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# Selective interactions between different species of mycorrhizal fungi and *Rhizobium meliloti* strains, and their effects on growth, $N_2$-fixation ($^{15}N$) and nutrition of *Medicago sativa* L.

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SUMMARY

Three isolates of vesicular–arbuscular (VA) mycorrhizal fungi, belonging to the species *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, *G. fasciculatum* (Thaxter *sensu* Gerd.) Gerd. and Trappe, and *G. caledonium* (Nicol. and Gerd.) Trappe and Gerd, were inoculated in dual combinations with six strains of *Rhizobium meliloti* with the aim of testing these combinations for functional compatibility with their common host plant, the legume *Medicago sativa* L. Symbiotic efficiency (promotion of plant growth and N and P nutrition) was found to be dependent on the particular combination of *Rhizobium* strain and *Glomus* species indicating selective and specific compatibilities between strains and isolates of the two types of microsymbiont, but also between them and the common host plant. Observed effects on plant growth were in general, though not always, related to the extent of VA mycorrhizal colonization. Although the different mycorrhizal and/or rhizobial treatments produced different effects on plant growth, the rate of nodule formation on *M. sativa* roots remained constant. Most mycorrhizal treatments increased the concentration and/or content of N in plant shoots but effectiveness was in the order: *G. fasciculatum* > *G. mosseae* > *G. caledonium*. In some cases, this increase in N-content may be a consequence of a P-mediated stimulation of $N_2$-fixation by VA mycorrhiza, as ascertained using $^{15}N$. In other instances, however, the increase seems to reflect a VA mycorrhizal-mediated enhancement of N-uptake from soil. VA mycorrhizal inoculation decreased the concentration of Ca and Mg in plant shoots and a buffering effect of VA mycorrhiza in situations of nutrient excess in soil is proposed.

Key words: Vesicular–arbuscular mycorrhiza, *Rhizobium*–legume symbiosis, $N_2$ fixation, $^{15}N$, Ca–Mg uptake.

## INTRODUCTION

Microbe–microbe interactions in the root–soil interfaces are key events within the dynamic processes concerning rhizosphere establishment and maintenance (Barea & Azcón-Aguilar, 1982; Linderman, 1988). Vesicular–arbuscular mycorrhizal (VA mycorrhizal) fungi are of particular interest in soil microbial interactions, since they actively affect the composition of microbial populations (Azcón, Barea & Hayman, 1976; Meyer & Linderman, 1986) and are in turn influenced by them in the context of mycorrhiza formation and function. These microbial activities also affect plant growth and nutrition. However, this mycorrhizosphere effect (Linderman, 1988) seems to be selective. Preferential stimulation of specific microorganisms (Ames, Reid & Ingham, 1984; Meyer & Linderman, 1986) was demonstrated to be dependent on the VA mycorrhiza fungal species involved (Secilia & Bagyaraj, 1987). Furthermore such selective interactions between establishment of microorganisms and VA mycorrhizal fungal species is reflected in plant growth and nutrient uptake (Azcón, 1989).

Microbial interactions involving VA mycorrhizal fungi are particularly interesting in the case of *Rhizobium* spp. since both microorganisms colonize the root system of a common host plant, a legume (Barea & Azcón-Aguilar, 1983; Hayman, 1986). The interactions between both endophytes are well documented (see Azcón-Aguilar & Barea, 1991), and show nutritional, as well as non-nutritional, host-mediated effects (Harris, Pacovsky & Paul, 1985; Ames & Bethlenfalvay, 1987; Brown & Bethlenfalvay, 1987; Brown, Thamsurakul & Bethlenfalvay, 1988).

**Table 1.** *Shoot dry weight (mg pot$^{-1}$) of alfalfa plants grown for 70 d, either non-inoculated or inoculated, singly or in dual combinations, with three* Glomus *spp. and six strains of* Rhizobium meliloti

| Rhizobial strain | Mycorrhizal fungus | | | |
|---|---|---|---|---|
| | None | *G. fasciculatum* | *G. mosseae* | *G. caledonium* |
| None | — | 1119b | 991ab | 989ab |
| Rm 104A14 | 1201bc | 1535d | 1036ab | 1376d |
| Rm 41 | 1186bc | 1455d | 1106ab | 887a |
| Rm 2 | 1218bc | 1055b | 1096b | 1065ab |
| Rm GR4 | 1063b | 1349c | 1378c | 939ab |
| Rm 309 | 1123b | 1349c | 1310c | 1078ab |
| Rm 2011 | 1056ab | 1410cd | 1217b | 1190bc |

Means (five replicates) not followed by a common letter differ significantly ($P \leqslant 0.05$) from each other (Duncan's multiple range test).

Information is, however, scarce concerning combinations of *Rhizobium* with different VA mycorrhizal fungus strain species (Bayne & Bethlenfalvay, 1987). Such information would be important from the basic point of view and also for practical purposes, since synergism between VA mycorrhizal colonization and nodulation can be decisive to nutrient uptake and $N_2$ fixation by the common host legume. Measurement of $N_2$ fixation, a critical parameter for this type of study, can be made by $^{15}N$ isotope dilution techniques. These are particularly suitable for estimation of the relative contribution of the atmosphere ($N_2$ fixation), added fertilizer, or endogenous soil N to the N content of the plant. Additionally, these methods indicate whether any particular treatment influences N nutrition by acting on $N_2$ fixation or on N uptake from soil (Danso, 1988). When quantitative estimates are needed only to rank the effects of several treatments, the method does not require a reference crop. Using this procedure, information is presented on compatibility between three species of VA mycorrhizal fungi of the genus *Glomus* and six strains of *Rhizobium meliloti*, as determined by their effects on growth, $N_2$ fixation and nutrient uptake by *Medicago sativa* L. In addition, we report studies to determine whether these effects are associated with changes in overall VA mycorrhizal colonization and/or nodulation.

## MATERIALS AND METHODS

Alfalfa (*Medicago sativa* L., cv. Aragón) was the test plant. After 5 d, seedlings (two per pot) obtained from surface sterilized seeds were transplanted to a soil:sand mixture (5/2, v/v), previously steam-sterilized (100 °C for 1 h during three consecutive days) and distributed into pots (300 ml). A clay-loam calcareous soil collected from Granada province (Spain) was used. The characteristics of this test soil were as follows: pH, 7·8; available ($NaHCO_3^-$-extractable) P, 11 mg l$^{-1}$; total N, 1180 mg l$^{-1}$; exchangeable K, 5·27 mequiv l$^{-1}$; exchangeable Ca, 345 mequiv l$^{-1}$; Mg, 86 mequiv l$^{-1}$. At transplanting, seedlings were inoculated with three VA mycorrhizal fungal species: *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *Glomus fasciculatum* (Taxter *sensu* Gerd.) Gerd. & Trappe and *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerd. This was achieved by placing 2 g per pot of V–A mycorrhizal fungus inoculum, obtained from our own stock culture collection and maintained by storage for 3–6 months in polyethylene bags at 5 °C. Inocular consisted of thoroughly mixed rhizosphere samples containing spores, hyphae and VA mycorrhizal root fragments. The level of application was saturating and, in a preliminary test, produced a similar level of infectivity with onion plants (*Allium cepa* L.). Control pots were prepared in the same way. Each mycorrhizal treatment and controls were assayed singly and/or in dual *Glomus/Rhizobium* combinations. The *Rhizobium meliloti* strains used were: Rm 104A14, Rm 41, Rm 2, Rm GR4, Rm 309 and Rm 2011. These strains were maintained in our own rhizobia culture collection. There were also control pots, uninoculated (with either endophyte). Inocula of *Rhizobium* strains were grown in Allen medium (Allen, 1957) and applied (1 ml containing $10^8$ cells per pot) at sowing time into both mycorrhizal and non-mycorrhizal pots. The resulting 28 treatments (3 VA mycorrhiza; 6 *Rhizobium*; 18 VA mycorrhiza + *Rhizobium*; 1 uninoculated control) were replicated 5 times. After 10 d of plant growth each pot received a solution of $(^{15}NH_4)_2SO_4$ with 10% $^{15}N$ atom excess, which supplied 2 mg N kg$^{-1}$ soil, equivalent to 5 kg N ha$^{-1}$.

Plants were grown for 70 d under greenhouse conditions with temperatures ranging from 19 to 25 °C, 16/8 light/dark photoperiod and a relative humidity of 70–90%. A photosynthetic photon flux density of 400–700 $\mu$mol m$^{-1}$ s$^{-1}$ was applied as supplementary light. During the assay plants were fertilized (10 ml w$^{-1}$ pot$^{-1}$) with an N and P-free nutrient solution (Hewitt, 1952).

Once plants were harvested, the weight of shoots and roots was recorded and the shoot tissues were analysed for N, P, K, Ca and Mg (Lachica, Aguilar & Yañez, 1973). The extent of root colonization was assessed by the staining method described by Phillips & Hayman (1970). The percentage of total root length that became mycorrhizal was calculated by a gridline intersect technique (Giovannetti & Mosse, 1980). The N isotopic composition of plant shoots was determined by mass spectrometry, as described by Fiedler & Proksch (1975), at the FAO/IAEA Agricultural Biotechnology Laboratory, Seibersdorf, Austria. It is assumed that when several treatments are being tested for their effects on $N_2$

**Table 4.** *Atom percent $^{15}N$ excess in the shoots of alfalfa plants grown for 70 d, either non-inoculated or inoculated, singly or in dual combinations, with three* Glomus *spp. and six strains of* Rhizobium meliloti

| Rhizobial strain | Mycorrhizal fungus: None | *G. fasciculatum* | *G. mosseae* | *G. caledonium* |
|---|---|---|---|---|
| Rm 104A14 | 0·053b | 0·045a | 0·048ab | 0·045a |
| Rm 41 | 0·055b | 0·043a | 0·052b | 0·058b |
| Rm 2 | 0·058b | 0·051b | 0·051b | 0·059b |
| Rm GR4 | 0·059b | 0·047ab | 0·051b | 0·063b |
| Rm 309 | 0·058b | 0·046ab | 0·044a | 0·058b |
| Rm 2011 | 0·054b | 0·046ab | 0·054b | 0·058b |

Means (five replicates) not followed by a common letter differ significantly ($P \leq 0·05$) from each other (Duncan's multiple range test).

**Table 6.** *Shoot K, Ca and Mg concentration in alfalfa plants grown for 70 d, either non-inoculated or inoculated, singly or in dual combinations, with three* Glomus *spp. and six strains of* Rhizobium meliloti

| Rhizobial treatments | Mycorrhizal treatments: None | *G. fasciculatum* | *G. mosseae* | *G. calcedonium* |
|---|---|---|---|---|
| | | K (mg/g) | | |
| None | — | 1·48rs | 1·55st | 1·34rs |
| Rm 104A14 | 1·34rs | 1·55s | 1·48st | 1·37rs |
| Rm 41 | 1·53rst | 1·63st | 1·57st | 1·55st |
| Rm 2 | 1·56st | 1·54rst | 1·64st | 1·65rst |
| Rm GR4 | 1·40rs | 1·60st | 1·44rs | 1·48rs |
| Rm 309 | 1·63st | 1·61st | 1·68st | 1·58st |
| Rm 2011 | 1·31rs | 1·59s | 1·62st | 1·57st |
| | | Ca (mg/g) | | |
| None | — | 2·04a | 2·26ab | 2·83b |
| Rm 104A14 | 3·83c | 2·26ab | 2·04ab | 2·77b |
| Rm 41 | 4·29c | 2·86b | 2·77b | 2·86b |
| Rm 2 | 4·24c | 2·25ab | 2·42ab | 2·75b |
| Rm GR4 | 3·96c | 2·33ab | 2·0a | 2·77b |
| Rm 309 | 3·84c | 2·21ab | 2·29ab | 2·68ab |
| Rm 2011 | 3·89c | 2·18ab | 2·19ab | 2·45b |
| | | Mg (mg/g) | | |
| None | — | 0·35xyz | 0·31xy | 0·38xy |
| Rm 104A14 | 0·43z | 0·31xy | 0·29xy | 0·36xy |
| Rm 41 | 0·50z | 0·33xy | 0·33y | 0·36xy |
| Rm 2 | 0·48z | 0·32y | 0·34y | 0·37y |
| Rm GR4 | 0·47z | 0·34y | 0·32xy | 0·39yz |
| Rm 309 | 0·48z | 0·30xy | 0·34xy | 0·38y |
| Rm 2011 | 0·48z | 0·32xy | 0·29xy | 0·39xyz |

Means (five replicates) not followed by a common letter differ significantly ($P \leq 0·05$) from each other (Duncan's multiple range test).

rhizobial treatments producing a differing degree of effectiveness nevertheless induced the same extent of nodulation on alfalfa roots (Tables 1, 2).

Table 3 records the effects of the different mycorrhizal × rhizobial treatments on N concentration and content of alfalfa plants. The presence of both *G. fasciculatum* and *G. mosseae* enhanced N concentration and content in plant shoots. In comparing the effect of the different *Rhizobium* strains within the same *Glomus* spp. treatment, only Rm 104A14 and Rm 41 (for *G. fasciculatum*) and Rm 309 (for *G. mosseae*), significantly enhanced the total N content, though not always the concentration, over other rhizobial treatments. Inoculation with *G. caledonium* did not change N concentration relative to that in non-mycorrhizal controls and improved the total N content in only one case (Rm 104A14).

Some of the effects of specific combinations of rhizobia and mycorrhizal fungi at enhancing N nutrition in alfalfa appear to be exerted through $N_2$ fixation, as shown by $^{15}N$ enrichment studies (Table 4). Lowering of the $^{15}N/^{14}N$ ratio in shoots of plant inoculated with certain *Rhizobium* × mycorrhizal combinations may indicate a relative improvement of $N_2$ fixation, as induced by the treatment.

Inoculation with *G. fasciculatum* and *G. mosseae*, but not with *G. caledonium*, improved the con-

**Table 5.** *Shoot P (concentration and content on a per pot basis) in alfalfa plants grown for 70 d, either non-inoculated, singly or in dual combinations, with three* Glomus *spp and six strains of* Rhizobium meliloti

| Rhizobial strain | Mycorrhizal fungus: None | | *G. fasciculatum* | | *G. mosseae* | | *G. caledonium* | |
|---|---|---|---|---|---|---|---|---|
| | P mg/g) | P (mg) | P (mg/g) | P (mg) | P (mg/g) | P (mg) | P (mg/g) | P (mg) |
| None | — | — | 0·16b | 1·79y | 0·17b | 1·68y | 0·11a | 1·09xy |
| Rm 104A14 | 0·11a | 1·32xy | 0·17cb | 2·60z | 0·16b | 1·65y | 0·11a | 1·51y |
| Rm 41 | 0·11a | 1·30y | 0·16b | 2·33z | 0·15b | 1·64y | 0·11a | 0·97x |
| Rm 2 | 0·13a | 1·58y | 0·19cd | 2·02z | 0·20d | 2·75z | 0·13a | 1·38y |
| Rm GR4 | 0·10a | 1·06x | 0·18c | 2·43z | 0·17bc | 2·34z | 0·11a | 1·03xy |
| Rm 309 | 0·14ab | 1·75y | 0·18c | 2·43z | 0·19cd | 2·48z | 0·11a | 1·18xy |
| Rm 2011 | 0·11a | 1·16xy | 0·18c | 2·54z | 0·16b | 1·94z | 0·11a | 1·31y |

Means (five replicates) not followed by a common letter differ significantly ($P \leq 0·05$) from each other (Duncan's multiple range test).