[1]Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic
[2]Institute for Environmental Studies, Faculty of Science of the Charles University, Czech Republic

Different effect of mycorrhizal inoculation in direct and indirect reclamation of spoil banks

J. Rydlová[1], D. Püschel[1], M. Vosátka[1], K. Charvátová[2]

Summary

Spoil banks generated during coal mining are usually reclaimed by layering of fertile soil over original barren clay (co called indirect reclamation). This well-proven method is effective from the aspect of vegetation establishment and production, but it is very expensive. Direct reclamation of spoil bank clay promises much cheaper approach, yet its success is uncertain and the process might be rather long-term.

This two-year field study aimed to assess the effect of application of commercially produced inoculum of arbuscular mycorrhizal fungi (AMF) Symbivit® on growth of two plant species commonly used for reclamation (*Lotus corniculatus* and *Arrhenatherum elatius*) sown on three different substrates: organic substrate (mixture of papermill waste, tree-bark and compost) and loess (both substrates typical for indirect reclamation) and original spoil bank clays (simulation of direct reclamation). On organic substrate and loess, *A. elatius* out-competed the legume and established 100 % cover in all treatments. The effect of mycorrhizal inoculation was not observed. In contrast, on clay both species established successfully. The produced biomass and cover were, however, substantially lower compared to organic substrate and loess. In clay the positive effect of introduced AMF on plant was observed.

Mycorrhizal inoculation was useful for supporting plant growth at direct reclamation. Direct reclamation in itself seems suitable for small-scale application, i.e. in patches where indirect reclamation is inconvenient or more diverse vegetation is required.

Key words: arbuscular mycorrhizal fungi; inoculum; clay; papermill waste; loess; *Arrhenatherum elatius*; *Lotus corniculatus*

Introduction

Large areas of spoil banks are created as a consequence of the extensive open-cast coal mining in the northern part of the Czech Republic. Spoil banks consist mostly of grey Miocene clays, which had to be put aside in order to access coal. While the majority of spoil banks created in past decades have already been successfully reclaimed, new spoil banks are still created as the mining continues. Natural spontaneous revegetation is not only surprisingly swift, but the established ecosystems are more "close to the nature" (in terms of species composition and general look of the landscape) than the sites reclaimed by human (PRACH, 2003; HODACOVÁ and PRACH, 2003). Although the reclamation procedures could, therefore, seem unnecessary, two reasons justify it. Firstly, a legislative obligation requires the mining companies to reclaim the areas disturbed by their mining activity within certain time. Secondly, the mining-affected sites differ in their characteristics, e.g. soil properties. While some substrates enable fast establishment of plant cover, other substrates are less suitable for spontaneous revegetation. Some of spoil bank Miocene clays have low pH, fertility and drainage ability and high vulnerability to erosion. Therefore, spontaneous plant succession is limited on these sites. For both reasons, the reclamation of spoil banks will probably remain an important restoration procedure in mining regions.

Importantly, reclamation of spoil banks is quite costly process. The current reclamation practice usually avoids direct reclamation (i.e. reclamation of original spoil bank clays). Before the standard planting of trees or agricultural management is performed, the original substrates are usually covered by a layer of organic matter (a mixture of lignocellulose papermill waste, tree-bark and compost). This material is not only suitable for plants in terms of nutrients availability, but it provides also anti-erosion stabilisation of slopes due to its physical structure. Alternatively, loess is applied as a topsoil horizon on original clays. Loess is soil of relatively high quality, which was stockpiled when the coal seams were exposed for mining and which could be later re-applied to the sites when the spoil banks are re-claimed. Despite the majority of spoil bank surface is treated as demonstrated, marginal sites and non-reclaimed patches of original clays are exposed to the process of natural plant succession.

Spontaneous plant succession on various mining-affected sites, including coal mine spoil banks, has been monitored for decades (PRACH, 1987; SKOUSEN et al., 1994; JOCHIMSEN, 1996; WIEGLEB and FELINKS, 2001). The first plant colonizers can be found already one year after spoil bank formation. After approximately 12 years, the annuals or biennials (belonging mostly to the families Chenopodiaceae, Brassicaceae, Polygonaceae and Asteraceae) are replaced by perennial communities with dominant forbs. Later, communities with dominant grasses are established (PRACH, 1987). Such progress of plant succession on spoil banks could be significantly affected by AMF – arbuscular mycorrhizal fungi (ALLEN and ALLEN, 1980; RYDLOVÁ and VOSÁTKA, 2001), which can provide important benefits for survivorship and growth of plants, mostly due to the increased uptake of nutrients (SMITH and READ, 1997). Furthermore, specific composition of AMF can affect the structure of plant communities on spoil banks (PÜSCHEL et al., 2007).

Initially, the population of AMF on fresh spoil banks or other disturbed ecosystems is very low (ALLEN and ALLEN, 1980; WAALAND and ALLEN, 1987; MOTT and ZUBERER, 1987). The speed of subsequent development of AMF is probably determined by multiple factors, e.g. by the distance of the sources of AMF propagules and possible means of their dispersion (wind, animals etc.), by the climate and by the presence of suitable host plants. The development of AMF and progress of plant succession are likely contingent on each other. It can be generalised that plant species of later stages of succession can profit from mycorrhizal symbiosis in contrast with certain species of the early stages (mostly of the non-mycorrhizal families Chenopodiaceae and Brassicaceae). In the consequence of the development of AMF in the soil, non-mycorrhizal plants are out-competed by mycorrhizal plants that pre-dominate the site (ALLEN and ALLEN, 1984).

A two-year field experiment was designed to verify the hypothesis whether mycorrhizal inoculation or application of slow-release biologic fertiliser guarantees sufficient survivorship and growth of sown plants during direct reclamation of spoil banks. If this practice turned to be successful, it could become an alternative approach to

standard costly indirect reclamation, when huge quantities of soil or organic material are transported and applied on spoil banks.

Material and Methods

The two-year field experiment on the coal-mine spoil bank Vršany (North-Bohemian coal basin, the Czech Republic) was set up in September 2004 just after the technical phase of reclamation was finished. The experimental area was divided into three equal blocks with different substrates. While the first block was left with original spoil bank Miocene clay, the second and third block were covered by 20 cm thick layer of either organic substrate (a mixture of lignocellulose papermill waste, tree-bark and compost) or loess, respectively. Chemical characteristics of the substrates are given in Tab. 1.

Tab. 1: Chemical characteristics of the substrates in the field experiment. Clay – original Miocene clay from spoil bank, organic substrate – consists of lignocellulose papermill waste, tree-bark and compost, loess – original overburden soil.

	Clay	Organic substrate	Loess
pH (H$_2$O)	6.25	7.32	7.92
pH (KCl)	6.13	7.15	7.64
C $_{total}$ [%]	2.58	6.80	12.48
N [%]	0.06	0.12	0.40
C/N	42	58	31
[a] P [mg·kg^{-1}]	9.7	56.1	17.2
[b] Mg [mg·kg^{-1}]	778	992	775
[b] Ca [mg·kg^{-1}]	1 804	8 004	5 820
[b] K [mg·kg^{-1}]	238	403	161

[a] Olsen (1954)
[b] Mehlich (1978)

On each of three blocks, four plots (36 m^2 each) with different amendment treatments were established: 1) fertilised with 0.69 kg·m^{-2} of a long-term, slow release complete natural fertilizer Conafer®, which is composed mostly of extracts of sea organisms, natural humates, ground minerals and rocks (producer Symbio-M Ltd., Czech Republic); 2) inoculated with AMF in the form of 1.7 l·m^{-2} of Symbivit® (producer Symbio-M Ltd., Czech Republic) containing reproductive particles (spores, mycelium and colonised root fragments) of 6 different *Glomus* species on an inert carrier (mixture of slate, zeolite and expanded clay), 3) inert carrier lacking propagules of AMF was applied in the same dose as in the previous plot (this amendment was used in order to distinguish the net effect of AMF on the development of the plant cover and the effect of abiotic components of the inoculum), 4) unamended control plot. All plots were sown with seeds of the grass *Arrhenatherum elatius* (L.) Presl, and the legume *Lotus corniculatus* L. at the dose of 2000 seeds of either species per one m^2. The experiment was sampled and harvested in September 2005 and September 2006. The following parameters were examined:

Plant biomass

In both years, vegetation growing in blocks covered either with organic substrate or with loess was very dense. The vegetation was represented only by the grass *A. elatius*, which out-competed *L. corniculatus* (see Results). Due to the dense cover, the plants were harvested in five randomly distributed sampling squares (0.5×0.5 m

each) in each plot. In each sampling square, plants were cut 2 cm above the soil surface, dried at 70 °C to constant weight and weighed. However, this methodology could not be applied for the third block (original clay). Due to extremely poor growth of both sown plant species in clay in the first year, the total plant biomass of each plot had to be harvested in order to obtain at least basic data (though statistically untreatable). In the second year, plant growth performance on clay improved, but the vegetation was very heterogeneous and it was still impossible to use the method of randomly distributed sampling squares. For that reason the plots on clay block were divided into 36 square "sub-plots" (1 m^2 each) and the biomass was completely harvested from each sub-plot and statistically treated as replicates.

Cover

Vegetation cover was visually estimated either for the whole plot (for both years in blocks layered with loess or organic substrate, for 2005 in block with original clay), or the cover was individually scored for each of 36 sub-plots (on clay block in 2006).

Mycorrhizal colonisation

For assessment of mycorrhizal colonisation of plants on organic substrate and loess, one mixed root sample (consisting of roots of five plants) was obtained for each of five sampling squares per plot. On clay, 5 root samples for both *A. elatius* and *L. corniculatus* were collected from each plot. The diversified character of vegetation in 2006 allowed more complex sampling methodology: the two species were sampled not only when growing individually (in patches isolated from each other) but also when growing together in close communities. In the laboratory, the roots were carefully washed from the soil, stained with 0.05 % Trypan blue in lactoglycerol (KOSKE and GEMMA, 1989) and the percent of root length colonised with AMF was evaluated using a segment method under the compound microscope (GIOVANNETTI and MOSSE, 1980).

Mycorrhizal inoculation potential

At the beginning of the experiment, all three studied substrates were sampled for assessment of initial mycorrhizal inoculation potential – MIP (defined as the ability of soil to induce mycorrhizal colonisation of plant roots). Then the sampling for MIP was performed during each harvest. For organic substrate and loess, the samples of soil were obtained from the rhizosphere of plants in analogous process described for mycorrhizal colonisation of roots (see above). For clay, 25 soil samples per plot were randomly collected from the rhizosphere of established plants in 2005. Diversified character of vegetation on clay in 2006 allowed evaluating possible effects of different types of vegetation on MIP. Therefore, in 2006 soil samples (5 samples per each plot) were collected from the rhizosphere of *A. elatius* and *L. corniculatus* growing separately, from the community of these plant species and from bare spaces without vegetation.

The MIP bioassays were performed on *Zea mays* as the host plant with five replicates in each treatment. Plants were grown in 180-ml pots in a greenhouse, watered daily with deionised water and supplemented with light from 400 W metal halide lamps for 12 hours a day. Plants were harvested after four weeks, the roots were washed from the soil and stained with 0.05 % Trypan blue in lactoglycerol (KOSKE and GEMMA, 1989). The percentage of mycorrhizal colonisation of roots was evaluated under the stereomicroscope according to the gridline intersect method (GIOVANNETTI and MOSSE, 1980).

Statistical analysis

Data showed gamma distribution (biomass) or binomial distribution (colonisation, MIP) and were analysed using Generalised Linear Models (S-Plus 2000; MathSoft Inc., USA) with substrate and amendment as independent variables. For evaluation of main effects of factors on mycorrhizal colonisation, only the data representing colonisation of *A. elatius* (as a plant species present on all three substrates) were included into the analysis. For evaluation of main effects of factors on MIP, the data of vegetation-free spots were excluded from the analysis, because the presence of host plants is necessary precondition for AMF development.

Tab. 2: Effect of the substrate (original clay, organic substrate or loess) and its amendment (natural slow-release fertiliser Conafer®, commercial mycorrhizal inoculum Symbivit®, carrier of mycorrhizal inoculum without living propagules of AMF, unamended) on plant biomass according to Generalised Linear Models. For year 2005, only the results for organic substrate and loess were included into statistic analysis due to poor growth of plants on clay (see text). Chi-square values and probability levels are presented (*** P<0.001, ns – non-significant effect).

	Year	Substrate (A)	Amendment (B)	A × B
Chi square value	2005	0.48	0.46	0.45
Significance		ns	ns	ns
	2006	255.07	254.3	212.9
		***	ns	***

Results

Plant biomass

Although the seeds of both species germinated successfully already in the autumn 2004, no plants of *L. corniculatus* were found on organic substrate or loess at either of the two harvests (2005 and 2006). Because *A. elatius* developed very dense cover already during the 1st growing season, *L. corniculatus* was out-competed by the fully engaged grass cover.

At the end of the first growing season (2005), there was no effect of substrate or amendment on plant biomass; due to extremely low biomass, the plots in clay block were excluded from the analysis (Tab. 2). In organic substrate and loess *A. elatius* developed 100 % cover and produced high amount of biomass (the average shoot dry weight was 653 $g \cdot m^{-2}$ and 853 $g \cdot m^{-2}$, respectively). In contrast, only isolated individual plants of *A. elatius* and *L. corniculatus* (mostly less than 5 % cover) with very low biomass grew on clay plots. The total biomass in clay ranged from 5 $g \cdot m^{-2}$ (unamended plot) to 59 $g \cdot m^{-2}$ (amendment with Symbivit).

In 2006, grass on organic substrate and loess formed similar biomass (average values 576 $g \cdot m^{-2}$ and 705 $g \cdot m^{-2}$, respectively), which was significantly higher as compared to clay (44 $g \cdot m^{-2}$). There was also a significant effect of interaction between substrate and amendment on biomass production (Tab. 2). Although the effect of amendment on plant biomass was not significant when evaluated for all 3 substrates together, for clay alone a strong significant effect of amendment on plant biomass and vegetation cover was found (Chi square 211.87, P<0.001 and 86.21, P<0.001, respectively). Both these parameters were highest after Symbivit application and reached low values in unamended treatment (Fig. 1a, b).
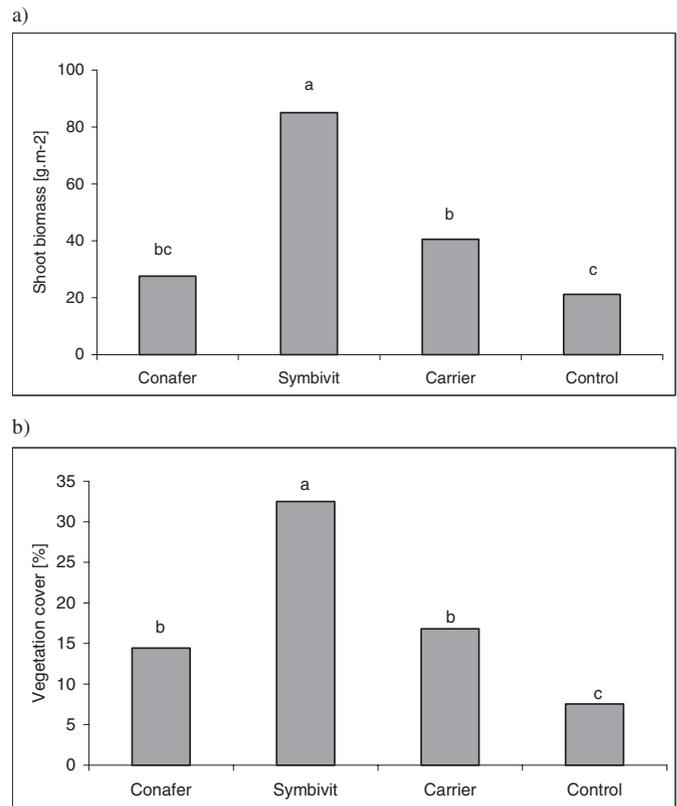
a)

b)

Fig. 1: Effect of amendment (natural slow-release fertiliser Conafer®, commercial mycorrhizal inoculum Symbivit®, carrier of mycorrhizal inoculum without living propagules of AMF, unamended control) on shoot biomass (a) and vegetation cover (b) of plants growing in clay at the end of the second growing season. Columns marked with the same letter are not significantly different according to Chi square test (P<0.05). Data are means of 36 replicates.

Mycorrhizal colonisation

Mycorrhizal colonisation was significantly affected by substrate, by amendment and also by interaction of these two factors in both years; because *L. corniculatus* was present only in clay, the data of this legume were not included into the evaluation of the effect of factors (Tab. 3). In 2005, root colonisation in organic substrate was negligible to zero. In loess and clay in 2005 and in all three substrates in 2006, colonised roots of plants were observed in all treatments, i.e. also in those plots that were not inoculated with Symbivit. None the less, the inoculation with Symbivit still generally increased mycorrhizal colonisation of *A. elatius*. In contrast, colonisation of *L. corniculatus* was not positively affected by Symbivit amendment (Tab. 3).

In carrier-amended treatment on clay, the colonisation level of both *A. elatius* and *L. corniculatus* was significantly increased if those species grew in engaged community, as compared to isolated patches of individual plant species.

Mycorrhizal inoculation potential

Initial MIP of the substrates, i.e. before the substrates were amended and sown with plants, was zero for organic substrate and clay and 4 % (of colonised roots in bioassay) for loess. During the experiment, MIP was significantly affected by substrate, by amendment and by interaction of these two factors in both years (Tab. 4). Generally, organic substrate showed the lowest MIP in both years, while the highest MIP was found in clay. There was no clear trend in the effect of amendments on MIP (Tab. 4) due to strong interaction of factors.

Tab. 3: Effect of treatment (natural slow-release fertiliser Conafer®, commercial mycorrhizal inoculum Symbivit®, carrier of mycorrhizal inoculum without living propagules of AMF, unamended) on mycorrhizal colonisation of plants growing on different substrates. For the evaluation of effect of factors, the data for *L. corniculatus* in clay were excluded, because this species was not present in organic substrate and loess. Values in columns within each substrate or plant marked with the same letter are not significantly different according to Chi square test (P<0.05). Effect of factors according to Generalised Linear Models (*** P<0.001). Data are means of five replicates. The empty boxes mean that the data were not available due to the absence of plants on relevant sites.

Substrate	Plant	Amendment	Mycorrhizal colonisation [%]			
			Year 2005		Year 2006	
Organic substrate	*A. elatius*	Conafer	2	a	8	b
		Symbivit	0	b	33	a
		Carrier	0	b	7	bc
		Unamended	0	b	5	c
Loess	*A. elatius*	Conafer	9	b	74	b
		Symbivit	33	a	81	a
		Carrier	8	c	49	c
		Unamended	4	d	78	ab
Clay	*A. elatius*	Conafer	19	b	61	b
		Symbivit	31	a	78	a
		Carrier	12	c	32	c
		Unamended	5	d	57	b
	A. elatius (community)	Conafer			53	c
		Symbivit			76	b
		Carrier			94	a
		Unamended			-	
	L. corniculatus	Conafer	36	c	93	a
		Symbivit	27	c	84	ab
		Carrier	56	b	56	c
		Unamended	85	a	76	b
	L. corniculatus (community)	Conafer			80	b
		Symbivit			88	ab
		Carrier			90	a
		Unamended			-	
Factor	Chi square value/	Substrate (A)	845.66		1086.45	
			***		***	
	Significance	Amendment (B)	486.49		859.08	
			***		***	
		A×B	429.76		610.06	
			***		***	

The structure of plant cover significantly influenced MIP of clay substrate (Chi square 1536.41, P<0.001). In most treatments, presence of *L. corniculatus*, either alone or as a component of a community, increased MIP of the substrate in comparison either with the patches of *A. elatius* alone, or with the bare areas without vegetation. *A. elatius* even negatively affected MIP in plot with inert carrier amendment and in unamended plot.

Discussion

Relatively high values of mycorrhizal colonisation and MIP in treatments without application of AMF, especially on clay, are indicative of surprisingly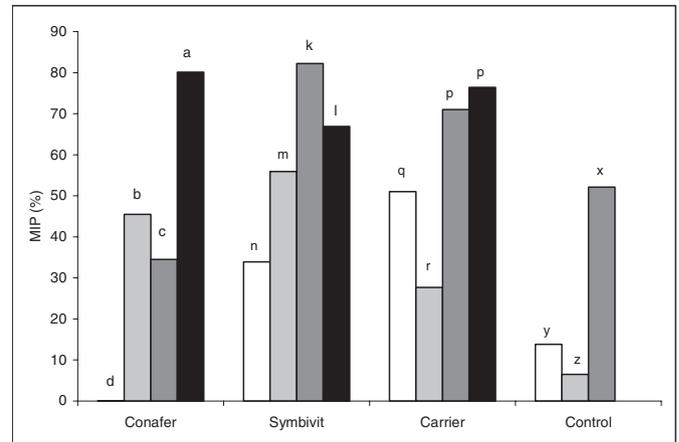 rapid natural dispersion of AMF propagules to freshly created spoil banks. Wind, soil erosion and small mammals are the possible dispersal agents that enable the AMF to invade new areas (WARNER et al., 1987; ALLEN and ALLEN, 1984). The transport by wind was probably the most important way by which AMF propagules dispersed on the experimental site. Strong winds are very frequent on spoil banks and small soil particles containing AMF propagules can be easily moved through the air from surrounding areas with established plant cover, especially during dry weather season. Moreover, despite the experimental area was fenced to prevent access of deer and rabbits, smaller mammals (or insect) could migrate across the plots and transport soil particles containing spores or mycelium of AMF not only from outside the plots, but also between the treatments. This factor, unfortunately, cannot be effectively eliminated in the field experiment.

Tab. 4: Effect of the substrate (original clay, organic substrate or loess) and its amendment (natural slow-release fertiliser Conafer®, commercial mycorrhizal inoculum Symbivit®, carrier of mycorrhizal inoculum without living propagules of AMF, unamended) on mycorrhizal inoculation potential (MIP). MIP is expressed as percentage of mycorrhizal colonisation of roots of bioassay plants. Values in columns within each substrate marked with the same letter are not significantly different according to Chi square test (P<0.05). Effect of factors according to Generalised Linear Models (*** P<0.001). Data are means of five replicates.

Substrate	Amendment	MIP [%]			
		Year 2005		Year 2006	
Organic substrate	Conafer	33	a	11	c
	Symbivit	13	b	27	a
	Carrier	0	d	2	d
	Unamended	3	c	14	b
Loess	Conafer	40	b	60	a
	Symbivit	47	a	45	b
	Carrier	23	c	37	c
	Unamended	10	d	45	b
Clay	Conafer	90	a	53	c
	Symbivit	79	ab	68	a
	Carrier	81	b	61	b
	Unamended	34	c	29	d
Factor Chi square value / Significance	Substrate (A)	4327.87 ***		2955.26 ***	
	Amendment (B)	2211.85 ***		2670.64 ***	
	A×B	999.46 ***		2014.38 ***	

Fig. 2: Effect of different vegetation cover on mycorrhizal inoculation potential (MIP) of clay substrate in different amendments (natural slow-release fertiliser Conafer®, commercial mycorrhizal inoculum Symbivit®, carrier of mycorrhizal inoculum without living propagules of AMF, unamended control) at the end of the second growing season. MIP is expressed as percentage of mycorrhizal colonisation of roots of bioassay plants. White columns – bare places with no plants, light grey columns – *Arrhenatherum elatius*, dark grey columns – *Lotus corniculatus*, black columns – community of *A. elatius* and *L. corniculatus*. In the control treatment, the two plant species grew separately and did not form a community. Columns marked with the same letter are not significantly different within each amendment according to Chi square test (P<0.05). Data are means of five replicates.

Despite rapid natural colonisation of the study site by AMF, the application of Symbivit inoculum containing selected strains of AMF significantly increased mycorrhizal colonisation of grass roots in all substrates. The introduced fungi were probably more infective as concerns the grass roots. However, quite different data were obtained for the legume, which was extensively colonised regardless of inoculation (i.e. AMF naturally invading clay substrate successfully colonised roots of this plant species). Yet, the effectiveness of the introduced AMF was probably still higher as shown by significantly highest biomass and plant cover on clay plots treated with Symbivit. Application of the inert inoculum carrier and the fertilizer Conafer also increased plant growth parameters on clay, but to a lower extend as compared to Symbivit. Inoculum carrier consisting of slate, zeolite and expanded clay might probably improve physical properties of the clay substrate, especially its aeration, while the fertilizer could contribute the to better plant performance due to its content of nutrients.

Organic substrate and loess showed substantially higher concentration of nutrients (especially P and N) than clay, thus the application of the fertilizer or inoculation with Symbivit had no effect on plant growth. The amount of nutrients was probably sufficient to cover plant demands, thus mycorrhizal symbiosis was not beneficial for plants (BETHLENFALVAY et al., 1983). At the same time, mycorrhizal colonisation of *A. elatius* in organic substrate was very low and might have negligible effect on plant growth. The development of AMF in organic substrate was restricted, which was also obvious from a low level of mycorrhizal inoculation potential. It is well documented that when applied in high doses, organic additives (such as compost, manure or sewage sludge) can be harmful to AMF (SÁINZ et al., 1998; JENSEN and JAKOBSEN, 1980; THORNE et al., 1998), possibly due to toxic components contained in the organic matter (LAMBERT and WEIDENSAUL, 1991). Moreover, the establishment of mycorrhizal association and the development of AMF could be also restricted by high concentrations of available P in this substrate (DOUDS and SCHENCK, 1990; VIVEKANANDAN and FIXEN, 1991). Both of these explanations are probably applicable for our study, where plants were seeded and AMF inoculum subsequently introduced directly into the layer of organic substrate. In other studies where positive effects of organic amendments on development of mycorrhizal symbiosis were detected (MUTHUKUMAR and UDAIYAN, 2000, 2002; MÄDER et al., 2000; PALENZUELA et al., 2002), much lower (by one order) doses of organic matter were applied as compared to the dose used for reclamation of our spoil bank site.

The initial MIP of loess in our study, though very low, could be probably ascribed to the presence of infective AMF propagules in this soil already before it was layered on the spoil bank. The AMF can colonise loess during its storage in stockpiles, which are often overgrown with vegetation. In association with suitable host plants AMF can proliferate in loess during this stockpiling stage and consequently can be dispersed on spoil banks. In both years, the lowest MIP was detected in the organic substrate, where the development of AMF was suppressed. In contrast, the highest MIP was found in clay where any amendment probably led to AMF proliferation and subsequent increase of MIP. Surprisingly, the application of Symbivit had not always result in the highest MIP in relevant plots. This could be probably related to rapid dispersion of AMF propagules to the spoil bank from its surroundings (see above; PÜSCHEL et al., unpublished results), which was reflected in increased ability of soil to induce mycorrhizae. However, the total number of prapagules is probably not the only important factor affecting MIP of soil. Based on results of a long-term field trial comparing organic and conventional farming systems, MÄDER et al. (2000) summarised that also soil properties such as nutrient content, biological activity, soil structure etc. are important factors, which can substantially affect the capacity of soil to initiate AM symbiosis.

There was a strong effect of plant cover on MIP. Presence of *L.*

corniculatus positively influenced the development of AMF as compared to *A. elatius*. The different influence of the two plant species was probably caused by higher mycorrhizal dependency (VAN DER HEIJDEN, 2003) of the legume.

Both substrates commonly used for spoil bank reclamation, loess and the organic substrate, are rich in nutrients, which provide suitable conditions for very rapid establishment of plant cover and formation of large plant biomass. However, plants with high competition abilities are likely to dominate such sites. Sowing a mixture of several plant species can thus result in monoculture plant cover. Direct reclamation of Miocene clays cannot guarantee comparable high yields, regardless the application of AMF inoculum. On the other hand, more diverse vegetation cover establishes on clay and the positive effect of inoculation on plant growth is evident in this substrate. While the results of this study would not change the prevalent trend of indirect reclamation into direct one, they indicate that, at some circumstances, the examined method of mycorrhizal inoculation can be applied in small-scale. In the patches of spoil banks, where layering of loess or organic substrate is inconvenient, the inoculation with AMF will help to establish the cover of vegetation, which will probably have more diverse character compared to surrounding reclaimed sites.

Acknowledgement

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., 1984: Competition between plants of different successional stages: mycorrhizae as regulators. Can. J. Bot. 62, 2625-2629.

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.

DOUDS, D.D., SCHENCK, N.C., 1990: Increased sporulation of vesicular-arbuscular mycorrhizal fungi by manipulation of nutrient regimes. Appl. Environ. Microbiol. 56, 413-418.

GIOVANETTI, M., MOSSE, B., 1980: An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84, 489-500.

HODACOVÁ, D., PRACH, K., 2003: Spoil heaps from brown coal mining: technical reclamation versus spontaneous revegetation. Restor. Ecol. 11, 1-7.

JENSEN, A., JAKOBSEN, I., 1980: The occurrence of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertilizer treatments. Plant Soil 55, 403-414.

JOCHIMSEN, M.E.A., 1996: Reclamation of colliery mine spoil founded on natural succession. Water Air Soil Poll. 91, 99-108.

KOSKE, R.E., GEMMA, J.N., 1989: A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92, 486-505.

LAMBERT, D.H., WEIDENSAUL, T., 1991: Element uptake by mycorrhizal soybean from sewage sludge-treated soil. Soil Sci. Soc. Am. J. 55, 393-398.

MÄDER, P., EDENHOFER, S., BOLLER, T., WIEMKEN, A., NIGGLI, U., 2000: Arbuscular mycorrhizae in long-term field trial comparing low-input (organic, biological) and high-input (convencial) farming systems in a crop rotation. Biol. Fertil. Soils 31, 150-156.

MEHLICH, A., 1978: New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese, and zinc. Commun. Soil Sci. Plan. 9, 477-492.

MOTT, J.B., ZUBERER, D.A., 1987: Occurrence of vesicular-arbuscular mycorrhizae in mixed overburden mine spoils of Texas. Reclam. Rev. Res. 6, 145-156.

MUTHUKUMAR, T., UDAIYAN, K., 2000: Influence of organic manures on arbuscular mycorrhizal fungi associated with *Vigna unguiculata* (L.) Walp. in relation to tissue nutrients and soluble carbohydrate in roots under field conditions. Biol. Fertil. Soils 31, 114-120.

MUTHUKUMAR, T., UDAIYAN, K., 2002: Growth and yield of cowpea as influenced by changes in arbuscular mycorrhiza in response to organic manuring. J. Agron. Crop Sci. 188, 123-132.

OLSEN, R.S., 1954: Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dept. Agric. Stat., No. 939.

PALENZUELA, J., AZCÓN-AGUILAR, C., FIGUEROA, D., CARAVACA, F., ROLDÁN, A., BAREA, J.M., 2002: Effects of mycorrhizal inoculation of shrubs from Mediterranean ecosystems and composted residue application on transplant performance and mycorrhizal developments in a desertified soil. Biol. Fertil. Soils 36, 170-175.

PRACH, K., 1987: Succession of vegetation on dumps from strip coal mining. Folia Geobot. Phytotax. 22, 339-354.

PRACH, K., 2003: Spontaneous succession in Central-European man-made habitats: What information can be used in restoration practice? Appl. Veg. Sci. 6, 125-129.

PÜSCHEL, D., RYDLOVÁ, J., VOSÁTKA, M., 2007: Mycorrhiza influences plant community structure in succession on spoil banks. Basic Appl. Ecol. 8, 510-520.

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.

SÁINZ, M.J., TABOADA-CASTRO, M.T., VILARIÑO, A., 1998: Growth, mineral nutrition and mycorrhizal colonisation of red clover and cucumber plants grown in a soil amended with composted urban wastes. Plant Soil 205, 85-92.

SKOUSEN, J.G., JOHNSON, C.D., GARBUTT, K., 1994: Natural revegetation of 15 abandoned mine land sites in West-Virginia. J. Environ. Qual. 23, 1224-1230.

SMITH, S.E., READ, D.J., 1997: Mycorrhizal Symbiosis. Academic Press, London.

THORNE, M.E., ZAMORA, B.A., KENNEDY, A.C., 1998: Sewage sludge and mycorrhizal effects on secar bluebunch wheatgrass in mine spoil. J. Environ. Qual. 27, 1228-1233.

VAN DER HEIJDEN, M.G.A., 2003: Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search of underlying mechanisms and general principles. In: van der Heijden, M.G.A., Sanders, I.R. (eds.), Mycorrhizal Ecology, 225-242. Springer, Berlin.

VIVEKANANDAN, M., FIXEN, P.E., 1991: Cropping systems effects on mycorrhizal colonisation, early growth, and phosphorus uptake of corn. Soil Sci. Soc. Am. J. 55, 136-140.

WAALAND, M.E., ALLEN, E.B., 1987: Relationships between VA mycorrhizal fungi and plant cover following surface mining in Wyoming. J. Range Manage. 40, 271-276.

WARNER, N.J., ALLEN, M.F., MACMAHON, J.A., 1987: Dispersal agents of vesicular-arbuscular mycorrhizal fungi in a disturbed arid ecosystem. Mycologia 79, 721-730.

WIEGLEB, G., FELINKS, B., 2001: Predictability of early stages of primary succession in post-mining landscapes of Lower Lusatia, Germany. Appl. Veg. Sci. 4, 5-18.

Address of the authors:

Dr. J. Rydlová, Dr. D. Püschel, Dr. M. Vosátka, Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Průhonice, Czech Republic.

Bc. K. Charvátová, Institute for Environmental Studies, Faculty of Science of the Charles University, Benátská 2, 128 43 Praha 2, Czech Republic.