Mycorrhiza (1993) 4:85–88

Mycorrhiza



# Infectivity of mine soils from Southeast Spain

## II. Mycorrhizal population levels in spoilt sites

**G. Díaz, M. Honrubia**

Departamento Biología Vegetal (Botánica), Facultad Biología, Universidad de Murcia, Campus Espinardo, E-30071 Murcia, Spain

**Abstract.** A bioassay was carried out to measure the mycorrhizal population levels in five soils disturbed by mining activities. Mycorrhizal infection of *Medicago sativa* (as test plant) was always less than 56%, and in some cases there was no mycorrhization. Thus degradation of soil by mining brings about a decrease in mycorrhizal inoculum potential. No relationship was found between the number of spores and either infectivity or soil characteristics.

**Key words:** Endomycorrhizae – Infectivity – Mining – Mycorrhizal infection

### Introduction

Arbuscular mycorrhizae (AM) have a broad ecological range; they are found in most ecosystems, including rain forests, grasslands, heaths, tropics, dunes, semi-deserts.

The occurrence of AM in mine soils has been documented in studies by Ponder (1979), Zak and Parkinson (1982), Kiernan et al. (1983), Waaland and Allen (1987), and Louis (1990). Several studies have also pointed out the important role of mycorrhizae in the rehabilitation of such disturbed soils, because of the contribution of mycorrhizal symbiosis to plant establishment and survival (Daft and Nicolson 1974; Khan 1978, 1981). The abundance of AM may to some extent also influence the pattern of plant succession (Allen 1984; Gemma and Koske 1990; Molina 1993).

Since natural endophytes are commonly present in most ecosystems, including disturbed lands, knowledge of the population levels in soil is necessary for planning reclamation strategy; this involves the isolation, maintenance and selection of mycorrhizal fungi. Propagules of mycorrhizal fungi may be either spores, mycelium or infected root fragments, and these can be measured by several methods. Measurements of AM levels by counting the number of extracted spores may also include dead or inviable spores, and as infected root or mycelium are important sources of inocula, the correlation between spore number and infection is usually low. Estimation of all mycorrhizal propagules in soil makes use of the most probable number (MPN) method (Porter 1979; Powell 1980; Adelman and Morton 1986). The value obtained depends up on the experimental conditions (Wilson and Trinick 1982), e.g. temperature, time of harvest, P level in the soil, or the morphological characteristics of the root system (Huisman 1982). Although both the number of spores and MPN are quantitative estimates of propagule number, neither reflects the infectivity of the soil. The term "soil infectivity" (Bouhot 1980; Plenchette et al. 1989) means the ability of a natural soil with AM to initiate a mycorrhizal infection on a host plant, and can be measured by a bioassay that determines mycorrhizal colonization in a test plant.

The aim of this present study was to estimate the infectivity of several disturbed mine soils, in order to check the effect of soil degradation on mycorrhizal populations. The study was carried out in La Unión (Murcia), SE Spain. Mining activities have been conducted in this area for 3000 years and this exploitation intensified towards the end of the last century. Between 1959 and 1990, 8000 tonnes of washed waste was poured into the sea daily and later sedimented onto the coast; Portman Bay thus became completely filled with waste. Huge amounts of solid mine refuse were also deposited at adjacent sites. The physical disruption of natural soil strata, the microbiological degradation of soil and heavy metal contamination have led to large denuded and devastated areas. Díaz and Honrubia (1989) studied the infectivity of several undisturbed, reclaimed and damaged soils; we now report results obtained from other spoilt sites.

*Correspondence to:* M. Honrubia

## Materials and methods

Five soils were chosen from spoilt sites. The location and physical-chemical characteristics are shown in Tables 1 and 2.

Twenty randomly selected samples from each site were collected to a depth of 20 cm, mixed and passed through a 4-mm sieve to remove stones and coarse fragments. Three subsamples from each bulked soil were sieved and decanted (Gerdemann and Nicolson 1963) and processed for spore number under a stereomicroscope.

Soil infectivity was determined by a bioassay based on that of Moorman and Reeves (1979). Three dilutions of each soil were made by mixing the original soil with sand sterilized for 1 h at 100°C on three consecutive days in the proportions of 1/0, 1/4 and 1/40. The soil was transferred to pots (8 cm in diameter) with twelve replicates per soil dilution. *Medicago sativa* was selected as the test plant because of its ability to serve as host to many AM fungi and its good response to mycorrhizal inoculation. Seeds were surface sterilized by soaking in $HgCl_2$ for 5 min and then washing with sterile water. Two seedlings per pot were planted. The pots were inoculated with a pure culture of *Rhizobium leguminosarum* and with natural soil washings without AM propagules to provide the natural microflora. Nutrient solution (Hewitt 1952) lacking phosphorus was added every 2 weeks. For mycorrhizal assessment, plants were harvested at 15, 30 and 60 days. The whole root system was collected, washed and stained (Phillips and Hayman 1970) and examined for mycorrhizal colonization using the grid-line intersect method (Giovannetti and Mosse 1980).

## Results and discussion

In general, the number of spores was very low, with fewer than 50 spores/100 g at each site (Table 3). A similar study of other sites in the same area revealed higher spore densities in damaged soils, and 894 spores/100 g were counted in an adjacent soil covered with natural vegetation and unaltered by mining activities that was taken as the control (Díaz and Honrubia 1989).

These differences cannot easily be attributed to one particular cause; although several attempts have been made to correlate, in natural conditions, spore production with the physical-chemical characteristics of the

**Table 1.** Site description

| | Locality | Soil type | Soil condition | Vegetation |
|---|---|---|---|---|
| 1 | El Portazgo 30S XG8864 | Xeric Torrifluvent | Disturbed soil | Residual vegetation and pioneer plants |
| 2 | La Unión 30S XG8763 | Xeric Torrifluvent | Mine spoil | Pioneer plants |
| 3 | Portman Bay 30S XG8962 | Typic Salorthid | Waste sediment | Salt grassland community: *Phragmites australis*, *Lygeum spartum*, *Limonium* sp. |
| 4 | Portman Bay 30S XG9062 | Typic Salorthid | Waste sediment | Salt grassland community more advanced: *Phragmites australis*, *Lygeum spartum*, *Anthyllis cytisoides*, *Dorycnium pentaphyllum*, *Dittrichia viscosa* |
| 5 | Portman Bay 30S XG8962 | Xeric Torriorthent | Watercourse with occasional refuse dumped | Giant reed community: *Arundo donax*, *Avena* sp. *Piptatherum miliaceum* |

**Table 2.** Physical and chemical characteristics of the soils

| Parameter | Site | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| pH | 7.62 | 7.28 | 7.86 | 7.79 | 7.52 |
| Organic matter (%) | 0.53 | 1.31 | 0.67 | 0.53 | 0.53 |
| $Co_3Ca$ equivalent total (%) | 2.0 | 34.0 | 6.0 | 9.0 | 7.0 |
| Electrical conductivity (ds/M) | 2.86 | 4.56 | 7.50 | 6.54 | 2.25 |
| Available P (mg/kg) | 0.62 | 1.86 | 0.93 | 2.09 | 7.13 |
| Available K (mg/100 g) | 4.31 | 30.11 | 35.97 | 23.85 | 4.91 |
| Total N (mg/100 g) | 32 | 74 | 35 | 47 | 55 |
| Na (mg/100 g) | 6.9 | 349.6 | 448.5 | 324.3 | 11.0 |
| Cl (cmol/kg) | 0.33 | 0.15 | 21.75 | 17.50 | 0.48 |
| $SO_4$ (cmol/kg) | 7.56 | 6.40 | 9.65 | 7.15 | 6.98 |
| DTPA extractable Zn (mg/kg) | 62.7 | 8.8 | 130.3 | 236.7 | 178.2 |
| DTPA extractable Pb (mg/kg) | 311 | 31 | 300 | 368 | 456 |

**Table 3.** Spore densities in the soils studied. Means of three replicates and standard errors

| Site | Spores/100 g dry soil |
|---|---|
| 1 Disturbed | 45±6 |
| 2 Spoil | 25±6 |
| 3 Waste sediment | 12±5 |
| 4 Waste sediment | 17±4 |
| 5 Watercourse | 15±2 |

soil, most studies found no such correlation. Abbot and Robson (1977), Hayman (1978) and Medina et al. (1988) observed no correlation between spore production and pH or the level of phosphorus available in the soil, nor was there any relation to nutrient content (Ho 1987) or organic matter (Gemma et al. 1989). On the other hand, humidity does seem to be linked with spore production (Sward et al. 1978; Walker et al. 1982). Seikh et al. (1975) and Ho (1987) found that the number of spores was related to pH, and Abbott and Robson (1977) correlated pH with the distribution of certain species of mycorrhizal fungi. In dune ecosystems, a negative correlation was observed between sand grain size and spore production (Koske and Halvorson 1981).

In the case of the soils studied by our assay, no correlation was found between spore density and physical-chemical characteristics, although low production might be partly connected with the high degree of substrate salinity, the high concentrations of Na, Cl and gypsum, and high electrical conductivity, which are characteristics absent from soils with higher spore densities in the same area (Díaz and Honrubia 1989). It is also possible that the vegetation influences the number of spores. Spore density in the soils of Portman Bay were below those of the other two localities studied. These recently deposited soils were covered with sparsely scattered vegetation, so the probability of taking rhizospheric soil at sampling was lower, with low spore counts.

The infectivity of the soils is shown in Fig. 1. The percentages of mycorrhizal colonization were generally low, always below 56%, and in some cases there was no mycorrhization of the host plant at all (1/40 of sites 2 and 3).

There does not seem to be any correlation between the number of spores and infectivity; the most infective soil (site 4) showed a low spore density. The failure of the number of spores to indicate the capacity of a soil to provoke infections might be explained by the presence of non-spore-forming fungi and by the survival of these fungi in the form of another type of propagules, such as infected root fragments or mycelium. Indeed, site 4 had a somewhat more advanced vegetation, with a greater proportion of mycorrhizal plants such as legumes or grasses than adjacent soils, which have non-mycorrhizal and facultative mycorrhizal plants. Therefore this soil probably contained more viable or infective propagules in the form or mycorrhizal roots and hence a higher degree of infection in the test plant.

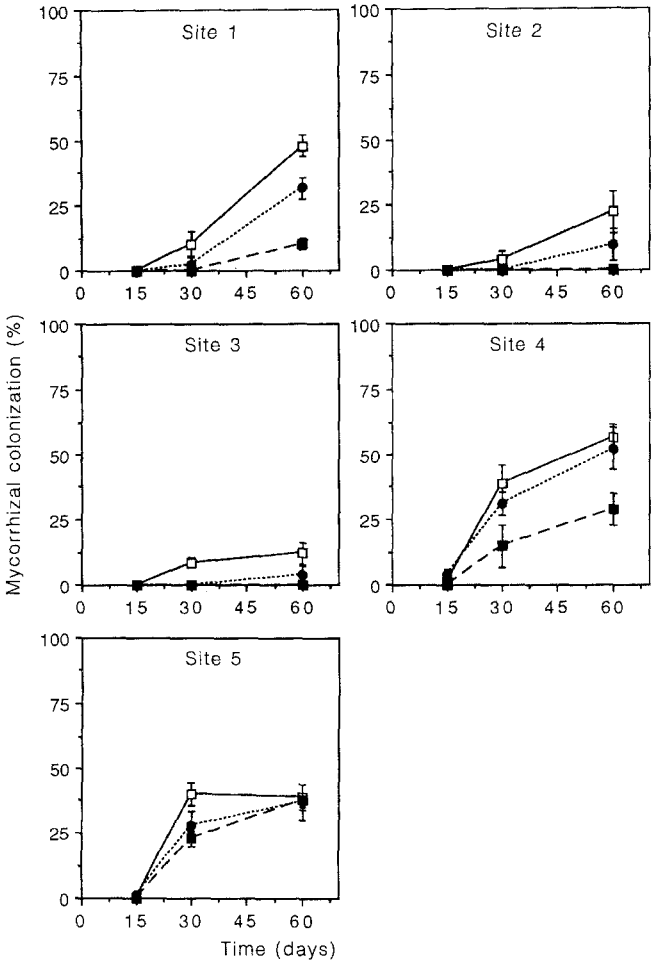


**Fig. 1.** Percentage infection of *Medicago sativa* grown for 15, 30 and 60 days in several soils at three dilutions: 1/0 (□), 1/4 (●), 1/40 (■). *Vertical bars* represent standard errors

It can be concluded that soil degradation by mining brings about a decrease in the mycorrhizal population, although differences in infectivity and number of spores may be observed between soils depending upon physical-chemical properties, degree of disturbance or vegetation. It is well known that soil degradation negatively affects microbiological populations, particularly mycorrhizal fungi (Williams and Allen 1984; Allen and Allen 1990). However, in spite of unfavourable conditions, the fungi do not totally disappear; this suggests an adaptation to stress that can facilitate the establishment of micotrophic plants capable of surviving in these disturbed soils.

Since reduction of the population of mycorrhizal fungi may be significant in the re-establishment of ecosystems on degraded soils, more research is needed to determine the impact of mine disturbance on mycorrhizal inoculum potential.

## References


Abbott LK, Robson AD (1977) The distribution and abundance of vesicular-arbuscular endophytes in some Western Australian Soils. Aust J Bot 25:515–522

Adelman MJ, Morton JB (1986) Infectivity of vesicular-arbuscular mycorrhizal fungi: influence of host-soil diluent combinations on MPN estimates and percentage colonization. Soil Biol Biochem 18:77–83

Allen MF (1984) VA mycorrhizae and colonizing annuals: implications for growth, competition, and succession. In: Williams SE, Allen MF (eds) VA mycorrhizae and reclamation of arid and semiarid lands. University of Wyoming Agricultural Experimental Station Science Report No SA 1261, Laramie, pp 41–51

Allen EB, Allen MF (1990) Carbon source of VA mycorrhizal fungi associated with *Chenopodiaceae* from semiarid shrubsteppe. Ecology 71:2019–2021

Bouhot D (1980) Le potentiel infectieux des sols: un concept, un modèle pour sa mesure, quelques applications. Thèse de doctoral, Université de Nancy I, Nancy, France

Daft MJ, Nicolson TH (1974) Arbuscular mycorrhizas in plants colonizing coal wastes in Scotland. New Phytol 73:1129–1138

Díaz G, Honrubia M (1989) Infectivity of mine soils from South-East-Spain. Agric Ecosyst Environ 29:85–89

Gemma JN, Koske RE (1990) Mycorrhizae in recent volcanic substrates in Hawaii. Am J Bot 77:1193–1200

Gemma JN, Koske RE, Carreiro M (1989) Seasonal dynamics of selected species ov VA mycorrhizal fungi in a sand dune. Mycol Res 92:317–321

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–499

Hayman DS (1978) Mycorrhizal populations of sown pastures and native vegetation in Otago, New Zealand. J Agric Res 21:271–276

Hewitt EJ (1952) Sand and water culture methods used in the study of plant nutrition. (Technical Communication 22, 2nd edn) Commonwealth Agriculture Bureau, London

Ho I (1987) Vesicular-arbuscular mycorrhizae of halophytic grasses in the alvord desert of Oregon. Northwest Sci 61:148–151

Huisman OC (1982) Interrelations of root growth dynamics to epidemiology of root-invading fungi. Annu Rev Phytopathol 20:303–327

Khan AG (1978) Vesicular-arbuscular mycorrhizas in plants colonizing black wastes from bituminous coal mining in the Illawarra region of New South Wales. New Phytol 81:53–63

Khan AG (1981) Growth response of endomycorrhizal onions in unsterilized coal wastes. New Phytol 87:363–370

Kiernan JM, Hendrix JW, Maronek DM (1983) Endomycorrhizal fungi occurring on orphan strip mines in Kentucky. Can J Bot 61:1798–1803

Koske RE, Halvorson WL (1981) Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. Can J Bot 59:1413–1422

Louis I (1990) A mycorrhizal survey of plant species colonizing coastal reclaimed land in Singapore. Mycologia 82:772–778

Medina OA, Sylvia DM, Kretschmer AE Jr (1988) Response of Siratro to vesicular-arbuscular mycorrhizal fungi. II. Efficacy of selected vesicular-arbuscular fungi at different phosphorus levels. Soil Sci Soc Am J 52:420–423

Molina R (1993) Specificity phenomena in mycorrhizal symbioses. Community-ecological consequences and practical implications. In: Allen M (ed) Mycorrhizal functioning. An integrative plant-fungal process. Chapman & Hall, London

Moorman T, Reeves FB (1979) The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. Am J Bot 66:14–18

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Plenchette C, Perrin R, Duvert P (1989) The concept of soil infectivity and a method for its determination as applied to endomycorrhizas. Can J Bot 67:112–115

Ponder F Jr (1979) Presence of endomycorrhizal fungi in recently graded coal mine spoil. J Soil Water Conserv 34:186–187

Porter WM (1979) The "most probable number" method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi. Aust J Soil Res 17:515–519

Powell CLL (1980) Mycorrhizal infectivity of eroded soils. Soil Biol Biochem 12:247–250

Seikh NA, Saif SR, Khan AG (1975) Ecology of *Endogone*. II. Relationships of *Endogone* spore population with chemical soil factors. Islamabad J Scie 2:6–9

Sward RJ, Hallam ND, Holland AA (1978) *Endogone* spores in a heathland area of South-eastern Australia. Aust J Bot 26:29–43

Waaland ME, Allen EB (1987) Relationships between VA mycorrhizal fungi plant cover following surface mining in Wyoming. J Range Manage 40:271–276

Walker C, Mize CW, McNabb HS Jr (1982) Populations of endogonaceous fungi at two locations in central Iowa. Can J Bot 60:2518–2529

Williams SE, Allen MF (1984) VA mycorrhizae and reclamation of arid and semiarid lands. Proceedings of a conference, 1982, Dubois, Wyoming. University of Wyoming Agricultural Experimental Station Science Report No SA 1261, Laramie

Wilson JM, Trinick MJ (1982) Factors affecting the estimation of numbers of infective propagules of vesicular-arbuscular mycorrhizal fungi by the most probable number method. Aust J Soil Res 21:73–81

Zak JC, Parkinson D (1982) Initial vesicular-arbuscular mycorrhizal development of slender wheatgrass on two amended mine spoils. Can J Bot 60:2241–2248