of sporulation by stress has been found in many fungi (Moser 1993), and has been indirectly demonstrated for arbuscular mycorrhizal fungi (Pacioni 1985; Kurle and Pfleger 1994).

The relationship found between certain species in the PCA suggests that these species are similar or complementary at the biological or ecological levels. Lloyd-Macgilp et al. (1996), studying genetic diversity of the ribosomal transcribed spacers within and among six isolates of *G. mosseae* and related mycorrhizal fungi, reported that sequences of two isolates of *G. fasciculatum*, one from the United Kingdom and one from Canada, placed them firmly within the range of diversity found in *G. mosseae* isolates, in spite of clear differences in spore morphology. Interestingly, *G. mosseae* and *G. fasciculatum* were also related in our analysis. A lack of methodologies has limited the study of AM fungal ecology. The current adaptation of molecular techniques to AM fungus research (e.g. Claassen et al. 1996) should open a very important new approach and shed light on aspects crucial for AM fungus management in agricultural soils: the complex relationship between soils, plant management and natural AM fungus populations.

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