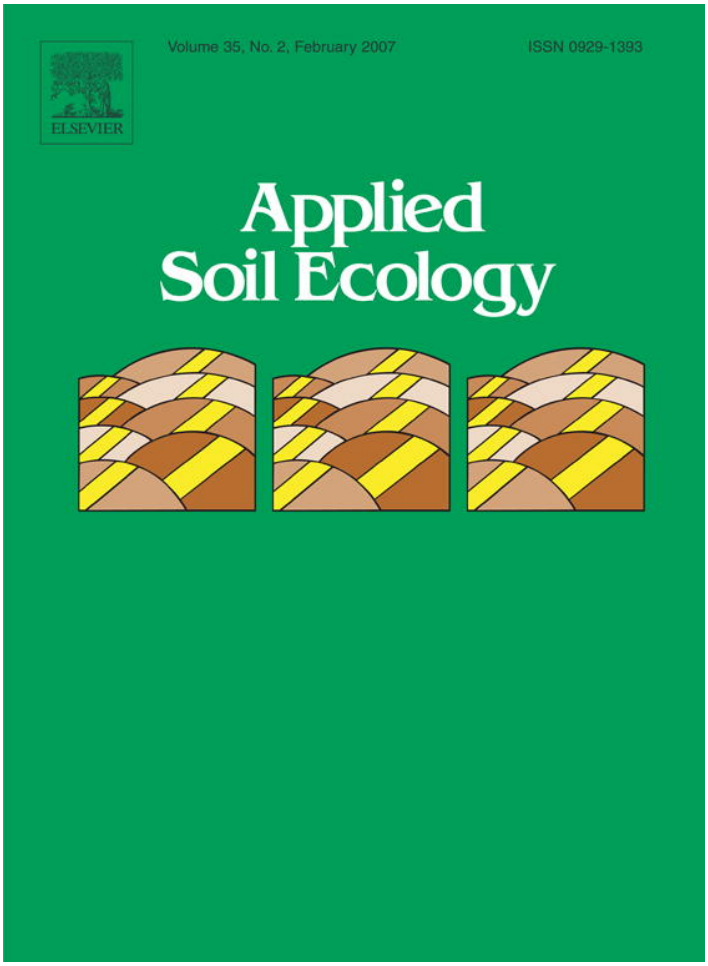

Available online at www.sciencedirect.com
ScienceDirect
Applied Soil Ecology 35 (2007) 363–369
Applied Soil Ecology
www.elsevier.com/locate/apsoil

The development of arbuscular mycorrhiza in two simulated stages of spoil-bank succession

David Püschel *, Jana Rydlová, Miroslav Vosátka

Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Průhonice, Czech Republic

Received 13 March 2006; received in revised form 17 July 2006; accepted 18 July 2006

Abstract

A greenhouse experiment based on a dual mode of mycorrhizal inoculation simulated the formation of mycorrhizal symbiosis at two different stages of plant succession on coalmine spoil banks. The model plants were inoculated either with propagules of the arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* BEG95, which represented the initial stages of succession, or were provided with the pre-established extraradical mycelium (ERM) network of the same AMF isolate, which simulated later succession stages. The plant species used – non-mycotrophic *Atriplex sagittata* and *Sisymbrium loeselii*, and mycotrophic *Tripleurospermum inodorum*, *Calamagrostis epigejos* and *Elytrigia repens* – represented succession dominants at those sites. Even though the grasses were colonised in both mycorrhizal treatments, the presence of an established ERM network increased the intensity of their colonisation and arbuscular abundance. No trace of colonisation of non-mycotrophic plants was found in the treatment inoculated with propagules. Surprisingly, marked colonisation, including abundant arbuscules, was observed when non-mycotrophic plants were grown in the presence of a pre-established ERM network. In *A. sagittata*, arbuscules were found at maturity and senescence of the plants after 16 weeks of growth. In *S. loeselii*, however, the arbuscules were found at the vegetative stage of the leaf rosette after 8 weeks and then completely disappeared during the following weeks. When the ability of propagules and ERM to induce mycorrhizal colonisation is compared, it seems that the established mycelium probably has an enhanced potential to colonise roots of plants, even if the plants belong to species usually not hosting mycorrhizal fungi.

Keywords: Arbuscular mycorrhizal fungi; ERM network; Propagules; Primary succession; Non-mycotrophic plants; Spoil banks

1. Introduction

Opencast coal mining in the Most coal basin (NW Bohemia, the Czech Republic) is accompanied by the establishment of spoil banks covering thousands of hectares of landscape. The spoil banks are composed mainly of grey Miocene clays mined from a depth of about 200 m. This substrate is characterised by adverse physical properties (e.g. vulnerability to erosion and low drainage ability) and is, therefore, usually covered by a layer of another soil (e.g. loess) that facilitates spoil bank reclamation.

In the Most coal basin, pioneer plants colonise spoil banks as early as the first year after deposition, but the process of establishing a complete herbaceous vegetation cover can take up to 15 years (Prach, 1987). The first invaders of spoil banks are species that do not usually form mycorrhiza; later in succession plants associated with AMF are found at these sites (Janos, 1980; Reeves, 1985). The typical representatives of non-mycotrophic plants are members of families such

* Corresponding author. Tel.: +420 271 015 333; fax: +420 271 015 332.
E-mail address: puschel@ibot.cas.cz (D. Püschel).

0929-1393/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2006.07.001

as Chenopodiaceae or Brassicaceae. Plant communities with dominance of these species can occur even between the 7th and 12th year after spoil-bank establishment. After approximately 12 years, perennial plants replace communities of annuals and biennials (Prach, 1987).

The role of mycorrhiza in plant succession remains rather unclear and not much detailed experimental data have been reported. The spores or resting propagules are the main sources of AM associations in freshly man-made sites without vegetation. Although spores can maintain their viability in soils for long periods of adverse conditions (Rives et al., 1980), they have very low mobility (e.g. their transport by air is very limited). The mycorrhizal inoculation potential (the ability of mycorrhizal propagules in the soil to initiate mycorrhizal associations) of soils without an initial spore population can increase only very slowly (Allen and Allen, 1980). Thus, the predominance of non-mycotrophic plants in the early stages of succession has been attributed to the absence of infective mycorrhizal propagules (Reeves et al., 1979; Janos, 1980).

Nevertheless, species from plant families usually indicated as non-mycotrophic (Chenopodiaceae, Brassicaceae, Amaranthaceae, etc.) also can become colonised by AMF. Mycorrhizal colonisation of non-mycotrophic plants on spoil banks was found, e.g. by Rydlová and Vosátka (2001). The occurrence of mycorrhizal colonisation in the roots of non-mycotrophic plants has been explained as the mere presence of AMF in the roots of ageing plants that has, nevertheless, no benefit for those plants (Hirrel et al., 1978; Janos, 1980). On the other hand, an efficient AM symbiosis in non-mycotrophic plants may occur in the highly competitive environment of grasslands (Lovera and Cuenca, 1996). Miller (1979) suggested a close relationship between the occurrence of AMF colonisation and plant life strategy: while perennial woody Chenopodiaceae with a stress-tolerant strategy formed arbuscular mycorrhiza, annual ruderals from the same family were non-mycorrhizal. However, DeMars and Boerner (1996) found no relationship between the formation of arbuscular mycorrhiza and the length of the life cycle after inoculation of 646 representatives of Brassicaceae with AMF in a pot experiment.

Mycorrhizal colonisation of non-mycotrophic plants is usually found in the roots of plants growing in the vicinity of colonised neighbours (Miller et al., 1983; Stejskalová, 1989; Lovera and Cuenca, 1996). This phenomenon was described as a ‘‘nurse-plant effect’’ (Ocampo et al., 1980). The network of extraradical mycelium (ERM) spreading through the soil from the colonised roots creates an effective matrix that can initiate colonisation of the roots of surrounding plants or emerging seedlings probably more quickly and efficiently than spores (Malcová et al., 2001; Sýkorová et al., 2003; Enkhtuya et al., 2005).

This study presents the results of a greenhouse experiment based on two different modes of inoculation of model plants. Each mode simulated a different stage of plant succession with specific conditions in the soil: (1) early stage when only first propagules of AMF were present and (2) later stage when the whole ERM network was already developed and thus more effective colonisation of seedlings’ roots was enabled. By planting the seedlings of five model plant species (typical non-mycotrophic and mycotrophic representatives of spoil bank succession) into those two different conditions, we aimed to find the possible difference in the establishment and temporal dynamics of the development of mycorrhizal symbiosis with particular plant species.

2. Materials and methods

The establishment of mycorrhizal symbiosis within two simulated stages of spoil-bank succession was compared in a pot experiment conducted under greenhouse conditions. The substrate was loess (chemical characteristics presented in Table 1) collected from the freshly formed spoil bank of the Vršany coal mine (North-Bohemian coal basin, near the town of Most, the Czech Republic). The loess was originally located in the upper layer of the overburden of coal seams. Because this material was suitable for later reclamation of spoil banks composed of grey Miocene clays, loess was removed before coal mining and put aside for later utilization. For the purposes of the experiment it was mixed with perlite 1:1 (v/v) and sterilised by γ-irradiation (25 kGy).

Table 1
Chemical characteristics of the soil

pH (H_2O)	7.99
pH (KCl)	7.51
Conductivity (μS)	493
N (%)	0.25
C_{total} (%)	0.38
$C_{org.}$ (%)	0.38
C/N	1.54
[a]P (mg kg^{-1})	24.8
[b]Mg (mg kg^{-1})	1156
[b]Ca (mg kg^{-1})	2324

[a] 0.5 M sodium bicarbonate-extractable (Olsen-P).
[b] 1 M ammonium acetate-extractable.

The experiment consisted of two stages. In the first stage, plastic pots (volume 500 ml) were filled with the substrate and one pre-germinated maize seed was sown in each pot. One third of the pots were inoculated with 5 ml of inoculum suspension of *Glomus mosseae* BEG95—a native AMF species originally isolated from a spoil bank in the same region. The suspension contained colonised roots, spores and fragments of the ERM. The aim of this step was to develop the ERM network in the soil using maize as a ''nurse plant''. After 4 months, the shoots of maize were cut, while the root systems were left intact. At that time, the second third of the pots were inoculated with the same volume of the same inoculum as before, whereas heat-sterilised inoculum and 5 ml of inoculum-filtrate were added to the remaining pots to supply similar doses of organic matter and bacterial conditions in all treatments. Furthermore, 5 ml of filtrate from original soil containing indigenous soil bacteria were added to pots of all treatments.

This procedure formed three treatments with different mycorrhizal conditions: (1) ''ERM''—with the intact network of mycelium present in the soil; (2) ''propagules''—containing spores, mycelium fragments and root fragments colonised with AMF; (3) ''NM''—with no mycorrhizal propagules available (the control treatment).

In the second stage of the experiment, the seedlings of six model plant species (pre-cultivated on heat-sterilised sand for 10 days) were transplanted into the pots, one plant per pot. *A. sagittata* (Chenopodiaceae) and *Sisymbrium loeselii* (Brassicaceae) represented non-mycotrophic and *Tripleurospermum inodorum* a mycotrophic plant species of the early stages of succession on the spoil banks, while *Calamagrostis epigejos* and *Elytrigia repens* represented mycotrophic grasses that invade the spoil banks at the later successional stages. One half of the pots were harvested after 8 weeks (harvest 1), the remaining pots were harvested after 16 weeks (harvest 2). There were seven replicates for each treatment.

At both harvests, shoot biomass was cut, the roots were washed from the soil and shoot and root dry weights were determined after drying at 70 °C to constant weight. Root samples were stained with 0.05% Trypan blue in lactoglycerol (Koske and Gemma, 1989) and mycorrhizal colonisation was evaluated according to Trouvelot et al. (1986). At the second harvest, the total and the active lengths of the ERM were also evaluated. The total ERM length was assessed using a modified membrane filtration method (Jakobsen et al., 1992). The extracted ERM was stained with 0.1% Trypan blue in lactoglycerol. The total length of the ERM was assessed under a compound microscope at 100× magnification and expressed in meters of hyphae per gram of dry soil. The ERM extraction was also performed in the non-inoculated control treatment and this value was subtracted from the values of both inoculated treatments. The viability of ERM was estimated using staining for NADH-diaphorase activity (Sylvia, 1988; Hamel et al., 1990) in mycelium samples extracted by wet sieving. The proportion of ERM containing red precipitate (NADH-diaphorase activity) was evaluated using an ocular grid at 400× magnification.

Statistical analysis was carried out using ANOVA (STATISTICA 5.1′98 Edition). Data were checked for normality and significant differences were tested by Tukey honest significant difference (HSD) test.

3. Results

3.1. Development of arbuscular mycorrhizal symbiosis

Inoculation with a pre-established ERM network resulted in colonisation of both non-mycotrophic species. In the treatment inoculated with propagules, no colonisation of these plants was observed (Table 2). However, the time of mycorrhizal occurrence differed in the non-mycotrophic plants. While *S. loeselii* was colonised and arbuscules were present at the first harvest after 8 weeks, colonisation of roots of *A. sagittata* was not observed until the second harvest after 16 weeks. Meanwhile, the intensity and characteristics of colonisation of the *S. loeselii* roots changed; although a few AMF hyphae were still found at the second harvest, arbuscules were no longer present. When arbuscules were present in the roots of non-mycotrophic plants, their abundance in the whole root system was lower than in mycotrophic plants (Table 2). In colonised root fragments, however, the abundance of arbuscules almost reached the values observed for mycotrophic species (*A. sagittata* 29%, *S. loeselii* 59% versus *T. inodorum* 86%, *E. repens* 71% and *C. epigejos* 59%).

The colonisation of both grasses generally was induced more effectively by the presence of ERM compared with propagules (Table 2). With the ERM inoculation, we found higher intensity of colonisation and higher abundance of arbuscules in the roots of both grasses; the frequency of colonisation was higher for *E. repens* only. In *E. repens*, the positive effect on colonisation gained from the ERM inoculation was observed throughout the experiment, while in *C.*

Table 2
Effect of inoculation mode (propagules or pre-established ERM network) on root colonisation of model plant species

Plant	Inoculation	*F* (%)		*M* (%)		*A* (%)	
		8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks
A. sagittata	Propagules	0 ns	0 b	0 ns	0 b	0 ns	0 b
	ERM	0 ns	41 a	0 ns	25 a	0 ns	7 a
S. loeselii	Propagules	0 ns	0 ns	0 ns	0 ns	0 ns	0 ns
	ERM	14 ns	3 ns	8 ns	1 ns	6 ns	0 ns
T. inodorum	Propagules	98 ns	97 ns	68 ns	57 ns	55 ns	25 ns
	ERM	94 ns	96 ns	60 ns	54 ns	52 ns	24 ns
E. repens	Propagules	79 b	78 b	25 b	28 b	13 b	2 b
	ERM	94 a	95 a	57 a	50 a	40 a	6 a
C. epigejos	Propagules	79 ns	74 ns	21 b	31 ns	11 b	1 ns
	ERM	86 ns	74 ns	40 a	34 ns	25 a	1 ns
Plant (A)		***		***		***	
Harvest (B)		ns		ns		***	
Inoculation (C)		***		***		***	
A × B		***		**		***	
A × C		***		***		**	
B × C		ns		ns		*	
A × B × C		***		**		**	

F, frequency of mycorrhiza in the root system; M, intensity of the mycorrhizal colonisation of the root system; A, arbuscular abundance in the root system. The values in columns marked by different letters are significantly different within particular plant species and harvest times at the level $P < 0.05$ according to Tukey honest significant difference (HSD) test. Effects of factors according to ANOVA: ns, non-significant effect; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are means of seven replicates.

epigejos it was lessened during the second half of the experiment. *T. inodorum* similarly responded to both modes of inoculation and exhibited similar values of frequency and intensity of colonisation and arbuscular abundance for both inoculation modes and for both harvests.

The ERM in the treatment with mycotrophic plant species tended to develop better (both total and active length) as compared to non-mycotrophic plants although the differences were not always significant (Table 3). For most plant species, both total and active ERM lengths were significantly higher in the treatment inoculated with ERM in comparison with the inoculation with propagules.

3.2. Plant growth

Inoculation with AMF had either no effect or a negative effect on the biomass production of experimental plants (Table 4). After the first 8 weeks, plant growth either decreased in the presence of ERM (*A. sagittata*) or in both mycorrhizal treatments (*S. loeselii*, *T. inodorum*), or was not negatively affected by AMF (both grasses). The response of these plants changed during the second half of the experiment. After 16 weeks, the biomass of both non-mycotrophic plants and *T. inodorum* was similar for all treatments, while the grasses prospered in the absence of AMF.

The production of root biomass was either not affected or negatively affected by mycorrhizal inoculation (Table 4).

Table 3
The total ERM length and active ERM length at final harvest time

	Total ERM length (cm g^{-1} soil)		Active ERM length (cm g^{-1} soil)	
	Propagules	ERM	Propagules	ERM
A. sagittata	23 b	53 c	0 c	7 c
S. loeselii	5 b	89 bc	0 bc	35 bc
T. inodorum	204 a	212 ab	194 a	148 ab
E. repens	153 ab	279 a	111 ab	200 a
C. epigejos	189 a	206 ab	71 bc	176 a
Plant (A)	***		***	
Inoculation (B)	*		*	
A × B	ns		*	

The values in columns marked by different letters are significantly different within a particular inoculation mode (propagules or pre-established ERM network) at the level $P < 0.05$ according to Tukey honest significant difference (HSD) test. Effects of factors according to ANOVA: ns, non-significant effect, $^{*}P < 0.05$, $^{***}P < 0.001$. Data are means of seven replicates.

Table 4
Effect of the inoculation mode (propagules or pre-established ERM network) on shoot and root dry weight of plant species

	Inoculation mode	Shoot dry weight (g)		Root dry weight (g)	
		8 weeks	16 weeks	8 weeks	16 weeks
A. sagittata	Non-inoculated	1.76 a	1.02	0.21 a	0.37 a
	Propagules	1.69 a	1.35 ns	0.18 ab	0.18 b
	ERM	1.13 b	1.10	0.13 b	0.27 ab
S. loeselii	Non-inoculated	0.61 a	0.69	0.23	0.33
	Propagules	0.23 b	0.41 ns	0.07 ns	0.15 ns
	ERM	0.43 ab	0.65	0.16	0.19
T. inodorum	Non-inoculated	0.77 a	1.54	0.47 a	1.39
	Propagules	0.48 b	1.43 ns	0.23 b	1.18 ns
	ERM	0.60 ab	1.17	0.29 ab	0.94
E. repens	Non-inoculated	0.77	1.12 a	1.03 a	2.31 a
	Propagules	0.61 ns	0.70 b	0.54 b	1.05 b
	ERM	0.56	0.79 b	0.41 b	1.31 b
C. epigejos	Non-inoculated	0.47	1.01 a	0.48	1.36
	Propagules	0.55 ns	0.88 ab	0.40 ns	0.99 ns
	ERM	0.44	0.70 b	0.33	0.77

The values in columns marked by different letters are significantly different within one harvest for a particular plant species at the level $P < 0.05$ according to Tukey honest significant difference (HSD) test. ns, non-significant difference. Data are means of seven replicates.

4. Discussion

Although the roots of mycotrophic plant species in our experiment easily became colonised both in treatments inoculated with propagules and with the developed ERM, inoculation with ERM resulted in significantly higher parameters of mycorrhizal colonisation of both grasses. Furthermore, the presence of an intact ERM network caused the formation of AMF structures in the roots of both non-mycotrophic plant species, while inoculation with propagules did not. Although the frequency of mycorrhiza in these plants was significantly lower than in the mycotrophic plant species, the intensity of colonisation and the arbuscular abundance (when considered in the colonised parts of roots) almost reached the values observed for mycotrophic plants.

Examining the roots of several representatives of the genus *Thlaspi* from diverse locations, Regvar et al. (2003) occasionally observed mycorrhizal colonisation including arbuscules in three species of *Thlaspi* growing on meadows. Also Orłowska et al. (2002) reported the presence of arbuscules during the flowering period in *Biscutella laevigata* colonising heavy-metal-contaminated and non-contaminated sites. Authors of both the latter studies, however, were not able to establish colonisation of the studied plant species in greenhouse experiments. Studying weed–crop interactions, Stejskalová (1989) found arbuscules in *Chenopodim album* cultivated with a mycotrophic crop plant (wheat, maize, lettuce) both in pot and field experiments. The magnitude of the ''nurse-plant effect'' was dependent mainly on the level of mycorrhizal colonisation of the crop. Also Allen et al. (1989) observed the formation of arbuscules in the roots of a non-mycotrophic annual *Salsola kali* in a pot experiment after inoculation with a mixture of several AMF. However, after 1–2 days localised lesions formed around penetration points and repeated penetration sometimes resulted in the death of individual roots and consequently the whole *S. kali* seedlings. In contrast, no lesions in the roots of non-mycotrophic plants were observed in our experiment. Francis and Read (1994) found severely reduced growth of non-mycotrophic plant species (*Arabis hirsuta* and *Arenaria serpyllifolia*) in the presence of the ERM network even without any evidence of root penetration by hyphae.

A question remains about the significance and function of the observed mycorrhizal structures, particularly arbuscules, in the roots of non-mycotrophic plant species. Hirrel et al. (1978) and Janos (1980) concluded that the occurrence of AMF in aged roots does not provide any benefits to these plants. By contrast, AM symbiosis in perennial non-mycotrophic Juncaceae and Cyperaceae was considered to be fully efficient in a highly competitive grassland environment (Lovera and Cuenca, 1996). Assessing stable carbon isotope ratios of AMF spores from the root space of

Salsola kali and *Atriplex gardneri*, Allen and Allen (1990) found that spores gained the majority of carbon from the plant under which they were isolated. Similar isotope ratios were found for both species, indicating a larger than expected contribution by *S. kali*. Because spores were produced, the fungus probably obtained adequate carbon from each host plant to complete its life cycle. The authors suggest a symbiotic interaction between the supposed non-mycotrophic plants and AMF.

Although plants in our experiment were colonised successfully by the AMF, no positive growth response to inoculation either with propagules or with the pre-established ERM network was observed in any plant species. This lack of positive growth response could be ascribed to three reasons.

Firstly, the content of nutrients, especially phosphorus (nearly 25 mg/1 kg of soil), in the cultivation substrate was probably sufficient to cover plant demands, thus mycorrhizal symbiosis was not beneficial for plants (Bethlenfalvay et al., 1983).

Secondly, the carbon cost for the establishment of mycorrhizal colonisation from propagules was probably too high and exceeded any potential benefits resulting from the mycorrhizal symbiosis. This has been estimated to be between 4 and 20% of the total net carbon fixed by the host plant (Smith and Read, 1997). The lack of positive growth response after inoculation with the pre-established ERM network, however, did not confirm the observations of other authors that the cost for development and maintenance of mycorrhizal symbiosis should be significantly decreased when plants are inoculated with ERM. Malcová et al. (2001) found a positive growth response of *C. epigejos* seedlings to the inoculation with the ERM outgrowing from a mature plant of the same species in the substrate from a spoil bank. In the study of Francis and Read (1995), *Plantago lanceolata* showed a highly significant increase in yield and grew vigorously in the presence of AMF mycelial network but was weak and showed high mortality when the inoculum was absent. McGee (1985) described the importance of a mycelial network for the survival and the establishment of mycorrhizal colonisation in *Centaurium erythraea*. Seedlings of *C. erythraea* even died in the absence of the AMF inoculum in soil with low nutrient levels.

Finally, the lack of positive growth response to the inoculation with ERM in our experiment may be due to the removal of maize as a living nurse plant before planting the seedlings of model plants. This resulted in the newly planted seedlings bearing the cost of maintaining the previously developed ERM network and consequently in the reduction of their growth in comparison with non-inoculated controls.

5. Conclusion

In summary, our results revealed that a developed ERM network in the later stage of succession compared with propagules at early successional stages has an enhanced potential to colonise roots of plants and, moreover, to initiate arbuscule formation in the roots of non-mycotrophic plants. Nevertheless, the general growth response of plants to mycorrhiza is probably determined by other factors as well, mainly by the nutrient content of the soil, other plants connected to the ERM network and sharing the costs of network maintenance.

Acknowledgements

Financial support of the Grant Agency of the Czech Republic (grant 526/04/0996 and 206/03/H137) and Grant Agency of the Academy of Sciences of the Czech Republic (grant AV0Z60050516) is gratefully acknowledged.

References

Allen, E.B., Allen, M.F., 1980. Natural re-establishment of vesicular–arbuscular mycorrhizae following stripmine reclamation in Wyoming. J. Appl. Ecol. 17, 139–147.

Allen, E.B., Allen, M.F., 1990. The mediation of competition by mycorrhizae in successional and patchy environments. In: Grace, J.B., Tilman, G.D. (Eds.), Perspectives on Plant Competition. Harcourt Brace Jovanovich. Academic Press, New York, pp. 367–389.

Allen, M.F., Allen, E.B., Friese, C.F., 1989. Response of the non-mycotrophic plant *Salsola kali* to invasion by vesicular–arbuscular mycorrhizal fungi. New Phytol. 111, 45–49.

Bethlenfalvay, G.J., Bayne, H.G., Pacovsky, R.S., 1983. Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: The effects of phosphorus on host plant–endophyte interactions. Physiol. Plant 57, 543–548.

DeMars, B., Boerner, R.J., 1996. Vesicular arbuscular mycorrhizal development in the Brassicaceae in relation to plant life span. Flora 191, 179–189.

Enkhtuya, B., Pöschl, M., Vosátka, M., 2005. Native grass species facilitates mycorrhizal colonisation and P uptake of tree seedlings in two anthropogenic substrates. Water Air Soil Poll. 166, 217–236.

Francis, R., Read, D.J., 1994. The contribution of mycorrhizal fungi to the determination of plant community structure. Plant Soil 159, 11–25.

Francis, R., Read, D.J., 1995. Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. Can. J. Bot. 73, S1301–S1309.

Hamel, C., Fylez, H., Smith, D.L., 1990. Measurement of development of endomycorrhizal mycelium using three different vital stains. New Phytol. 115, 297–302.

Hirrel, M.C., Mehravaran, H., Gerdemann, J.W., 1978. Vesicular–arbuscular mycorrhiza in Chenopodiaceae and Cruciferae: do they occur? Can. J. Bot. 56, 2813–2817.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L 1. Spread of hyphae and phosphorus inflow into roots. New Phytol. 120, 371–380.

Janos, D.P., 1980. Mycorrhizae influence tropical succession. Biotropica 12, 56–64.

Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92, 486–505.

Lovera, M., Cuenca, G., 1996. Arbuscular mycorrhizal infection in Cyperaceae and Graminae from natural, disturbed and restored savannas in La Grand Sabana, Venezuela. Mycorrhiza 6, 111–118.

Malcová, R., Albrechtová, J., Vosátka, M., 2001. The role of the extraradical mycelium network of arbuscular mycorrhizal fungi on the establishment and growth of *Calamagrostis epigejos* in industrial waste substrates. Appl. Soil Ecol. 18, 129–142.

McGee, P.A., 1985. Lack of spread of endomycorrhizas of *Centaurium* (Gentianaceae). New Phytol. 90, 37–43.

Miller, R.M., 1979. Some occurrences of VA mycorrhizae in natural and disturbed ecosystems of the Red Desert. Can. J. Bot. 57, 619–623.

Miller, R.M., Moorman, T.B., Schmidt, S.K., 1983. Interspecific plant association effects on vesicular–arbuscular mycorrhiza occurrence in *Atriplex confertifolia*. New Phytol. 95, 241–246.

Ocampo, J.A., Martin, J., Hayman, D.S., 1980. Influence of plant interactions on vesicular–arbuscular mycorrhizal infections I. Host and non-host plants grown together. New Phytol. 84, 27–35.

Orłowska, E., Zubek, Sz., Jurkiewicz, A., Szarek-Łukaszewska, G., Turnau, K., 2002. Influence of restoration on arbuscular mycorrhiza of *Biscutella leavigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds. Mycorrhiza 12, 153–160.

Prach, K., 1987. Succession of vegetation on dumps from strip coal mining. Folia Geobot. Phytotax. 22, 339–354.

Reeves, F.B., 1985. Survival of VA mycorrhizal fungi—interactions of secondary succession, mycorrhizal dependency in plants, and resource competition. In: Molina, R. (Ed.), Proceedings of the Sixth North American Conference on Mycorrhizae, Bend. pp. 110–113.

Reeves, F.B., Wagner, D., Moorman, T., Kiel, J., 1979. The role of endomycorrhizae in revegetation practices in the semi-arid West I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. Am. J. Bot. 66, 6–13.

Regvar, M., Vogel, K., Irgel, N., Wraber, T., Hildebrandt, U., Wilde, P., Bothe, H., 2003. Colonization of pennycresses (*Thlaspi* spp) of the Brassicaceae by arbuscular mycorrhizal fungi. J. Plant Physiol. 160, 615–626.

Rives, C.S., Bajwa, M.I., Liberta, A.E., Miller, R.M., 1980. Effects of topsoil storage during surface mining on the viability of VA mycorrhiza. Soil Sci. 129, 253–257.

Rydlová, J., Vosátka, M., 2001. Associations of dominant plant species with arbuscular mycorrhizal fungi during vegetation development on coal mine spoil banks. Folia Geobot. 36, 85–97.

Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis. Academic Press, London, 605 pp.

Stejskalová, H., 1989. The role of mycorrhizal infection in the weed–crop interactions. Agric. Ecosys. Environ. 29, 415–419.

Sýkorová, Z., Rydlová, J., Vosátka, M., 2003. Establishment of mycorrhizal symbiosis in *Gentiana verna*. Folia Geobot. 38, 177–189.

Sylvia, D.M., 1988. Activity of external hyphae of vesicular–arbuscular mycorrhizal fungi. Soil Biol. Biochem. 20, 39–43.

Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V., 1986. Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson, V., Gianinazzi, S. (Eds.), Physiological and Genetical Aspects of Mycorrhizae. INRA, Paris, pp. 217–221.