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Assessment of Natural Mycorrhizal Potential in a Desertified Semiarid Ecosystem

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A survey of the natural mycorrhizal potential has been carried out in a representative area of a desertified semiarid ecosystem in the southeast of Spain. Many indigenous plants from the field site were mycorrhizal, including the dominant *Anthyllis cytisoides*, which had high levels of colonization by arbuscular mycorrhizal fungi (AMF). Low numbers of AMF spores were present in the soil, although a range of species, including *Scutellospora calospora*, *Glomus coronatum*, *Glomus constrictum*, and several *Acaulospora* species, was represented. Soil infectivities, as determined by a soil dilution method, were similar for most plants tested but were significantly lower for *Anthyllis cytisoides*. Nevertheless, when a less disruptive method to determine soil infectivity was used, the importance of the mycelial network in maintaining the infectivity of soil under perennial shrubs, such as *Anthyllis cytisoides*, was highlighted. Seasonal variations in the mycorrhizal infectivity showed that it was higher towards the end of the summer period than in midwinter. In screening trials in a greenhouse, the indigenous AMF did not significantly improve the growth of plants compared with that of noninoculated controls. Augmentation of the soil with an inoculum of *Glomus intraradices* resulted in improved growth of *Anthyllis cytisoides* in both sterile and nonsterile conditions, in contrast to results obtained following inoculation with *Glomus mosseae* or another *Glomus* sp. Our findings suggest that the indigenous inoculum levels of AMF are inadequate to support an extensive revegetation program in the absence of an additional mycorrhizal inoculum.

The indigenous flora of the Mediterranean region is usually dominated by characteristic semiarid shrub communities formed mainly from small woody plants. Mediterranean ecosystems are subjected to a set of particular climatic conditions in which scarce and irregular rainfall is a key determinant (4). There is often a characteristic, very long dry period in the summer, usually lasting for several months. Desertification can become a serious problem where this precipitation regime is particularly erratic and is combined with anthropogenic pressure exerted over a long time. Desertified Mediterranean ecosystems are very fragile and subject to progressive disturbance of the vegetation cover (12) and the rapid erosion of surface soils. This disturbance and the soil erosion generally result in the loss or reduction of mycorrhizal propagules present in the soil and thus in the subsequent reduction in the inoculum potential for mycorrhiza formation (5, 12, 16, 17). This can be critical because mycorrhizal symbioses are key components of natural systems, particularly in desertified Mediterranean landscapes (5, 7), because of their essential role in sustaining the vegetation cover (17). The low density of mycorrhizal propagules in damaged soils, such as semiarid ecosystems (20) or mining sites, may limit the successful reestablishment of native plants (25), and a rehabilitation approach for revegetation of these areas must begin with the evaluation of the mycorrhizal status of the soil (13). If the mycorrhizal inoculum potential is low or ineffective, revegetation must include the reconstitution of an appropriate mycosymbiont population (2, 13). Since the plant species show differences in the degree of dependency on mycorrhizae, revegetation strategies must also consider the level of dependency of the plants involved as well as the actual mycorrhizal potential of the soil.

The aim of this work was to determine qualitatively and quantitatively the mycorrhizal status and the spatial and temporal variations in propagules of arbuscular mycorrhizal fungi (AMF) in a semiarid ecosystem in the southeast of Spain. The site chosen is being used as a pilot study zone for revegetation of desertified Mediterranean ecosystems. It has a natural community of woody legume plants in which the shrub legume *Anthyllis cytisoides* dominates. This species has thus been chosen as a test plant to investigate whether mycorrhizal technology can be used to accelerate the natural process of revegetation of barren areas.

MATERIALS AND METHODS

The ecosystem. The area chosen for a general program of revegetation is situated in a sedimentary basin, about 600 to 800 m high, located in the Sierra de Filabres, Almeria (southern Spain). The mean annual precipitation is 230 mm. The soil is an Eutric Regosol, the main characteristics of which are recorded in Table 1. The natural vegetation of this area consists of three main shrub species, i.e., *Anthyllis cytisoides*, *Stipa tenacissima*, and *Retama sphaerocarpa*, and a variety of small graminaceous species, such as *Stipa capensis*. The most important of these shrubs is *Anthyllis cytisoides*, a drought-tolerant legume able to form symbioses with both *Rhizobium* and AMF species. This species accounted for more than 60% of the shrub vegetation. Several other shrub species, such as *Artemisia herba alba* and *Thimelaea hirsuta*, also grew in this area. A representative experimental area of 20 by 100 m which contained randomly spaced *Anthyllis*, *Artemisia*, *Retama*, and *Stipa tenacissima* plants was chosen. Most of the intervening area was occupied by annual grasses (dominated by *Stipa capensis*). The area had a slight slope and included plants of *Stipa tenacissima* towards the higher region of the incline. All soil and plant samples were taken from within the experimental area.

Field sampling. A number of samples from the rhizosphere of the plants present within the experimental area were collected. Six replicate samples of soil from the root zone of each species were analyzed. Samples were stored in plastic bags at 4°C until used. Roots were washed and stained for analysis of coloniza-

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TABLE 1. Soil components and characteristics

Soil component or characteristic	Value
Sand (%)	
Very coarse	13.96
Coarse	20.97
Medium	14.58
Fine	14.76
Very fine	4.65
Total	68.93
Clay (%)	5.10
Loam (%)	25.98
Organic carbon (%)	0.41
Nitrogen (%)	0.10
C/N	4.1
P (ppm)	2.64
K (ppm)	201.9
$CaCO_3$ (%)	0.28
pH	
H_2O	7.91
ClK	7.07

tion by AMF by a modified Phillips and Hayman procedure (21). The roots were kept in cold 10% (wt/vol) KOH for 24 h and then heated in 10% KOH for 1 h before being cleared with alkaline H_2O_2 for 30 min. The roots were then rinsed with tap water and neutralized with 10% (wt/vol) HCl for 5 min and stained with boiling glycerol-trypan blue solution (0.05%) for 10 min. Newman's intersection method was used to measure root colonization by AMF (11).

Spores of AMF were isolated by wet-sieving 100 g of rhizosphere soil into a 50-μm sieve. Spores were then concentrated from the sievings by centrifugation onto a 50% (wt/vol) sucrose solution. Spores were collected with a pipette from the water-sucrose interface and rinsed with tap water. The number and type of spores were recorded.

Measurement of the infectivity of soil samples by a soil dilution method. The infectivities of AMF in the root zone of *Anthyllis cytisoides*, *R. sphaerocarpa*, and both *Stipa* species were determined by a soil dilution method (10). This method consisted of making serial dilutions of the rhizosphere soil with soil pasteurized by steaming for 1 h on 3 consecutive days. Seedlings of a test plant species (either *Anthyllis* or *Sorghum* sp.) were placed individually into each of five replicates of each soil dilution treatment. Similar results were obtained with either of these trap plants, and *Sorghum* sp. was used subsequently as a test plant as recommended by Franson and Bethlenfalvay (10). After 15 days of growth, the number of entry points of AMF per root length was calculated after clearing and staining with trypan blue (21). Root lengths were measured with an image analyzer. The infectivity test was carried out twice during 1993 (13 January and 28 September), i.e., at the start of the wet season and at the end of the dry season.

Measurement of the infectivity of the soil by a less disruptive method than the soil dilution method. Because the network of hyphae in soil is more sensitive to soil disturbance than the relatively robust spores and mycorrhizal roots (14, 15), it was considered necessary to also assess the infectiveness of the soil by a less disruptive method. Soil was carefully collected from the root zone of *Anthyllis* or *Stipa capensis* plants growing in the field and placed in 1.5-kg pots with minimal disturbance. Four replicate pots were established for each source of soil. Four nylon mesh bags, two with a pore size of 53 μm and two with a pore size of 2 mm, were buried in each pot (Fig. 1). Each bag was filled with 130 g of steamed soil collected from the same area, and one *Sorghum* seed was set into each bag. After 4 weeks of growth, mycorrhizal colonization was estimated as before. It was assumed that within this short time period, colonization of *Sorghum* plants within the 53-μm-pore-size bags must have resulted only from growth of mycelium into the bags. In contrast, in the 2-mm-pore-size bags, roots of the *Sorghum* seedling were able to grow out of the steamed soil and into the unsterile soil and thus come into contact with other propagules such as germinating spores and infected root fragments.

Screening for AMF compatible with *Anthyllis cytisoides* for use in disturbed soil. *Anthyllis cytisoides* seeds collected in the experimental area were sacrificed, surface sterilized with 2.5% (wt/vol) $HgCl_2$ for 10 min, and rinsed five times with sterile deionized water. Seeds were then germinated on filter paper after being immersed in sterile tap water for 24 h. Seedlings (10 to 15 mm tall) were sown in pots containing 300 g of soil collected from the experimental site. Half of the pots contained sterilized soil (1 h of steaming for 3 consecutive days), and the other half contained nonsterile soil. Three AMF from the stock culture collection of the Estación Experimental del Zaidín were used, namely, *Glomus mosseae* (Nicol. et Gerd.) Gerd. et Trappe, a *Glomus* sp. (laboratory collection), and *Glomus intraradices* (Schenck et Smith). The inoculum was applied as 10 g of crude inoculum of onion roots per pot, containing an average of 70% of the root length colonized. Plants growing in sterile soil were also given 10 ml of a soil filtrate (Whatman no. 1) to partially replace the original bacterial microbiota. Ten replicates were used per treatment. Plants were grown in the greenhouse for 80 days under controlled conditions of light, moisture, and temperature and watered every 2 days with tap water to 70% soil water-holding capacity. After harvest, shoot and root fresh weights were determined, and the plants were then dried at 60°C for 24 h to determine the dry weight. Roots were stained for mycorrhizal colonization (11, 21).

Statistical methods. Data on soil infectivity obtained by the soil dilution method were analyzed by one-way analysis of variance for a completely randomized design. Significant differences between treatments were separated by use of least significant differences at a P of <0.05 when main effects were significant. Results from the infectivity test obtained with the less disruptive method were expressed with the standard error of the treatment means for 95% confidence limits. Data from the fungal screening were subjected to a randomized block analysis of variance (one-way analysis of variance), and significant results ($P < 0.05$) were analyzed by the Duncan test.

RESULTS

Natural mycorrhizal colonization. Examination of roots from the main mature plants present in the ecosystem (*Anthyllis cytisoides*, *R. sphaerocarpa*, *Stipa tenacissima*, and *Artemisia herba alba*) showed that all of them were mycorrhizal. *Stipa* and *Anthyllis* roots were the most densely colonized, whereas *Artemisia herba alba* had a relatively low level of colonization by AMF (Table 2). Seedling and young plants of *Anthyllis cytisoides* were also examined and were heavily mycorrhizal. In all cases, typical mycorrhizal structures (coils, arbuscules,

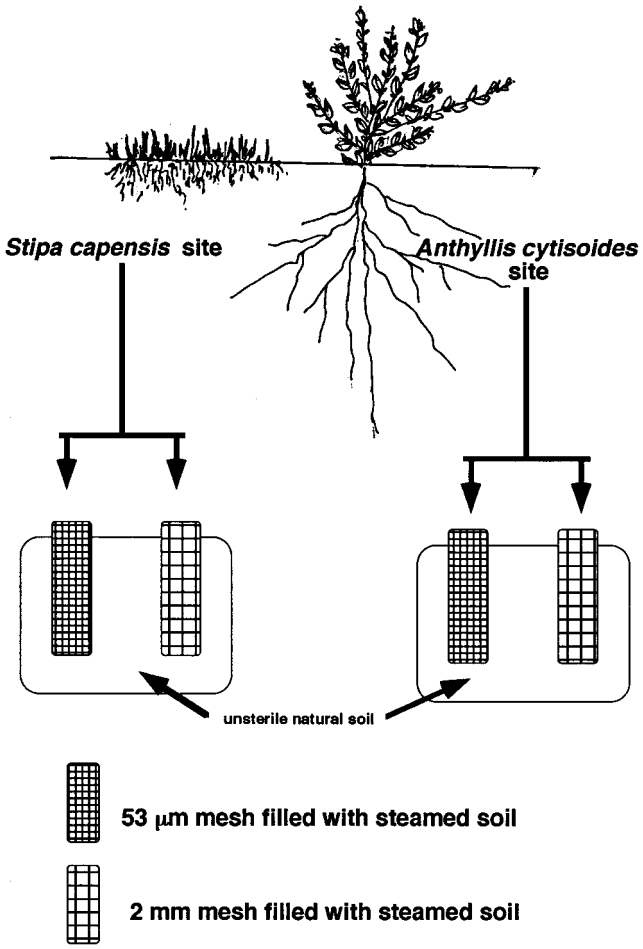

FIG. 1. Design of the experiment for the measurement of the mycelial infectivity.

TABLE 2. Main plant species in the experimental area and their range of AMF colonization

Plant species	Range (%) of AMF colonization
Family Leguminosae	
Anthyllis cytisoides	60–80
Retama sphaerocarpa	40–50
Family Compositae—*Artemisia herba alba*	0–5
Family Poaceae	
Stipa tenacissima	70–80
Stipa capensis	40–60

hyphae, and vesicles) were present, and in the case of *Anthyllis cytisoides*, entry points were frequently observed on root hairs.

Population of spores. The total number of spores of AMF recovered from the soil of the root zone was relatively low (ca. 20 to 40 spores per 100 g of soil), and many appeared degenerate. The number of spores found in the root zone of *Stipa* species was slightly higher than that found around *Anthyllis* plants. Spores of many different species of AMF were present, the most common being *Scutellospora calospora*, *Glomus coronatum*, *Glomus constrictum*, *Acaulospora* spp., an uncultured species with a white hyaline reticulate spore (previously described by Dodd and Krikum [8]), and a species with a small (50- to 80-μm-diameter) yellow spore (22). The species recorded are typical of other arid Mediterranean ecosystems (8, 18).

Spatial and temporal infectivity of the native AMF determined by the soil dilution method. There was no significant difference in levels of mycorrhizal infectiveness among samples collected from soil under *Stipa capensis*, *R. sphaerocarpa*, and *Stipa tenacissima* plants as determined by the soil dilution method. The soil from beneath *Anthyllis* plants, however, was significantly less infective in the *Sorghum* bioassay (Fig. 2) in terms of mycorrhizal colonization.

Differences in relative mycorrhizal infectivity at the two sampling times (28 September and 12 January) were recorded (Fig. 3). Infectivity was higher in September (end of the hot season) than in January (the coldest month). These differences were more noticeable in soil taken from under *Stipa capensis* plants. Plants grown in native soil collected in September had almost twice the number of entry points than those grown in soil collected in January.

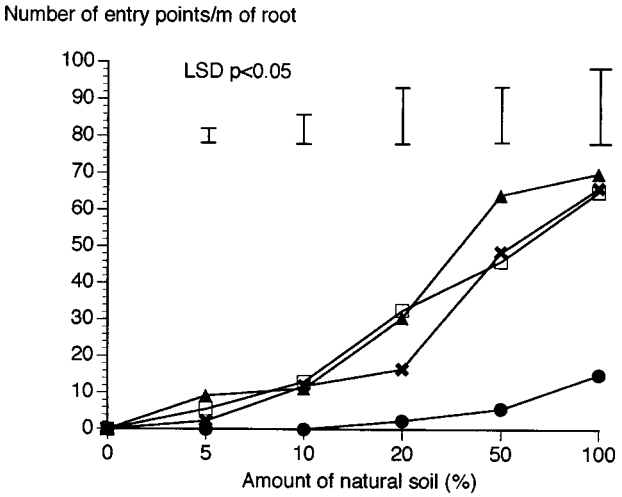

FIG. 2. Infectivity of soil samples from under four different plants collected in the area, determined by the soil dilution method. Results are expressed as the number of entry points per meter of root. Symbols: □, *Stipa capensis*; ●, *Anthyllis cytisoides*; ▲, *R. sphaerocarpa*; ×, *Stipa tenacissima*; LSD, least significant difference.

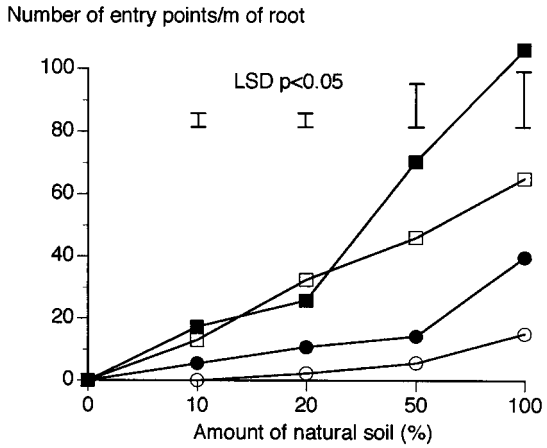

FIG. 3. Infectivity of soil from the *Anthyllis cytisoides* and *Stipa capensis* rhizosphere at two sampling times (I and II), determined by the soil dilution method. Results are expressed as the number of entry points per meter of root. Symbols: ■, *Stipa capensis* I; ●, *Anthyllis cytisoides* I; □, *Stipa capensis* II; ○, *Anthyllis cytisoides* II. LSD, least significant difference.

Relative contribution of the mycelia to the AMF infectivity. There were 8.5 ± 1.5 entry points per m of root for *Sorghum* plants grown in 53-μm-pore-size mesh bags in soil from below *Anthyllis* plants compared with 5 ± 1.5 entry points when grown in 53-μm-pore-size mesh bags in soil collected from below *Stipa* plants. In contrast, for *Sorghum* plants grown in 2-mm-pore-size mesh bags, the respective values were 14.5 ± 1 entry points per m of root in soil from below *Anthyllis* plants and 21 ± 2.5 entry points per m of root in soil from below *Stipa* plants. These *Sorghum* plants grown in soil bagged within the smaller-pore-size mesh bags had significantly fewer entry points than those grown in 2-mm-pore-size mesh bags, irrespective of the plant below which the rhizosphere soil was obtained. However, in soil obtained from *Anthyllis* rhizospheres, the infectivity within the 53-μm-pore-size mesh bags was 60% of that recorded for the 2-mm-pore-size mesh bags, whereas in soil from under *Stipa* plants, the respective value was only 24%.

Screening of AMF for use in disturbed soil with *Anthyllis cytisoides*. Results indicated that native fungi were ineffective at promoting growth of *Anthyllis cytisoides* despite colonizing a relatively large percentage of the roots (40%) (Fig. 4 and 5). Two of the fungi from the stock culture collection were also relatively ineffective under these dry, poor-nutrient growing conditions (i.e., *G. mosseae* and the *Glomus* sp.). Inoculation with *G. intraradices* enhanced shoot dry weight and biomass production when applied in either sterile or nonsterile soil. Inoculation with *G. mosseae* also caused a slight positive effect on shoot growth, but this was no longer evident in nonsterile soil conditions.

DISCUSSION

Mycorrhizal symbiosis is a key component in helping plants to establish in degraded soils. Before initiating a revegetation program, it is necessary to study the existing vegetation and its

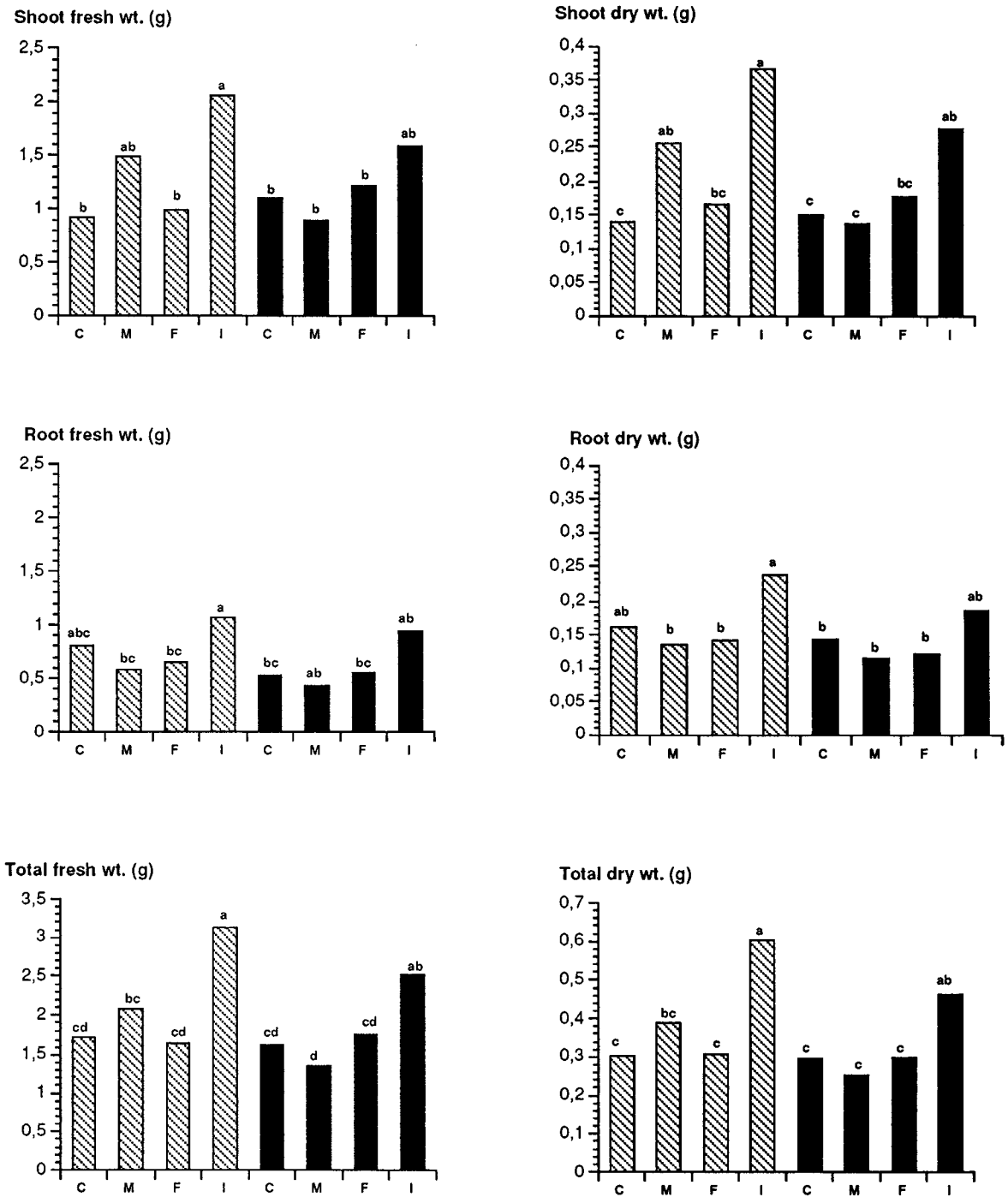

FIG. 4. Screening of *Anthyllis cytisoides*-compatible AMF under sterile and nonsterile soil conditions. Columns sharing any letter are not significantly different for a P of <0.05 by Duncan's multiple range test. Symbols: ▨, sterile soil; ■, nonsterile soil. Abbreviations: C, control; M, *G. mosseae*; F, the *Glomus* sp.; I, *G. intraradices.*

associated mycorrhizal propagules (13). Most of the plants examined in this study from the Almerian ecosystem were heavily mycorrhizal, indicating the high level of mycotrophy of the existing vegetation within this degraded ecosystem, where the lack of appropriate nutrient levels and water stress (<230 mm of annual mean precipitation) make it difficult for plants to survive. Woody legumes have already been recorded as being highly dependent on mycorrhizae, especially in stressed ecosystems (12). *Anthyllis cytisoides* is one of the plants that is most densely colonized by AMF in the area, including young plants (9). It is known to be very responsive to mycorrhizal inoculation (19) in nutrient-deficient conditions. In contrast, it was expected that *Stipa tenacissima*, a plant species with a graminoid type of root system, would be less mycotrophic. However, that was not the case. *Stipa* plants were extensively colonized by mycorrhizal fungi, perhaps because they grew where the arable soil layer is thinner and more rocky and because of the extremely low nutrient levels of the soil. *Artemisia* plants, how-

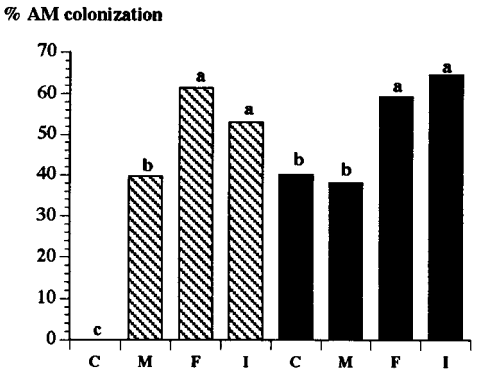

FIG. 5. Arbuscular mycorrhizal (AM) colonization rates expressed in percentage of colonized root for the different treatment assayed. Columns sharing any letter are not significantly different for a P of <0.05 by Duncan's multiple range test. Symbols: ▧, sterile soil; ■, nonsterile soil. Abbreviations: C, control; M, *G. mosseae*; F, the *Glomus* sp.; I, *G. intraradices.*

ever, seemed capable of surviving with a relatively low level of mycorrhizal colonization.

Under greenhouse conditions, the indigenous AMF appeared to be relatively ineffective in promoting growth of *Anthyllis cytisoides*. This may not be surprising since most of the AMF in the area probably became established during the long period of cultivation of agricultural plant species prior to land abandonment earlier this century. Once the land was no longer cultivated, native plants, such as *Anthyllis cytisoides*, began to recolonize the abandoned areas and would have become mycorrhizal from the existing AMF. Thus, the plant and AMF populations might not have developed together and may not be functionally compatible. Conversely, an exotic species, *G. intraradices*, promoted plant growth even in the presence of other fungi (i.e., under nonsterile soil conditions). This may be due to its faster ability to colonize plant roots, which makes it highly competitive, when compared for example with the colonizing ability of *G. mosseae*. Results must be interpreted with care, however, since those species of AMF which promote growth increases may not be the most appropriate for the long-term survival and competitiveness of the indigenous shrubs.

From a functional point of view, three forms of propagules of AMF could contribute to the infectivity of a given ecosystem, namely, (i) soilborne spores of AMF, (ii) mycorrhizal roots or fragments of these, and (iii) the network of mycelium of AMF. This mycelium is ultimately derived from germinating spores or from mycorrhizal roots, but once established, it can be considered a different propagule because of its position, structure, and spatial pattern of growth. In extreme cases, it has been considered that the infectivity of the hyphal network does not necessarily depend on attachment to host plant roots and can be maintained in the absence of spores (15). The relative importance of spores, mycorrhizal roots, and the mycelial network as propagule sources and the differences in their behavior in response to disturbance may determine how severely such disturbance affects the soil infectivity in a given ecosystem (16).

Soilborne spores of AMF are sometimes considered the main propagule reserve. There are usually only very low numbers of viable spores in soil from eroded ecosystems (6, 20). This is supported by the present study, in which a low number of spores was found in all samples, and the fact that many of these spores appeared empty or parasitized. This suggests that this propagule is not the main source of a mycorrhizal inoculum in this ecosystem, as suggested by McGee (20) for a semiarid site in Australia. On the other hand, the rapid initiation of colonization by AMF of young *Anthyllis* plants, grown in the soil from established *Anthyllis* root zones, indicates that the mycelium extending from mycorrhizal roots may be the main source of an inoculum.

With regards to the test determining the overall infectivity of soil samples, the higher infectivity level of the samples taken from the root zone of the grass relative to that of samples from *Anthyllis* plants could be explained by the different nature of the plants. Annual plants, such as the small grasses, complete their growth cycle within a single growing season. The roots are relatively superficial and grow mainly during the wet season. They maintain a large amount of a fine mycorrhizal root network, which persists after the growing season and is supplemented as an inoculum source by sporulation and infected root fragments. Perennial plants, such as *Anthyllis cytisoides*, have a much deeper root system, with long main roots poorly ramified near the topsoil. This would suggest that the *Anthyllis* root zone will support a relatively stable mycelial network deep in the soil that was established from an inoculum in the surface layers during seedling establishment.

Soil disturbance has two main effects on the mycelium of AMF (14, 15): one is the detachment of this hyphal network from the host plant, and the other is the disruption of this network. The former was demonstrated not to affect the overall infectivity, whereas the disruption of the mycelial network produced a reduction in soil infectivity. Both effects are inherent in the manipulation of samples for the soil dilution test in this experiment. It follows that when the growing mycelium in a soil sample is the main propagule source (i.e., in *Anthyllis* plants), the true infectivity level is underestimated, since the results are affected by the disturbance much more than in samples where a variety of propagules exists (i.e., in the grass root zone). In fact, fragmentation of the root system can give rise to an artificial increase in soil infectivity by increasing the number of viable propagules (26). Thus, the finding that infectivity was lower in soil under *Anthyllis* plants than under *Stipa capensis*, by a soil dilution test, can be explained by the characteristics of the different plant root systems. Alternatively, it might be that the annual *Stipa capensis* can establish only where it is rapidly colonized by AMF, i.e., where the soil inoculum potential is already inherently high.

The test carried out in the present study to evaluate soil infectivity with a minimal disturbance caused by the sampling procedure, i.e., that using the mesh compartmental system, gives a better indication of the relative importance of the mycelial network in the arbuscular mycorrhizal infectivity of the site. This test demonstrated that growing hyphae are the main source of mycorrhizal infection in the soil under *Anthyllis* plants, whereas their relative contribution to the total infectivity of the soil under grasses appears to be lower. Thus, because the overall infectivity is much higher in the grass root zone, it is assumed that the pool of fine mycorrhizal roots from the grasses is the main source of the inoculum. This agrees with the conclusions of other authors (16), who suggested that the overall infectivity is less affected by the disturbance of soils containing a high number of spores or mycorrhizal rootlets whereas the mycelial network is the form of propagule which is most sensitive to disturbance. In summary, it appears that, in the case of *Anthyllis* plants, the mycelium growing out from roots or growing from germinating spores outside the mesh bags appears to be the most important source of an inoculum; conversely, the mycelia arising from other propagule sources could be responsible for the infectivity in soil under the small grasses.

Spatial and temporal variations of mycorrhizal propagules occur not only quantitatively but also qualitatively in soils (1, 3, 23, 24). Production of new roots in the growing season (i.e., spring) results in an increase in the availability of vacant sites for establishment of arbuscular mycorrhizae and the activation of the life cycle of AMF (23). After rainfall, seeds of grasses and *Anthyllis* plants germinate, and older *Anthyllis* plants start producing new leaves and stems concomitant with root regrowth. These changes reflect the results obtained. For example, mycorrhizal infectivity of the grass site was greater at the end of the hot season than in the middle of winter. After summer, the small grasses have died, but their roots remain and maintain their infectivity. When winter progresses, infectivity decreases until the new growing season in spring. New roots then develop, and the mycorrhizal colonization occurs. In the *Anthyllis* root zone, there is no significant difference between the infectivities recorded in summer and in winter.

In conclusion, it is clear that a considerable level of mycorrhizal inoculum is present in this desertified ecosystem, and the type of mycorrhizal propagule present in the soil is dependent on the dominant plant species present in the area and on the season. However, because of the relative low effectiveness of the indigenous fungi in promoting plant growth of the target shrub, *Anthyllis cytisoides*, an inoculation strategy might be considered. AMF from stock culture collections, such as *G. intraradices*, could be used to improve the establishment and development of new plants in the area. The behavior of the introduced fungi under natural conditions, however, must be studied to predict the success of the reclamation strategy.

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