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Importance of mycorrhizal symbiosis for local adaptations of *Aster amellus*

Diplomová práce

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Prohlašuji, že jsem diplomovou práci zpracovala samostatně, pouze s použitím citované literatury

Abstract

The importance of arbuscular mycorrhizal (AM) symbiosis for survival and growth of many plant species is generally recognized. It has been repeatedly shown that symbiosis with mycorrhizal fungi can increase the fitness of many plant species. This increasing fitness is caused by increased uptake of phosphorus and other nutrients or pathogen protection. Most studies on mycorrhizal associations explore these types of relationship using single plant population and single fungal species. This contrasts with many studies that show local adaptations of plant populations to environmental conditions. Recently it has also been shown that fungal species may have themselves different adaptations at different localities. Inspite of this knowledge only few recent studies consider both differences between plant populations and their possible local adaptations to environmental conditions at their localities as well as differences in the abundance and composition of AM fungal communities and possible local adaptations of plants directly to these communities.

We studied interactions of plants from different populations of *Aster amellus* (an endangered species of the Czech Republic) with AMF in their natural habitats and in a pot experiment where plants from different populations were inoculated with two different AMF isolates (*Glomus mosseae* BEG25 or *G. intraradices* BEG75) and cultivated either in the soil of the population origin or in soil for other *A.amellus* population.

Results of both field sampling and pot experiment indicate that mycorrhizal colonisation of plant roots differs significantly depending on plant identity and the soil origin. In addition, growth response of plant populations differs between the AMF isolates. All this indicates that differences in root colonization are result of local adaptations of plants to AM symbiosis and that conclusion on species response to AM fungi must be based on studies at multiple sites. I repeated the pot experiment with all 3 populations from each region, their soils and indigenous fungal isolates. Due to the long process of fungal isolation, the experiment was started in the spring 2006, so the results are not complete yet. Preliminary results indicate that there are differences in growth between different fungi, soil and populations. There is, however, no agreement in conclusion between populations within regions.

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Figure 1. It was hard work to lead my master thesis.

Abbreviations

AM	arbuscular mycorrhizae, arbuscular mycorrhizal
AMF	Arbuscular mycorrhizal fungi
ERM	Extraradical mycelium
CS	České Středohoří
CK	Český Kras
Gm	*Glomus mosseae*
Gi	*Glomus intraradices*
BEG	Europishe bank for Glomeromycota
CS1	České Středohoří, lokalita Malíč
CS2	České Středohoří, lokalita Holý Vrch
CS3	České Středohoří, lokalita Encovany
CK1	Český Kras, lokalita Koda
CK2	Český Kras, lokalita Karlík
CK3	Český Kras, lokalita Lochkov
MIP	Mycorrhizal inoculation potential
PF	Complex population-fungi
PS	Complex population-soil
SF	Complex soil-fungi

Content

1. Introduction

1.1 Arbuscular mycorrhizal symbiosis

Mycorrhiza is often defined as symbiotic interaction between roots of plants and specific groups of soil fungi or as an apparatus produced by roots of plants and structures of symbiotic fungi (Wilcox 1991). Mycorrhiza occurs in nearly 95% of plant species in natural ecosystems, including some ferns, mosses or liverwort. Only a few of plant groups have no mycorrhizal associations, e.g. *Brassicaceae, Proteaceae, Caryophyllaceae* or *Chenopodiaceae* (Smith and Read 1997). However, recent studies show that in some cases even plants from these groups can establish mycorrhizal associations (Pattison and McGee 2002, Püschel et al. in press).

The classification of different forms of mycorrhiza is based on specific fungal structures created either directly in the roots of host plants or in their proximity. The most commonly used classification describes four basic groups of mycorrhizal symbiosis (Gianinazzi and Gianinazzi–Pearson 1988, Fig. 2).

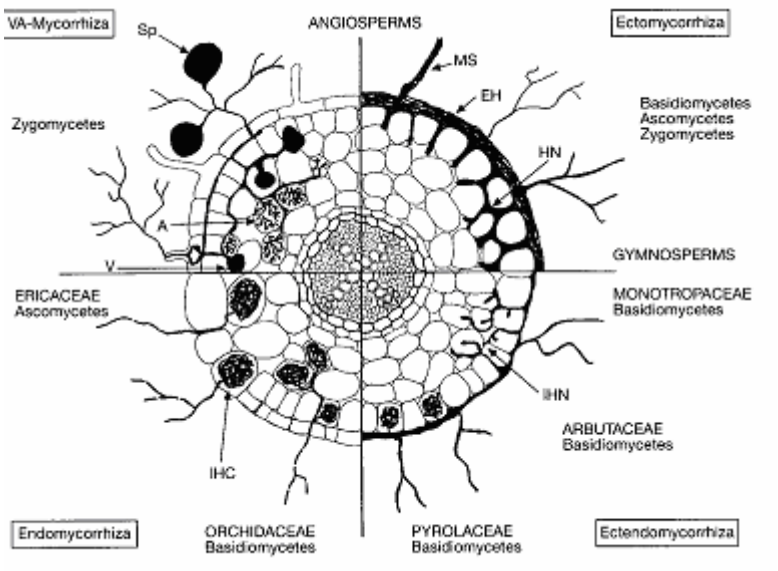


Fig.2. A schematic overwiev of the different forms of mycorrhiza. *MS*-Mycelial strands, *EH*- external hyphae, *HN*-Hartig net, *IHN*-intercellular hyphal net, *IHC*-intercellular hyphal complexes, *V*-fungal vesicle, *A*-arbuscule, *Sp*-spore (Gianinazzi and Gianinazzi–Pearson 1988).

One of these types is **arbuscular mycorrhiza** (AM), which occurs in more than 80% of plant species in all terrestrial ecosystems. It is especially common for temperate and tropical grasslands, plants in semi-deserts and deserts and especially for tropical forests (Smith and Read 1997).

The name of this group comes from **arbuscules** – characteristic structures that develop in deeper parts of primary core of colonised roots (Fig. 3). They are generated by repeated dichotomy branching of hyphae inside cells and in maturity fill great deal of root cells. These organs have probably a key role in transport of nutrients between plants and fungi (Alexander et al. 1989).

The **vesicles** (Fig. 4) are other remarkable structures that can be found in the roots colonised by AM fungi. However, not all AMF species form vesicles. Vesicles develop in latter phases of AMF growth and probable have reservoir-function, especially for fats (Carling and Brown 1982).

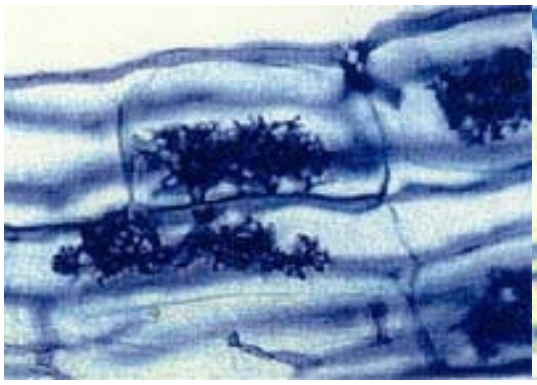


Fig.3. arbuscules (invam.caf.wvu.edu/)

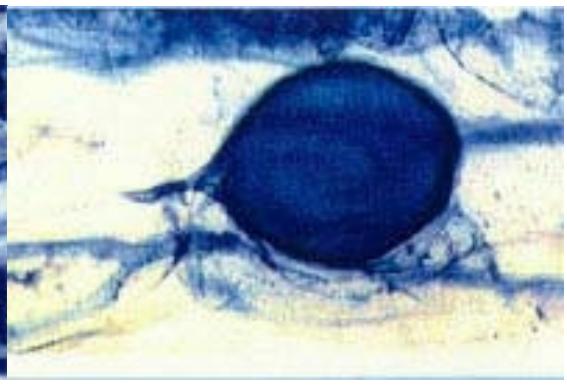


Fig. 4.vesicules(invam.caf.wvu.edu/)

Other characteristic organs of arbuscular mycorrhiza are hyphae. They create extensive network of polynucleus **extraradical mycelium** (ERM). Because this mycelial network can draw and transport nutrients from long distances, the absorption area from which plant can procure nutrients is greatly extended (Li et al. 1991). The length of the ERM in the soil can be from a few meters to more than tens of metres per gram of the soil and thus the ratio between AM hyphae length and the root length may be even 1: 100 (George et al. 1995). The growth rate of ERM varies from 0.7 to 3.1 mm per day depending on fungal species (Jacobsen et al. 1992a).

Spores and sporocarps are dormant and dispersal stadium of AM fungi. In favourable conditions (temperature, moisture, pH, nutrients and roots exudates) in the soil, the spores begin to germinate (Daniels and Hetrick 1984, Bowen 1987). If hyphae from germinated spore get to the proximity of a root of compatible plant, it can tack the root surface, create appressorium and penetrate inside the root (Giovannetti et al. 1993). It is assumed that root exudates importantly affect this process of colonisation. The combination of physical pressure and enzymatic mechanism enable penetration of AM hyphae to cortical cells of the roots (Bonfante and Perotto 1995), which is followed by formation of inter and intracellular hyphae in primary cortex (Carling and Brown 1982). Root colonization is regulated by cascade of genes of plants and fungi, but only one gene was described up to now (Requena et al. 2002).

The morphology of spores and type of mycorrhizal structures has been used as determinant of **taxonomic position of AMF**; these methods are supplemented by molecular analyses nowadays. In past AM fungi were classified in class *Zygomycetes* (Rosendahl et al. 1994). Modern molecular methods separated AM fungi from this class and a new class called *Glomeromycota* was established (Schüssler et al. 2001). Internal division of *Glomeromycota* is shown in Fig. 5.

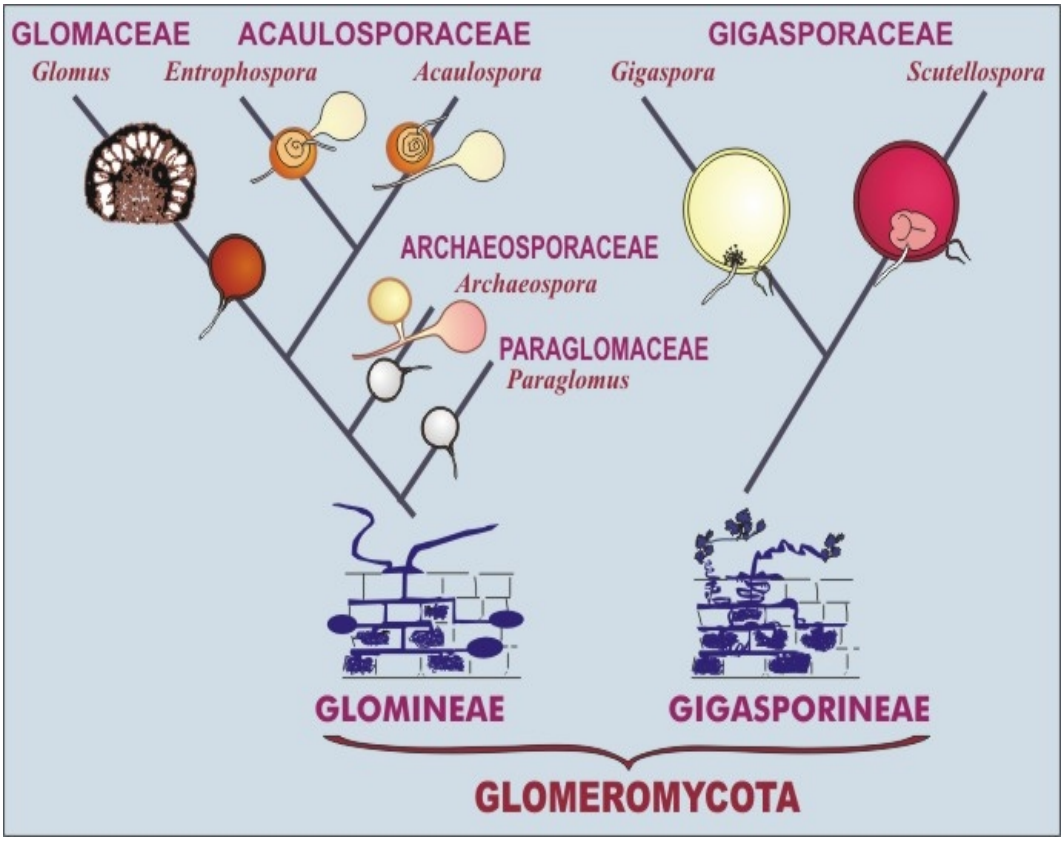


Figure 5. Internal division and classification of *Glomeromycota* (http://www.invam.caf.wvu.edu/).

AM symbiosis was probably the first type of mycorrhizal symbiosis that developed 400 mil years ago in Devonian period (Simon et al. 1993), when plants started to colonise terrestrial ecosystems (Gryndler 1992). This fact is supported by fossil findings of *Rhinia, Asteroxylon* or *Cordaites* with typical vesicules and arbuscules in their roots (Osborne 1909, sec. Mejstřík 1988). The genesis of AM symbiosis was probably a necessity for those early terrestrial plants to overcome stresses like new pathogens and nutrients and/or water deficiency.

At present, the AM fungi support plants in a similar way. AM symbiosis influences plants both directly and indirectly. One of the indirect influences is **protection against fungal pathogens**, e.g. *Pythium, Fusarium* or *Thielaviopsis* (Hooker 1994) or viruses (Dehne 1982). Mechanism of this protection was explained by Dehne (1982), who showed that roots colonized by AM fungi have higher content of lignin, which delay invasion of parasites. These roots have also increased chitinolytic activity of root cells. Another explanation was proposed by Baltruschat and Schoenbeck (1975), who explain this by production and accumulation of

antagonistic chemical compounds. Roots colonized by AM fungi are also less attacked by helminths (Hooker et al. 1994). On the other hand, increase of nutrients in leaves of mycorrhizal plants can lead to faster development of leave pathogens (Dehne 1982).

Decrease of heavy metal toxicity is another indirect effect of AM symbiosis on plants (Gali et al. 1994, Malcová et al. 2001). Although the mechanisms of this effect have not been fully clarified yet, it is assumed that metals are fixed in the roots of AM plants (Schuepp et al. 1987, Turnau et al. 1993).

Considerable **improvement of nutrient income** is considered the most important direct effect of mycorrhizal symbiosis on plants. Among all nutrients transported to plants by AMF mycelium, phosphorus is considered the most important (Sanders and Tinker 1973, Francis et al. 1986, Van der Heijden et al. 2003). Li et al. (1991b) estimated that more than 70-80% of total content of phosphorus in plants was transported by AM fungi. Thanks to their connection to the extensive mycelial network, mycorrhizal plants utilize much higher surface for phosphorus absorption. Furthermore, AMF have higher affinity for phosphorus and are able to acquire it at lower concentrations in the soil (Cress et al. 1979, Bolan 1991, Smith and Read 1997). Finally, the ERM has the ability to acquire phosphorus not only from anorganic sources, but also from organic matters in the soil (Joner and Jacobsen 1994). The absorbed phosphorus is transported through the mycelium in form of polyphosphate granules. The rate of transport can be affected by concentration gradient and flow of cytoplasm (Jacobsen 1992). The capacity of transport depends on fungal species (Jacobsen et al. 1992b, Joner and Jacobsen 1994) and also on environmental conditions, e.g. soil temperature (Hetrick et al. 1994) or activity of soil animals (Jacobsen et al. 1994).

Another nutrient, income of which is improved by AM symbiosis, is nitrogen. The importance of mycorrhiza for acquisition of **nitrogen** depends on the form and availability of this nutrient in the soil. If the dominant form is nitrate, which is highly mobile, the benefit of mycorrhiza is reduced (Johansen et al. 1992). In contrast, if the soil pH is low, most of anorganic nitrate is in the form of ammonium. It is absorbed by negatively charged clay minerals and the mobility is limited (Smith and Read 1997). In these conditions mycorrhizal symbiosis is very useful for improvement of nitrogen income. Ammonium can be assimilated by ERM and in organic form transported to the plants (Smith and Read 1997). It was also documented that AM

symbiosis can have positive effect on fixation of atmospheric nitrogen by *Rhizobium* (Barea et al. 1989) or *Frankia* (Jha et al. 1993).

Not only AM influences nutrient income to plants, it also affects whole **biogeochemical cycles**. Miller and Jastrow (1994) demonstrated that the mycelium of AMF is in fact an extensive sing of carbon and its turnover, therefore, represents a significant part of soil carbon cycle.

Second effect of ERM on biogeochemical cycles is caused by location of hyphae in the soil matrix, which allows acquisition of nutrients from spaces unavailable to roots (Read 1992). This assumption was supported also by Jacobsen (1992a), who showed that differences in income of phosphorus between fungal isolates were not dependent on amount of ERM, but on its distribution in the soil.

ERM can further change chemical **properties of the soil** in hyphosphere and thereby increase availability of mineral ions for plants or microorganisms. Li et al. (1991b) demonstrated that a zone with decreased content of phosphorus and lower pH is generated in proximity of hyphae. This acidification is caused by excretion of protons or production of low molecular weight organic acids such as oxalate. The increase in content of CO_2 as a result of root and fungal respiration is also an important factor (Jacobsen and Rosendal 1990).

ERM has also large impact on establishment of soil aggregates, which are necessary for accumulation of organic masses and nutrients in the soil. ERM creates mainly soil macroaggregates (average >250 μm) with big pores. They hold water and avoid drying of the soil in the dry period, but allow better movements of water in wet period (Miller 1987). This property of ERM is very important for stabilization of the soil and thus protection against soil erosion (Jeffries and Dodd 1991).

The last factor, by which AM fungi can affect biogeochemical cycles, is connection of two or more plants mediated by ERM. Existence of **mycelial connection between plants** is common in grasslands ecosystems and herbaceous brushwood (Newman 1988). These connections allow potential transport of nutrients directly between root systems of plants of the same or different species. Such transport was demonstrated for phosphorus (Wittingham and Read 1982), carbon (Francis and Read 1984) and nitrogen (Frey and Schuepp 1992). The ability of ERM to capture nutrients from dying roots and transport them directly to the roots of another plant is also very important (Heap and Newman 1980). During 3 weeks a dying plant can release about 60% of its nitrogen or 70% of phosphorus content.

When roots are colonized with AM fungi, most of nutrients from a dying plant were detected in neighbouring plants (Eason and Newman 1990).

Connenction of plants mediated by AMF have also important effect **on diversity of plants communities**. One study demonstrated this is from Grime et al. (1987). He established long-term experiment to demonstrate that presence of AM fungi contributed to suppression of dominant species *Festuca ovina* and increase of growth of accessory plants because of transport assimilates from dominant species to accessory plants by ERM. The effect of ERM on competition between plants and community structure can also be due to improvement of seedlings recruitment and growth facilitated by the mycelial network (Read et al. 1976, Grime et al. 1987, Francis and Read 1994). No such effect was, however, demonstrated in similar experiments by Moora and Zobel (1996) and Eissenstat and Newman (1990).

Plant competition can be affected by AM fungi both directly and indirectly: indirectly by modifying interaction between plants and other organisms (Zobel and Moora 1997) or directly by modification of plant properties, e.g. changing of some reproduction characteristic (Allen 1991), quality and quantity of the seeds, content of phosphorus or enhancement of clonal growth (Miller et al. 1987).

Other experiments showing effect of AMF on plant competition compare not only non-mycorrhizal and mycorrhizal plants, but also mycorrhizal plants with different mycorrhizal dependency (Allen and Allen 1984, Gange et al. 1990, Harnett and Wilson 1993, 2002, Harnet et al. 1993 or Zobel and Moora 1995). If non-mycorrhizal (or plants with lower dependency on mycorrhiza) plants were inoculated, their biomass decreased compared to mycorrhizal (more dependent on mycorrhiza) plant species (Harnett and Willson, 1993, Gange et al. 1990, Hart and Klinomoros 2002, Zobel and Moora 1995).

These results indicate that plants response to AM fungi depends on **mycorrhizal status of the plants**. In this context plants can be divided into obligatory mycorrhizal, facultatively mycorrhizal and non-mycorrhizal. Non-mycorrhizal plants do not need mycorrhizal symbiosis for successful growth and are usually not colonised by AMF. In contrast, obligatory mycorrhizal species are unable to grow without mycorrhizal colonization. This division is dependent also on content of nutrients in the soil or mycorrhizal inoculation potential of the soil (Janos 1980, Table 1). Since mycorrhizal dependency of plants seems to be an important indicator

of their response to AMF, this knowledge can be utilised for perpetuation of many rare or endangered species (Sýkorová et al. 2003).

Content of nutrients in the soil			
		Low	High
Mycorrhizal inoculation potential	Low	Non-mycorrhizal	Facultative mycotrophic
	High	Obligate mycotrophic	

Table 1. Relationship between content of nutrients in the soil, mycorrhizal inoculation potential and mycorrhizal status of the plants (Janos 1980)

The **specificity of associations** is another important factor influencing relationships between plants and AMF. In the past, it was declared that association between plant and fungi is non-specific. The first study rejecting non-specificity was a study of Streitwolf – Engel et al. (1997). They concluded that fungi behave relatively non-specifically in laboratory conditions but in natural ecosystem with stronger selection, the existence of specificity can be presumed. On the other hand, Šmilauer (2001) suggested that AM associations are not species specific, but they can differ in functions. It means that AMF probably colonize any plant species, but the interactions are quite specific.

The existence of specificity of AMF-plants association was supported by many studies (e.g. Stahl and Christensen 1991, Streitwolf – Engel et al. 1997, Hildebrandt et al. 1999). Some of them concern the adaptations of AMF species to the soil conditions. These experiments showed that AMF species from phosphorus rich agricultural soils have lower mutualistic ability in unmanaged soils than other AMF species. It indicates that these AMF species are adapted to their soil conditions (Stahl and Christensen 1991, Streitwolf – Engel et al. 1997, Hildebrandt et al. 1999).

Other studies tested the effect of different fungal species on plants growth. Streitwolf – Engel et al. (1997) examined response of *Prunela vulgaris* and *Prunela grandiflora* to inoculation with three different AM species and detected that different fungal species are able to support different plants differently. Because of large variability in the results between individuals, the author examined further effect of inoculation with 3 different fungal species on different genotypes of *Prunela*

vulgaris. The results confirmed the theory that not only the responses of different plants species vary, but also genotypes of the same plant respond to different AMF isolates differently (Streitwolf – Engel et al. 2001). Similar results were also found in experiments with agriculture crops (Lackie 1987, Douds 1993, Gange et al. 1990).

All the above results indicate the existence of plant adaptations to AMF. The establishment of such local adaptations and their importance for plant populations is described in the following chapter.

1.2. Local adaptation in plant populations

Many plant populations contain large amount of genetic variation, with quantitative genetic differences between individuals and single populations. Many of these polymorphisms are selectively neutral, but some are likely to be maintained by natural selection (Silvertown and Charlesworth 2001). Such selection pressure differs between habitats with different environmental conditions. Populations at these different types of habitats often remarkably differ also in phenotypic characters. These differences in phenotype may be due to phenotypic plasticity or local adaptations. Both of these phenomena allow plants persist in various environmental conditions, but each of them develops under different set of circumstances and there are differences between them.

Phenotypic plasticity is recognised as a major source of phenotypic variation in natural populations. When phenotypic responses to environment are functionally adaptive, plasticity allows individuals to maintain fitness under diverse environmental conditions (Sultan 2002). When environmental conditions are changed, plants are able to respond plastically to the new conditions and perform as well as in the old environment. An example of phenotypic plasticity in plants is heteromorphism such as differences between floating and submerged leaves of aquatic plants (Briggs and Walters 1997). In the most cases variation in phenotypes is more or less continuous. It is usually described by “reaction norms” (Silvertown and Charlesworth 2001). Bazzaz (1991) linked plastic response of plant to habitat-selection behaviour in animals, and therefore called it “foraging” responses. These “foraging” responses are often observed in clonal plants on both horizontal and vertical growth, e.g. branching frequency, stolon and internodes length, leaf length

and height of stolons (van Kleunen and Fischer 2001). Phenotypic plasticity is common in widespread species or species occurring at localities with unstable environments (Joshi et al. 2001, Hangelbroek 2003).

On the other hand, **local adaptations** are characterised by genetic differentiation and by formation of genotypes specialized to different conditions. Such genetic differentiation is expected to enhance performance in response to local environmental conditions (Sork et al. 1993, Galloway and Fenster 2000). When these plants grow in different conditions, their fitness is lower. Local adaptations of populations to specific environmental conditions on both small and broad scale are well documented not only in plant populations (e.g. Jordan 1992, Kindel 1996, Mc Cay 2001), but also for animals (e.g. Kimura 2004, Pulgar 2005, Terblanche et al. 2006) or soil bacteria (e.g. Belotte et al. 2003).

Development of phenotypic plasticity versus local adaptations in plants depends on several factors: (1) isolation (2) genetic diversity, (3) degree of environmental variability over time and space.

The reason of the first requirement is that continuous supply of new genes (gene flow) distracts establish of specialized genotypes. The **gene flow** can be simply defined as movement of genetic information between populations via pollen or seed dispersal (Brigs and Walters 1997). First studies on gene flow are related to agriculture, because the prevention of hybridisation of agriculture cultivars was in the main interest (Levin and Kerster 1974).

Gene flow mediated by pollen movement depends on the movements of pollinators (insect, wind etc.). Studies on relationship between plants and their pollinators show that some plant species are very well adapted to pollination by insect or other animals (e.g. Levin 1988, Falk and Holsinger 1991). However, it does not mean that pollination is effective. According to cost-benefit hypothesis (sometimes called races in armament), the pollinators (e.g. insect, butterflies) prefer localities with maximum density of food per area and so small populations have lower probability to be visited. In the large populations pollinators visit only plans close to each other, so such plants have higher probability to be pollinated with their relative (Jennersten 1988, Dafnii 1992). This economic behaviour is confirmed by recent studies, which show that most movements of pollinators are relatively local (to 15m), but with occasional very long distance movement (Isagi et al. 2000).

Surprisingly, similar low distance dispersal was observed for wind-pollinated plants (Brigs and Walters 1997).

Gene flow mediated by seed dispersal is affected not only by behaviour of animals, but also by wind dispersal characteristics. The situation is similar to pollen movement. Even in well adapted seeds for both wind and animal dispersal, only a few percentages of seeds are moved far from the parent plants (Nathan et al. 2002)

The above information indicates that plants have means to disperse to relatively long distances, even if the conditions are not optimal. This can maintain high level of gene flow. It has been shown that plants able of long distance dispersal have populations with very low levels of allelic differentiation (Williams and Guries 1994, Linhard and Grant 1996). Gene flow, therefore, controls the extent to which populations evolve independently on each another (García-Ramos and Kirkpatrick 1996, Stanton and Galen 1997, Alleaume-Benharira et al. 2006).

Genetic diversity of plant populations represents second factor that affects the possibility of establishment of local adaptations of plants. Both populations created by long-distance dispersal and isolation are usually small and suffer from similar problems. Because these populations are usually founded only by a few seeds or individuals, they carry only a small sample of alleles of the source population. This situation is known as **founder effect** (long-distance dispersal) or **bottleneck** (fragmentation of large source population). Because the selection of plants to new populations is random, the arisen populations are usually very different in their genotype structure. Establishment of genetically different populations does not mean that these populations are adapted to their environment. When is new population founded by few genotypes, low genetic variability in fact limits the possibility that some of the genotypes will perform well under current conditions. Therefore, losses of genetic variability are very important in the context of conservation biology and have important effect on the possibility of establishment of local adaptations (Barrett and Kohn 1991).

The establishment of specialized genotypes in populations is also complicated by **genetic drift** (Briggs and Walters 1997). This term represents random changes of allelic frequencies during transmission between generations. The occurrence of the same frequency of alleles in new generation is unlikely, so allele frequencies in real populations randomly fluctuate over the generations. Although genetic drift occurs both in small and large populations, in large populations its effect is negligible. In

large populations all alleles are presented in higher numbers, so it is expected that new generation will have similar pattern as parents. In contrast, in small populations many alleles occur in only one copy and may be easily lost. Small and isolated populations also tend to become genetically uniform over time, because after satisfactory generation every allele will be a descendent of just one of the alleles that were present in the ancestral populations. If one allele reaches frequency of 100%, it becomes fixed. Adequately, another allele must be lost, so these populations will be divergent in their allele frequency over time. These processes are random, the loss or fixation of allele is not dependent on their selective advantage, so this process may affect establishment of local adaptation both positively and negatively (Briggs and Walters 1997).

The third factor that influences establishment of local adapted genotypes is **environmental variability over time and space**. If the variabilityin environmental conditions at the localities occur relatively fast compared to the life cycle of the plant species, it is more advantages for plants to be plastic than to develop local adaptations. In this situation is establishment of local adapted genotypes for plant disadvantageous, because there is high probability that offspring will be live in other environmental conditions. Similarly, if the environments vary on small scale, the offsprings will also likely grow in different environments and local adaptations will not be favoured (Brigs and Waltes 1997).

As shown in previous text, plants are able to answer to changes of environmental conditions thanks to phenotypic plasticity (when are not genotypes specialized) or local adaptation (genotypes are specialized to different condition, this specialization is influenced by gene flow, genetic diversity and genetic drift). Distinction of these characteristics is very useful for prediction of plant population ability to acclimate to changes of environmental conditions and also for transfer of our knowledge from one population to another one. Local adaptations on following factors are currently recognised: light density (Chazdon and Pearcy 1991), competition (Fowler 1990), extreme nutrient poor or rich soils (Brady et al. 2005, Knight and Miller 2006, Wright et al 2006), moisture, temperature and elevation (Yamahira and Conover 2005, Broggi 2005), stress (Griffith and Watson 2005, Platenkamp and Shaw 1992), herbivory (Brigs and Walters 1997) and parasitism (Nuismer 2006). However, the evidence of local adaptations to symbiotic organisms is still missing.

1.3. Local adaptation to mycorrhizal fungi?

As shown in chapter 1.1, the importance of arbuscular mycorrhizal (AM) symbiosis for survival and growth of many plant species is generally recognized (e.g., van der Heijden 2002, Jeffries et al. 2003). It has been repeatedly shown that symbiosis with mycorrhizal fungi can increase the fitness of many plant species (e.g., Batty et al. 2001, Khurana et al. 2001, Panwar et al. 2002, McCormick et al. 2004, Pereira et al 2005). This increased fitness is caused mainly by higher uptake of phosphorus and other nutrients (Francis et al. 1986, Van der Heijden et al. 2003) or protection against pathogens (Perumal and Maun 1999, Bever 1994).

Mycorrhizal associations are, however, not always mutualistic. In some cases, this interaction can be marked as parasitism of fungi on their host plants. This type of relationship can be detected especially in situation, when plants are grown in soil with high content of nutrients, specifically phosphorus (Johnson et al. 1997, Ryan et al. 2005). In that case the benefits from mycorrhizal association are lower than the costs (Clapperton and Reid 1992, Al-Karaki 2001). Graham et al. (1991) showed that plant species with low mycorrhizal dependency can better regulate degree of mycorrhizal colonization than plant species with high mycorrhizal dependency. Switching between parasitism and mutualism in plant-AM fungi associations thus seems to depend on plant dependency on mycorrhiza and on nutrient content in the soil.

Most studies evaluating plant response to AMF use plants from a single population. While this may not be a problem for plant species with large, well-connected populations, it can be a problem for species in small and isolated populations (e.g. numerous rare and endangered species – see Chapter 1.2). The isolation resulting in lack of communication between these populations can lead to formation of local adaptations (Briggs and Walters 1997). Many studies have shown local adaptations of plant populations to environmental conditions (e.g., Jordan 1992, Kindell et al. 1996, Galloway and Fenster 2000). The existence of such adaptations in studies on plant-fungal interactions has been, however, largely ignored. This is surprising given both the common knowledge from plant ecology as well as studies

on adaptations of fungal species to different environments (Stahl and Christensen 1991, Hildebrandt et al. 1999).

The variation in response of different genotypes of the same plant species to mycorrhizal colonization was found by Hartnett and Wilson (1993) and Streitwolf – Engel et al. (1997, 2001) for grassland plant species. Similar results were obtained in experiments with agriculture crops (Lackie 1987, Gange et al. 1990, Douds 1993). These studies did not, however, consider the original habitat conditions of the populations. It is, therefore, not known, whether the observed differences are really due to adaptations of these plants to different AMF levels. The study of plant-AMF interactions under original and altered habitat conditions and the study of plant growth with fungi from the same/different localities are necessary steps to explore the adaptations of plants to AMF.

Only few recent studies attempted to study plant adaptations to AMF. Bever (1994) demonstrated negative response of four old-field perennial plant species to inoculation with fungal communities from the original soil in comparison with inoculation with non-indigenous fungal communities. On the contrary, Ronsheim et al. (2001) and Sylvia (2003) showed that plants performed better when inoculated with fungi from the localities of their origin. The only study that looked at the response of plants from different populations to AMF in different soils was that of Schultz et al. (2001). They have shown that populations of *Andropogon gerardii* originating from soils with high content of phosphorus cultivated in the soil with low P content benefited less from mycorrhiza in comparison with plants that originated from localities with low content of phosphorus. Their study, however, compared growth of plants from populations originating from soils with large difference in content of phosphorus and did not look at colonization of plants in the field. The results thus represent a very extreme case using a species with a very wide range of habitats.

Knowledge of such pattern from a narrower range of habitats, more relevant for most rare plant species, is missing. In my **diploma thesis** I explore adaptations of plants to AMF on localities with low but similar content of phosphorus. I used *Aster amellus*, a rare species occurring in fragmented dry grasslands, as a model species and studied six different populations distributed within two different regions.

In the first part I asked how does the response of plants to AMF differ between plant populations and how is it affected by origin of the soil in which plants

are cultivated. I compared patterns of root colonisation of plants grown in greenhouse conditions with plants collected in the field. In this experiment I used only two populations (one from each region). Because the isolations of native fungi is a long-term process, non-native AMF species (from International Bank for *Glomeromycota*) were used for inoculation.

The second part of my diploma thesis concerned the local adaptation of plant populations to their co-occurring AMF species. To answer this question a greenhouse experiment was launched; this time, pre-cultivated native fungal species were used for plant inoculation. In this experiment I asked 3 main questions: whether the species' response to mycorrhiza differs between its populations and depends on soil conditions. Then we estimated the effect of soil, AM fungal isolate and their interaction on local adaptations of the plant.

In the above experiment were used single fungal species as a surrogate for the whole mycorrhizal community. Plant growth in the field, however, depends on the whole mycorrhizal community as well as on other microorganisms in the soil and on abiotic conditions of the localities. Therefore, it is important to know, to what extent does our conclusions on local adaptations derived from the experiment described above hold in the field. We, therefore, explored this issue in non-sterile soil in greenhouse conditions.

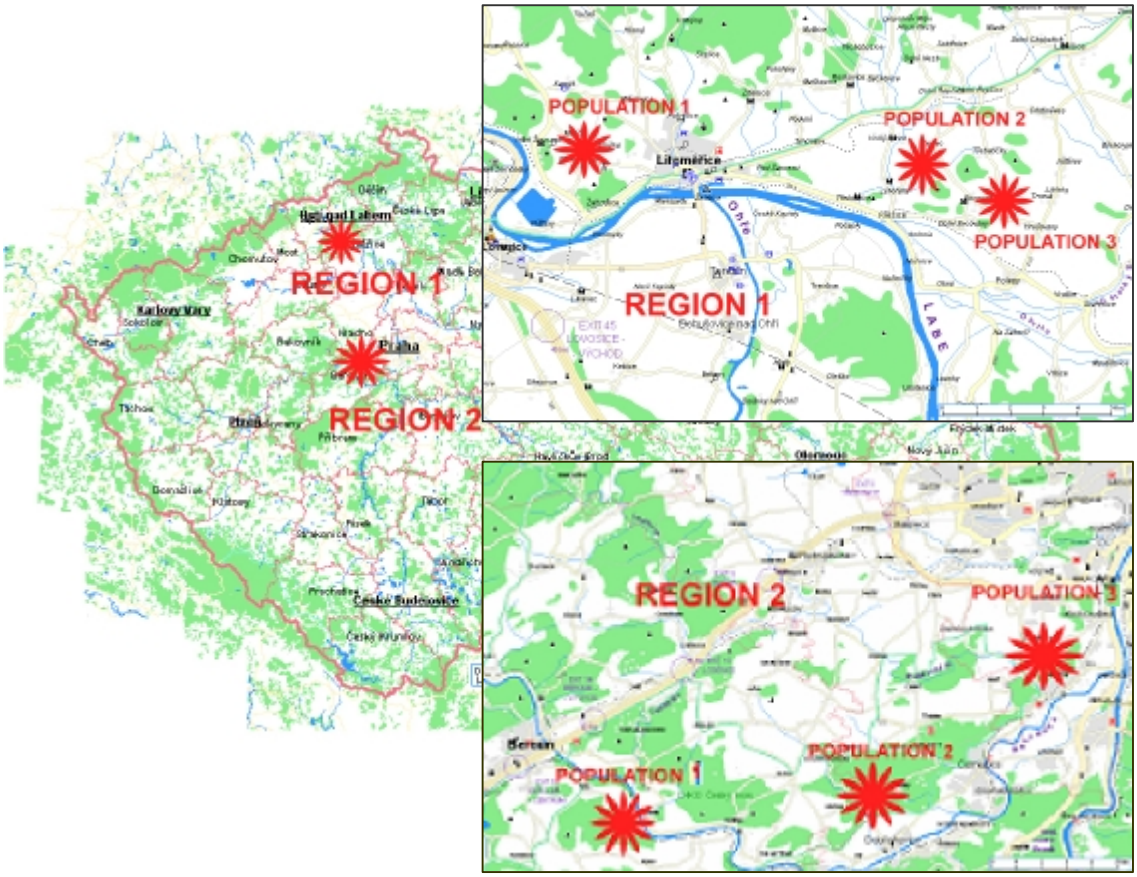
2. Material and methods

2.1 Study species and area

Aster amellus L. (*Asteraceae*) is an endangered perennial self-incompatible plant species (Holub and Procházka 2000, Fig.6), which grows in dry grasslands. This species has been subject of recent studies on Department of Botany and we thus have good knowledge on species population dynamics, population structure and ecology (Mandáková and Münzbergová in press, Münzbergová in press). In the Czech Republic, it occurs in two ploidy levels (Krahulcová 1990). We have, however, selected only diploid populations of the species for this study (based on data of Mandáková and Münzbergová in press).

Figure 6. *Aster amellus L.* (*Asteraceae*), an endangered plant species in the Czech Republic

We studied plants from two different regions, České Středohoří Mts (Fig 7) and Český Kras (Fig 8), in the central part of the Czech Republic. Populations from three localities were selected in each region: Malíč, Encovany and Holý Vrch in České Středohoří and Koda, Karlík and Lochkov in Český Kras (Tab. 2, Map 1).



Map 1.Position of Regions in the Czech Republic and selected population in each Region. Region 1 = České Středohoří, Region 2 = Český Kras. Region 1: Malíč (Population 1), Holý Vrch (Population 2) a Encovany (Population 3). Region 2: Koda (Population 1), Karlík (Population 2) a Lochkov (Population 3)

	Population	Code	Longitude	Latitude
České Středohoří	Malíč	CS1	14°07.8´	50°33.4´
	Encovany	CS2	14°13.3´	50°31.4´
	Holý Vrch	CS3	14°13.4´	50°31.4´
Český Kras	Koda	CK1	14°07.5´	49°56.0´
	Karlík	CK2	14°14.9´	49°56.9´
	Lochkov	CK3	14°20.2´	49°59.9´

Table 2. Geographical position of studied populations.

Here, dry grasslands often constitute small isolated patches. Consequently, plant populations at these localities are also small and isolated. The two regions are about 70 km apart and differ considerably in geology and type of dry grassland communities. Localities in České Středohoří are situated on marl (Studnička 1972) and *A. amellus* grows on open moderate slopes with vegetation belonging to the *Bromion* community; localities in Český Kras are located on limestone and the species grows on steep slopes at openings in oak-hornbeam forests belonging to the association *Querco pubescenti- petraeae* (Moravec 1995). Soils in both regions show lack of nutrients, mainly phosphorus, so the importance of mycorrhizal symbiosis for plant growth at these sites can be expected.

Figure 7. Region 1 (České Středohoří)

Figure 8. Region 2 (Český Kras)

2.2. Chemical analyses of the soils

Because it is generally recognized that importance of AM symbiosis depends on abiotic conditions (Smith and Read 1997, van der Heijden 2002), recording differences in soil conditions among the localities is the first necessary step of the study.

In September 2003, soil samples from all localities were taken (5 samples from each locality, 20 cm in depth, spread over the locality), sieved through the 2 mm sieve and used for chemical analyses. Following parameters were determined: pH, concentrations of Ca, Mg, K (methods of Moore 1986 and Dědina 1987) and available P (methods of Olsen 1954, 1982), total N content and total, organic and carbonate C contents (method ISO/DIS 10693: Soil quality – determination of carbonate content – volumetric method, International Organization for Standardization, 1993). Contents of elementary C and N were determined according to Monar (1972) and Ehrenberger and Gorbach (1972). All these parameters were determined for all soils also after sterilizing by γ-irradiation to confirm that sterilization does not have significant effects on content of nutrients in the soil.

2.3. Mycorrhizal inoculation potential of the soils

To estimate mycorrhizal background on the six sites and determine the potential of the fungal propagules in the soil to induce mycorrhiza I used bioassay approach that reveals amount of AM propagules in non-sterile soil. For determination of mycorrhizal inoculation potential (MIP – ability of soil to initiate mycorrhizal associations), soil from root systems of 10 plants taken at each sites in September 2003 was mixed, homogenized and diluted with autoclaved sand in ratio 1:10, 1:100, 1:1000 (v: v) or left undiluted. Prepared substrates were filled into 380 ml pots. One pre-germinated seed of maize (*Zea mays* L. cv. TATO) was planted into each pot. There were 7 replicates for each dilution series type. This experiment was conducted in a computer-controlled (25 °C day temperature, 21°C night temperature, 14 hours photoperiod) greenhouse for 7 weeks. At the harvest, 15 ml soil core was taken from the centre of each pot; roots were washed and stained as described below.

In all root samples, presence or absence of mycorrhizal colonization was determined under a compound microscope at 100× magnification.

2.4. Mycorrhizal colonization of the roots

To determine root colonization and its seasonal variability, roots of 10 plants from each locality were sampled randomly three times in the vegetation season (June, September and November 2003). Number of sampled plants was a compromise between satisfactory number of replicates and an attempt not to affect the populations. Roots from each plant were carefully removed from soil, washed, cut into segments and stained with 0.05% trypan blue in lactoglycerol (Koske and Gemma 1989). Root mycorrhizal colonization was quantified visually using modified segment method (Giovannetti and Mosse 1980) under a compound microscope at 100× magnification

2.5. First pot experiment

Two model localities, one from each region (CS1, CK1) were selected for the experiment testing whether the differences in mycorrhizal colonization observed previously in the field are caused by plastic response of the plants to environmental conditions or whether the plants are locally adapted to their environment. To do this, seeds from selected localities were combined with soils from these localities and two fungal isolates plus a non-inoculated control treatment in a fully factorial (2×2×3) design. Each treatment had 10 replicates. The soil was sieved through a 2 mm sieve, sterilized by γ-irradiation (25kGy) and filled into 500 ml plastic pots. Seeds of *A. amellus* were collected at each locality. These seeds were rinsed several times with deionized water and germinated in sterilized sand in a greenhouse (day temperature 25°C, night temperature 10°C, 14 hours photoperiod). One 4-week-old plant was transplanted to each pot. AM fungi used in the experiment were two reference AM fungal isolates from The International Bank for the Glomeromycota: *Glomus mosseae* BEG198 and *Glomus intraradices* BEG163. Each inoculated treatment received 5 ml of inoculum consisting of roots, extraradical mycelium (ERM) and

spores of one of the AMF. The inoculum was cultivated on maize in sand for 4 months prior to the experiment. Non-inoculated treatments received the same amount of heat-sterilized inoculum. To regenerate microbial community in sterilized soils, 5 ml of filtrate from non-sterile soil (30 g of soil from each locality was shaken with 300 ml of deionized water for 30 min and filtered two times in order to detain mycorrhizal propagules) was added to each pot. Furthermore, I added to all pots also 5 ml of the inoculum filtrate of both AMF to ensure similar microbial conditions throughout the experiment (5 g of soil was shaken with 150 ml of deionized water and filtered two times).

The experiment was conducted in the greenhouse for 12 months (from December 2003 to November 2004). During the experiment, number of leaves and length of the longest leaf were measured every month. At the harvest, shoot and root dry weights were evaluated after drying to constant weight at 70°C for 72 hours. Content of phosphorus was determined in the aboveground biomass by the method of Olsen. (1982).

Root samples were stained and mycorrhizal colonization was evaluated as described above. The length of ERM was assessed using a membrane filtration technique (Jacobsen et al. 1992). Soil core (15 ml) was taken from the central part of each pot and homogenized by hand. Three g of soil were put to a household blender with 500 ml of deionised water and blended for 30 s. One ml of the supernatant was pipetted onto a nitro-cellulose membrane filter (24 mm diameter, 0.40 μm pore size) and vacuum-filtered. 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× magnifications and expressed in m of hyphae per g of dry soil.

2.6. Isolation of one dominant AM fungus from each population

To capture maximum of fungal species I made trap cultures. These multispore cultures were gained from a mixed sample of soil collected at 5 sampling points placed over the whole locality. Using multispore, rather than single spore, isolates from each locality allowed us to capture the possible variation of fungi at each locality. We consider this approach an efficient solution to the need to take and

compare several single spore isolates from each locality. From each locality we selected one dominant fungal species and keep it in a culture with a host plant. We wanted to have all cultures with *Aster amellus* from their own populations to keep possible local adaptation, but this plant grows very slowly, so it was impossible to use it. Therefore, we decided to use maize *Zea mays* (Fig. 9) and African marigold (Fig. 10), what are in mycorrhizal studies usual host plants. Because gaining a sufficient amount of inoculum is a long process, it was not possible used this native species in the first pot experiment, but they were used in the big pot experiment.

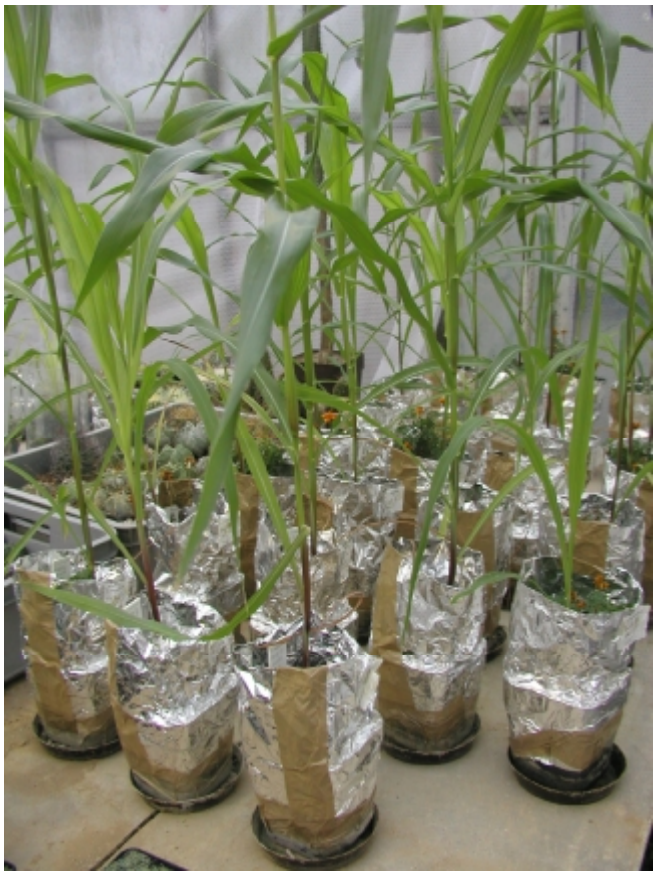


Figure 9. Trap cultures with host plant *Zea mays*

Figure 10. Cultures with host plants African marigold.

2.7. “Big” experiment

In the big experiment we used all 3 populations from each region, which were combined with all soils and with native fungal species isolated from each region. In this experiment we asked to 3 main questions: whether the species’ response to mycorrhiza differs between its populations and depends on soil conditions. Then we estimated the effect of soil, AM fungal isolate and their interaction on local adaptations of the plant.

The experiment is running in greenhouse conditions with plants grown from seeds collected from natural populations. Each treatment has 10 replicates. This number of replicates proved to be sufficient for detecting differences in response of plants to AMF in the pot experiment The soil was sieved through a 2 mm sieve, sterilized by γ-irradiation (25kGy) and filled into 500 ml plastic pots. Seeds of *A. amellus* were collected at each locality. These seeds were rinsed several times with deionized water and germinated in sterilized sand in a greenhouse (day temperature 25 °C, night temperature 10°C, 14 hours photoperiod). One 4-week-old plant was

transplanted to each pot. AM fungi used in this experiment were *Glomus mosseae*, what is multispore isolates from each locality. This inoculum was cultivated on maize in sand for 4 months prior to the experiment. Each inoculated treatment received 5 ml of inoculum consisting of roots, extraradical mycelium (ERM) and spores of one of the AMF. Non-inoculated treatments received the same amount of heat-sterilized inoculum. All soils were reinoculated with its soil microorganism obtained as filtrate from nonsterile soil (30 g of soil from each locality was shaken with 300 ml of deionized water for 30 min and filtered two times in order to detain mycorrhizal propagules). In all pots were added also 5ml microorganism filtrate from fungi inoculum (5 g of soil was shaken with 150 ml of deionized water and filtered two times), so difference between treatments will be caused only by fungal isolates and soil characteristics.

In the first comparison, we determine mycorrhizal responsiveness of plants from each of the six local populations when grown in their original soils and inoculated either with the original AM isolate or isolates originating from the other localities or non-inoculated. We thus plan to combine 6 plant populations grown in their original soils with 7 inoculation treatments (non-inoculated, inoculated with 6 different *G. mosseae* isolates, Task 1 in the Table 3).

Further we tested whether the response of plant populations to inoculation with their original AM isolate is related to soil conditions. In this part we grown each plant population with its own AM isolate and combine it with soils from all localities (Task 2 in the Table 3).

For answer to response of the plant populations to different complex of AM isolates plus soil we combined each plant population with each isolate in the AM isolate's original soil (Task 3 in Table 3).

	Each combination of corresponding	Combined with	No of treatments
Task 1	Plant population + Soil	All AM isolates + non-inoculated	
Task 2	Plant population + AM isolate	All soils	102
Task 3	Soil + AM isolate	All plant populations	

Table 3**.** Summary of treatments in Tasks 1, 2 and 3. Each treatment is replicated 10 times.

Figure 10.”Big” experiment at their beginning in summer 2006.

The experiment was established in summer 2006 and we plan to end it after flowering, so it is still running. During the experiment, number of leaves, length of the longest leaf and wide of the longest leaf are measured every month. After harvest we plan to evalued shoot and root dry weights after drying to constant weight at 70°C for 72 hours. In the aboveground biomass we will also determined content of phosphorus. We will also measure root colonization and length of ERM by methods described in the pot experiment.

2.8. Experiment in non-sterile soil

In this experiment we follow the fate of plants of the 6 target populations grown in non-sterile soils from all the six localities. The results of this experiment allow answering the question, whether the importance of local adaptation of plants to soil and fungi concluded from the big experiment can be confirmed if we use the whole

soil community. Similar experiment was performed also by Jana Raabová in the field.

This experiment thus helps to increase the realism of the conclusions on local adaptations in the system. It is important that in our big experiments we reinoculated the sterile soil with its soil microorganisms. The microbial conditions of the soils will be, therefore, the same in all experiments (and very similar to the field soil) and the difference between results of this experiment and the big experiments can be interpreted as due to one fungal species as compared to the whole fungal community. Soil microorganisms are, therefore, treated as part of the soil throughout the whole study.

For this experiment we used sieved mixed soil from each locality. In the experiment we combined all 6 populations with all 6 soils in full factorial design. This experiment will be evaluated in the same way as the big experiment and it was established also in summer 2006, so it is still running.

2.9 Statistical analysis

Differences in content of nutrients in the soil were tested using nested ANOVA. Independent variables were region and population nested within region; dependent variable was content of nutrients.

Data from the assessment of mycorrhizal inoculation potential of the soils were analysed using logistic regression. Dependent variable was presence of mycorrhiza in the pot; independent variables were regions, dilution, population nested within region and their interactions.

The differences in root colonization in the field were tested using nested ANOVA. Independent variables were region, population nested within region, time of sampling and their interactions.

Growth of plants in all pot experiment was analysed using repeated measures ANOVA. Length of the longest leaf was used to describe plant growth in this case (this parameter best correlated with biomass at the end of the experiment, $R^2 = 0.54$). We also performed in all experiments the analysis using number of leaves. Since the results of the analysis based on number of leaves were similar to those based on leaf length, only the latter are shown in the results. Independent variables were soil

origin, population and fungi in inoculated experiments and population and fungi in experiment with non-sterile soil.

After one month of the experiment, the data were used also to test differences in growth between inoculated and non-inoculated treatments in the first pot experiments. It was not possible to analyse these data in the subsequent censuses since most of the non-inoculated plants died within few months.

At the harvest, differences in shoot and root biomass, content of phosphorus in aboveground biomass, root colonization and length of the ERM were tested using ANOVA as well.

The data of the big experiment were divided to three parts testing effect of fungal isolates, soil and population (see Task 1, 2 and 3 in Table3). The dependent variables were number of leaves and length of the longest leaf. Since the results of the analysis based on number of leaves were similar to those based on leaf length, only the latter are shown in the results. The analysis was done using repeated measures ANOVA with several hierarchical levels. The error level for each tested term is in Table 4 using the analysis of effect of soil as an example.

Tested factor	Error level
Region of soil	Soil
Region of PF	PF
PF	Soil x PF
Soil	Soil x PF
Time	Residuals
Region of soil x Region of PF	Soil x PF
Soil x PF	Soil x PF x Time
Time x Region of soil	Soil x Time
Time x Region of PF	Time x PF
Time x Region of soil x Region of PF	Soil x PF x Time
Soil x PF x Time	Residuals

Table 4. The error level for each tested term in the tests of effest of the soil on the length of the longest leaf. PF=complex population and fungi. Similar error levels were used in tests of effect of population and fungi.

Similar repeated measures ANOVA with several hierarchical levels was used to analyse of results from experiment with non-sterile soil. The error level for each tested term is in Table 5.

Tested factor	Error level
Region of soil	Soil
Region of population	Population
Population	Soil x population
Soil	Soil x population
Region of soil x region of population	Soil x population
Soil x population	Residual

Table 5. The error level for each tested term in the tests of effest of the soil on the length of the longest leaf.

To gain normality, data on percentage root colonization in the field and in the experiment were arcsin transformed and data on length of the longest leaf, root biomass and length of the ERM were logarithmically transformed. All the analyses were done using S-plus (S-PLUS 2000). All graphs were drawn using program Statistica (StatSoft 1998).

3. Results

3.1. Chemical analyses of the soils

Our results show that there were no differences between sterilized and non – sterilized soils in all determined parameters (data not shown). Chemical analyses of the soils showed low content of phosphorus in both regions. There were no significant differences in C total and C/N ratio between the two regions, but there were significant differences between localities within the regions. There were significant differences between regions as well as between localities within regions in all other chemical characteristics. Soils from Český Kras had significantly lower pH, higher content of phosphorus and other nutrients (Ca, Mg, N and K; Table 6). Soils CK1 and CS1, which were used in the pot experiment, significantly differed in pH and content of all nutrients except for phosphorus. Content of Ca was very high in all soils but soil from CK1 had significantly lower values than CS1. Soil CK1 had significantly higher content of Mg, K and N than soil CS1.

Region	České Středohoří			Český Kras		
Localities	CS 1	CS 2	CS 3	CK 1	CK 2	CK 3
pH (KCl) *	7.3	7.5	7.3	4.5	5.7	7.4
pH(H2O) *	7.9	8.1	7,8	5.44	6.2	7.7
Ca (mg/g) *	8.3	9.4	9.7	4.7	5	10.9
Mg (mg/1000g)*	75	37	80	191	738	299
K (mg/1000g) *	168	83	180	271	217	283
P (mg/1000g) *	7.5	7.7	8.5	6.7	11.1	18.8
N (%)*	0.2	0.1	0.3	0.4	0.5	0.5
C Total (%)	8.2	8.3	8.9	6.6	6.5	10.2
C. Carbonate (%)*	6.2	6.4	5.2	0.02	0.03	3.0
C organ (%)*	2.0	1.9	3.7	6.5	6.5	7.3
C/N	9.9	15.7	14.1	15.0	14.2	13.6

Table 6. Chemical characteristics of the soils. * - significant differences between regions ($p<0.001$, Df error = 24, Df = 1), analyzed using nested ANOVA. There were significant differences between localities within region in all cases ($p<0.001$, Df error = 24, Df = 4).

3.2. Mycorrhizal inoculation potential of the soils

Mycorrhizal inoculation potential (MIP) did not significantly differ between regions, but there was marginally significant difference between single localities within regions. There were no interactions between region or locality within region and dilution (not shown). Roots were colonized by AMF also in dilution 1:1000, so MIP was very high.

3.3. Root colonization in the field

Root colonization differed significantly between regions, but not between single populations within each region. It varied between the three sampling periods and there was also a significant interaction between population and time and region and time (Table 7). Plants from the region České Středohoří had significantly higher colonization (about 85%, Fig. 9) as compared to plants from Český Kras (about 10%, Fig. 10 and Fig. 11). In both regions, the highest root colonization was detected in spring for all populations. In summer, the colonization decreased, in autumn, further decrease of colonization was found at localities CS1 and CK1 (Fig. 11).

	df	df Error	F	p-level
Sampling date	2	162	6.58	**<0.001**
Region	1	162	172.99	**<0.001**
Locality within region	4	162	12.31	**<0.001**
Sampling date × region	2	162	0.12	0.89
Sampling date × locality within region	8	162	1.15	0.33

Table 7. Effect of sampling date (spring, summer, autumn), region and locality within region on root colonization of plants in the field analysed using ANOVA. Significant effects are in bold.

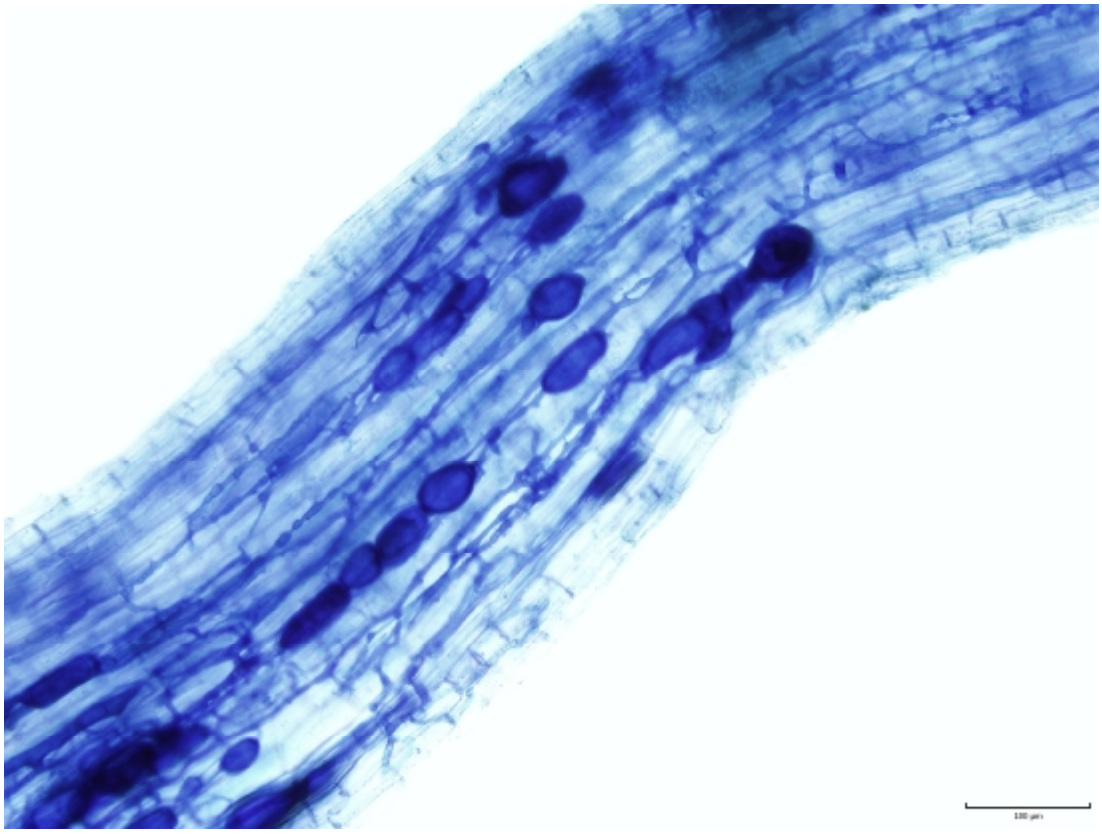


Figure 9. Colonization of the roots from Region 1 (České Středohoří)

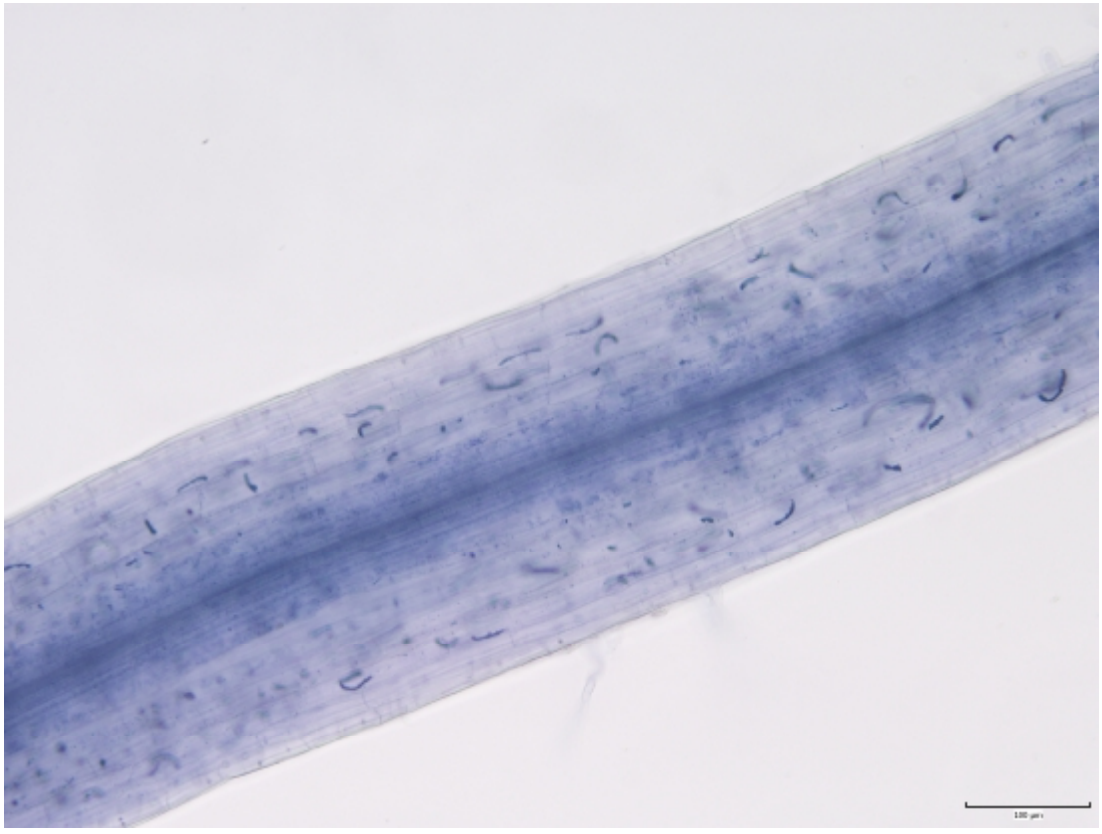


Figure 10. Colonization of the roots from Region 2 (Český Kras)

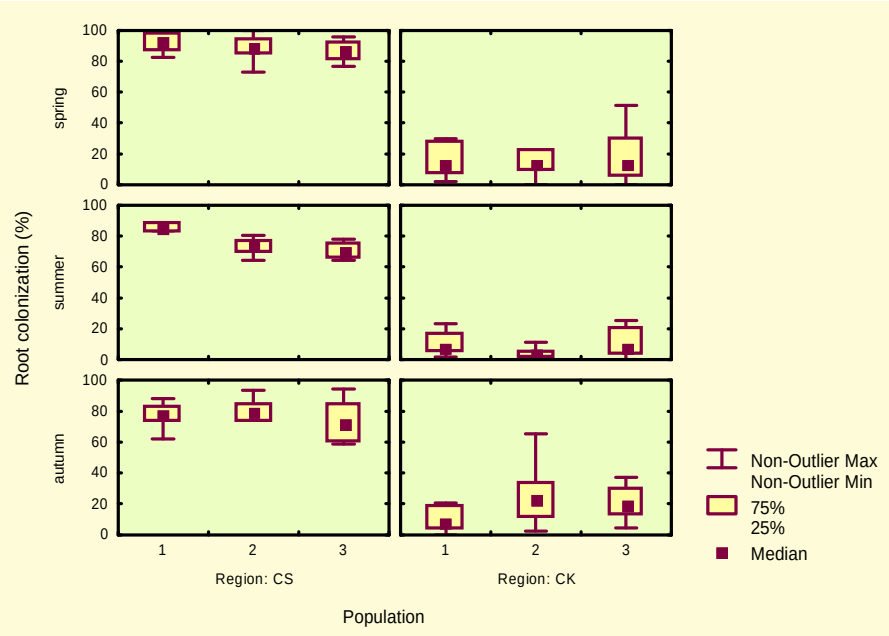


Figure 11. Seasonal changes of root colonization of plants from three populations from each of the two regions (CS – České Středohoří, CK – Český Kras). There were significant differences between populations over time and between regions. Table 4.

3.4. Pot experiment

After 1 month of growth, inoculated plants were significantly larger than non-inoculated plants (Table 8, Fig. 13). There were no significant differences in the length of the longest leaf between populations, but there was a significant difference between soils. Also there was a significant interaction between soil and inoculation; no other interactions were significant (Table 8).

Figure 12. Pot experiment – selected plants after 4 month of growth. S=soil, P=population, Gi= *Glomus intraradices*, Gm=*Glomus mosseae*, Nm=non-inoculated control

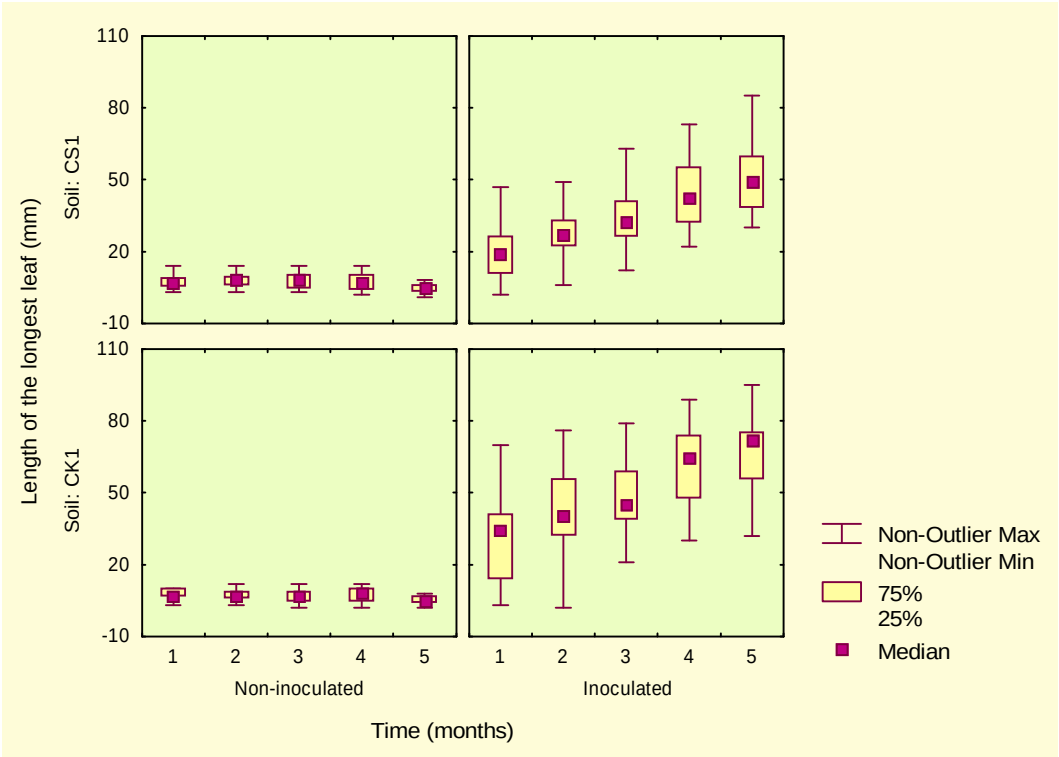


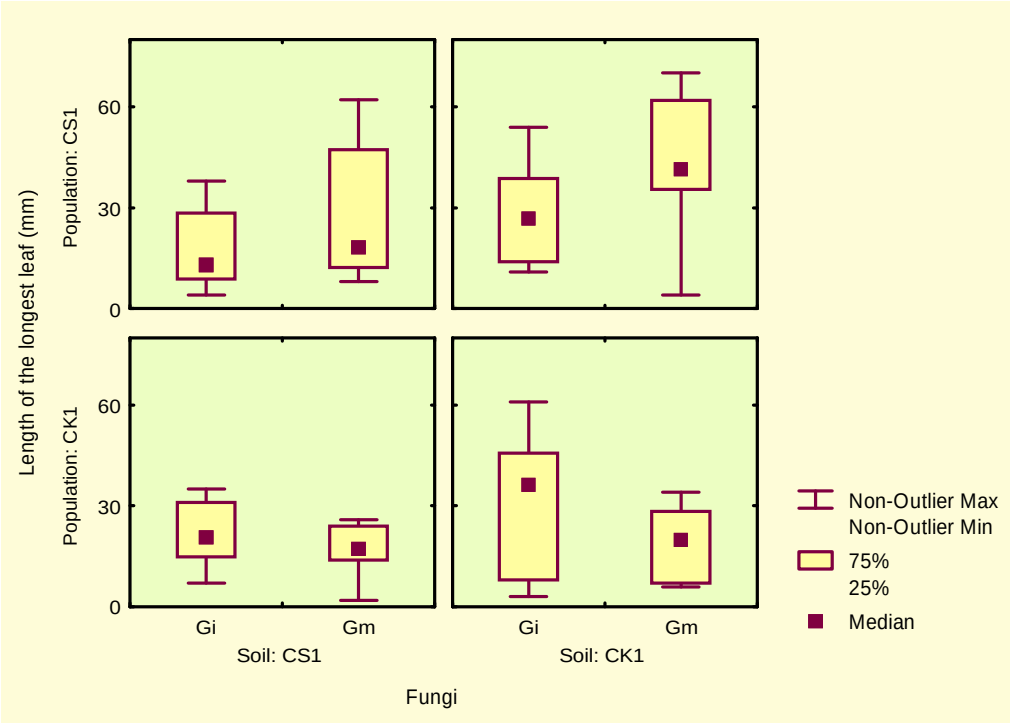
Figure 13. Effect of inoculation with AMF on the growth of *Aster amellus* in a pot experiment. Data are summarized over both plant populations and both AM fungi. There were significant differences between inoculated and non-inoculated plants

	df	Df Error	F	p-level
Soil	1	50,00	22,94	**<0.001**
Population	1	50,00	0,97	0,33
Fungi	1	50,00	0,03	0,86
Time	9	450,00	142,91	**<0.001**
Soil × Population	1	50,00	0,25	0,62
Soil × Fungi	1	50,00	0	0,99
Population × Fungi	1	50,00	5,78	**0,02**
Soil × Time	9	450,00	2,71	**<0.001**
Population × Time	9	450,00	0,37	0,95
Fungi × Time	9	450,00	0,49	0,88

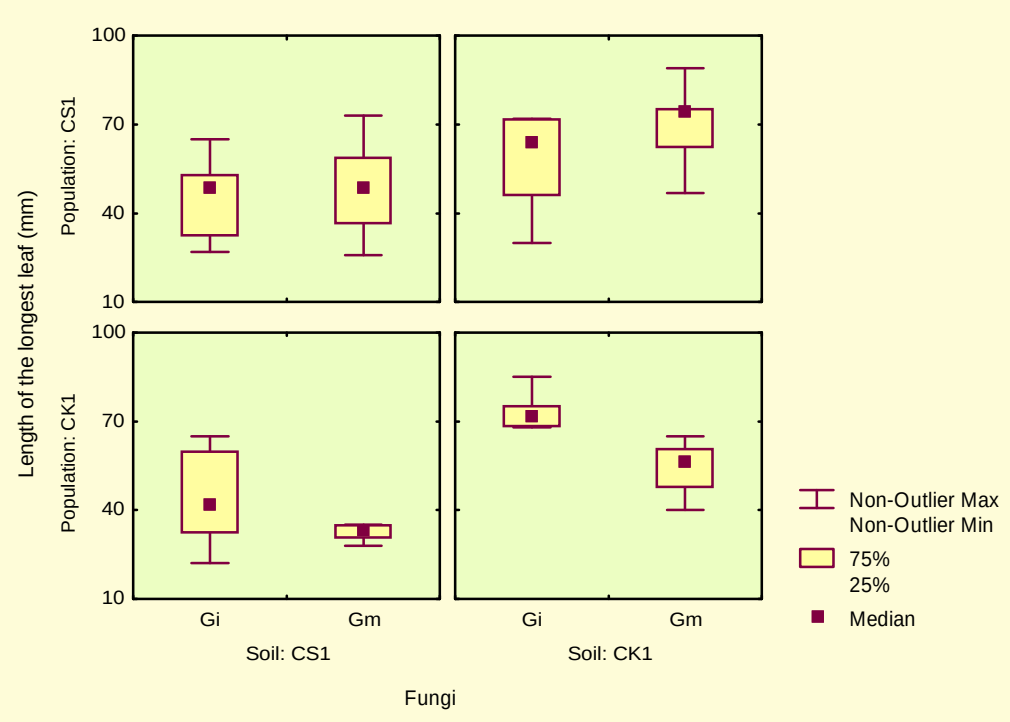
Table 8. Effect of soil, plant population, fungal isolate, time and their interactions on the length of the longest leaf of plants during the pot experiment (repeated measurements ANOVA). Significant are in bold.

Results of the analysis comparing only inoculated plants throughout the whole experiment showed significant differences in plant growth between soils (Table 8). Plants grown in CK1 soil were significantly larger as compared to plants cultivated in CS1 soil (Fig. 14a, b, c). There was no significant effect of population and AMF isolate on plant size, but there was a significant interaction between these factors. Plants from CK1 population grew better after inoculation with *Glomus intraradices* while growth of CS1 plants was better supported by *G. mosseae*. There was also a significant effect of the time and an interaction between soil and time. Plants grown in the soil CK1 grew faster than plants grown in the soil CS1. There was also a significant interaction between fungi, population and time (Table 8).

a)


Length of the longest leaf (mm)
Population: CS1
Population: CK1
60
30
0
Gi
Gm
Soil: CS1
Soil: CK1
Fungi
Non-Outlier Max
Non-Outlier Min
75%
25%
Median

b)


Length of the longest leaf (mm)
Population: CS1
Population: CK1
100
70
40
10
Gi
Gm
Soil: CS1
Soil: CK1
Fungi
Non-Outlier Max
Non-Outlier Min
75%
25%
Median

c)

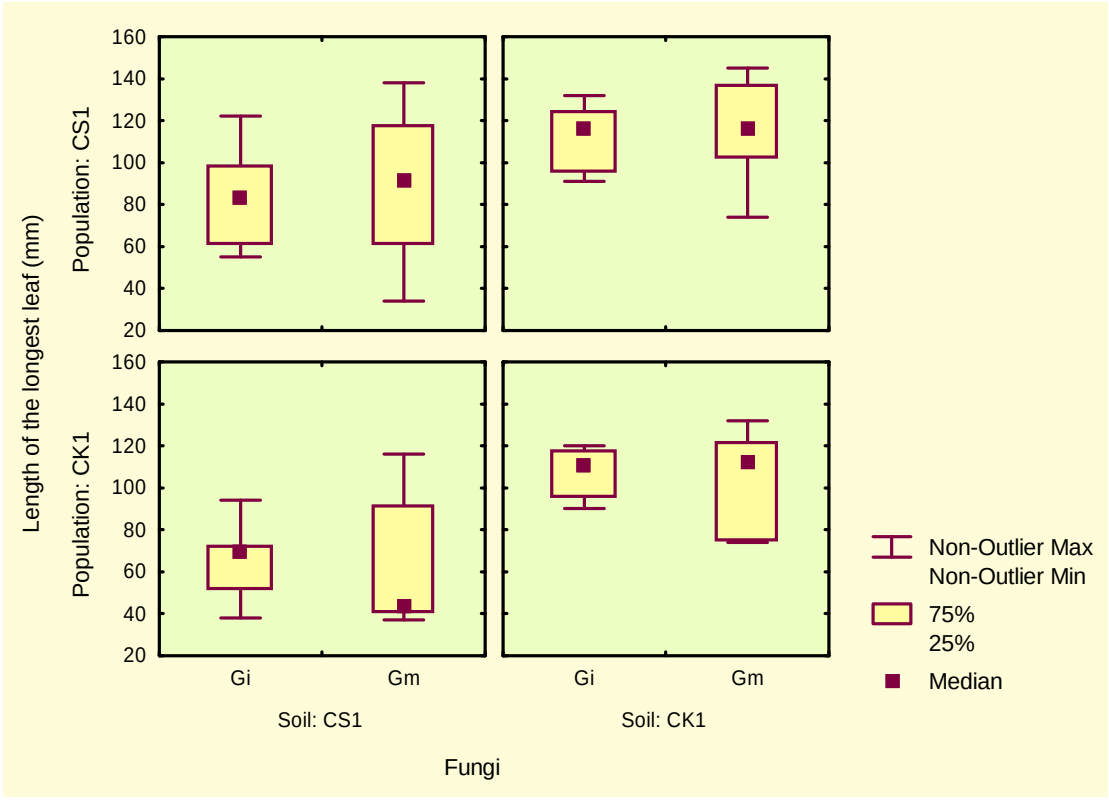


Figure14. Effect of soil, fungal isolate and plant population on length of the longest leaf after one month (a), five months (b) and 12 months (c) of plant growth in a pot experiment. CS1 – České Středohoří - Malíč, CK1 – Český Kras – Koda; Gi – G*lomus intraradices*, Gm – G*lomus mosseae.* There is significant effect of soil (a, b, c) and population (c). Table 8.

At the end of the experiment, aboveground and belowground biomass of plants differed significantly between soils (Table 9). Larger shoot and root biomass was observed in plants growing in CK1 soil (Fig. 15 a, b). There were, however, no significant effects of population and no significant interactions on aboveground and belowground biomass (Table 9).

Because of no differences in aboveground biomass between AMF, I determined content of phosphorus only for treatments inoculated with *Glomus mosseae*. The results showed that plants grown in the soil CK 1 have higher concentration of phosphorus in the aboveground biomass ($p= 0.051$, $F= 4.16$, $df=1$, df error=26). There was no significant effect of population or interaction on content of phosphorus in the aboveground biomass.

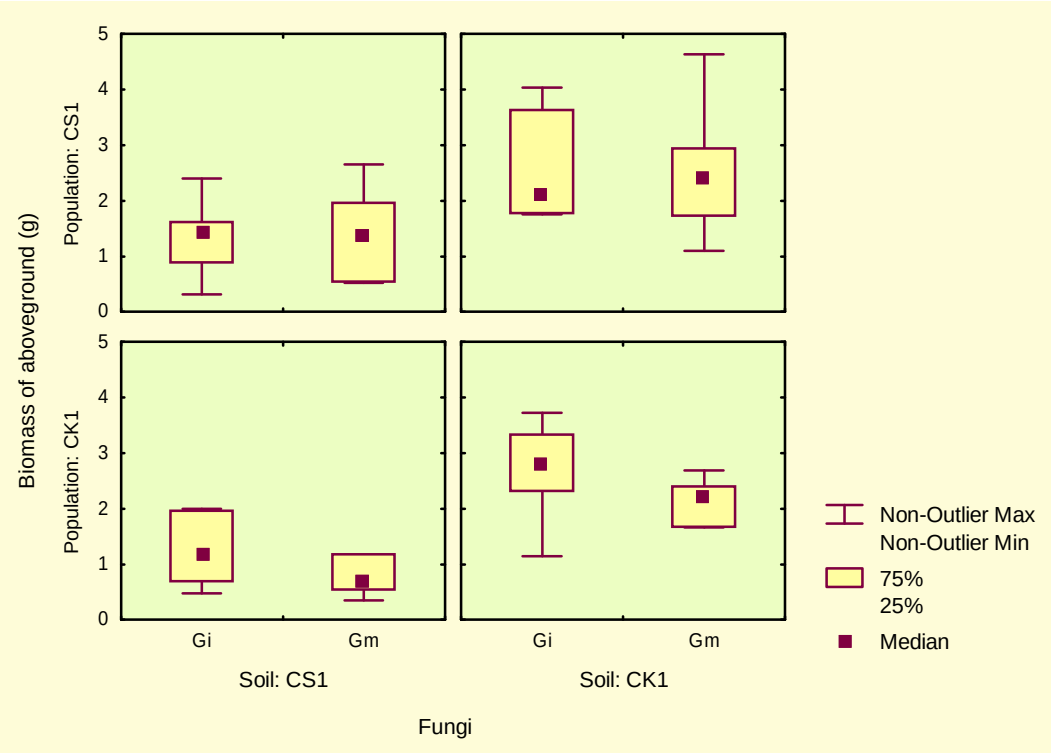
Soil and population significantly affected root colonization. There was also a significant interaction between population and soil (Table 9). Significantly higher colonization was found in roots of plants from CS1 population and plants grown in CS1 soil (Fig. 15c). Furthermore, root colonization of plants from CK1 increased when they were grown in CS1 soil. Root colonisation of CS1 plants was almost the same in both soils (Fig. 15c).

There were significant differences in length of ERM between soils and fungal isolates, but not between plant populations. Better development of ERM was observed in soil CK1 and in treatments inoculated with *G. mosseae* (Table 9, Fig. 15d).

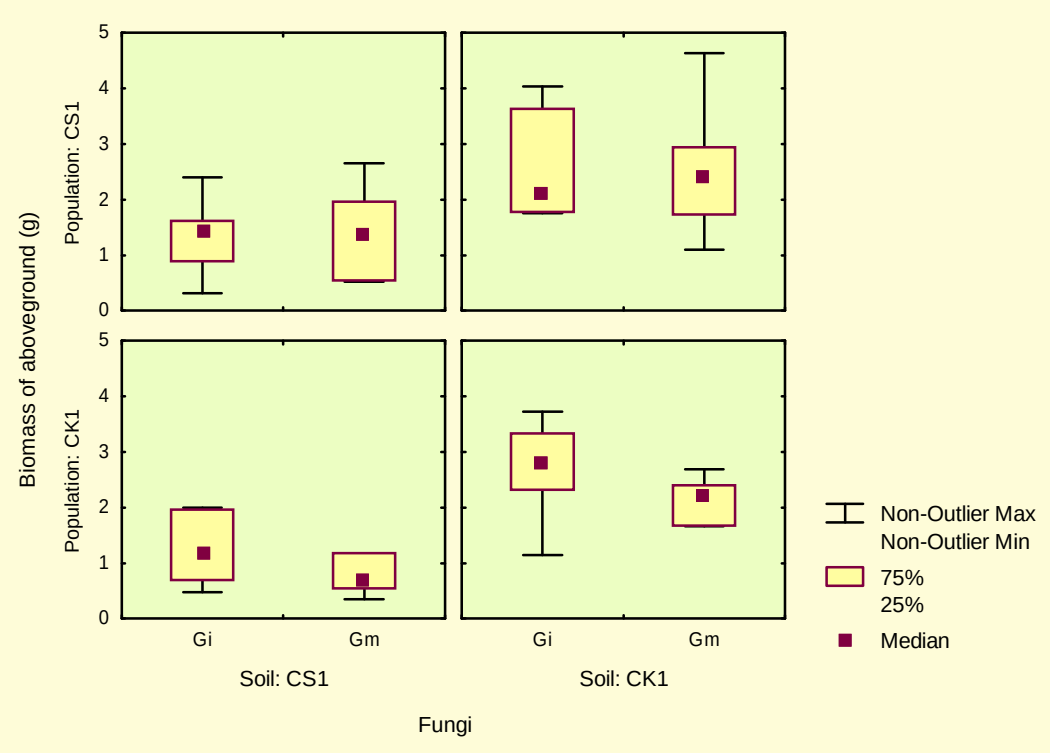
	Aboveground biomass		Belowground biomass		Root colonization		Length of ERM	
	F	p-level	F	p-level	F	p-level	F	p-level
Soil	25.60	**<0.001**	15.12	**<0.001**	14.05	**<0.001**	19.36	**<0.001**
Population	1.52	0.223	0.019	0.89	85.24	**<0.001**	1.74	0.192
Fungal isolate	2.01	0.161	0.76	0.387	1.49	0.228	16.53	**<0.001**
Soil × population	0.02	0.878	0.36	0.55	6.05	**0.017**	1.81	0.184
Soil × fungal isolate	0.25	0.62	0.01	0.902	0.35	0.556	1.71	0.196
Population × fungal isolate	0.76	0.388	0.55	0.461	0.52	0.474	2.03	0.161

Table 9. Effect of soil, plant population and fungal isolate and their interactions on aboveground and belowground biomass, root colonization and length of ERM at the end of the pot experiment estimated using ANOVA. Df error was 51 and Df effect was 1. Significant effects are in bold.

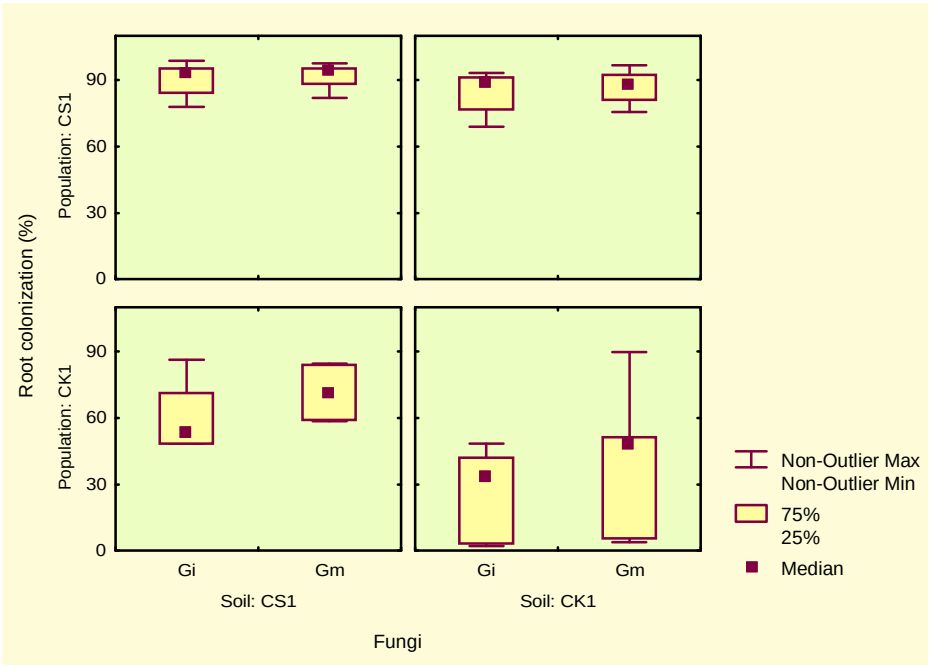
a)


Biomass of aboveground (g)
Population: CS1
Population: CK1
0
1
2
3
4
5
Gi
Gm
Soil: CS1
Soil: CK1
Fungi
Non-Outlier Max
Non-Outlier Min
75%
25%
Median

b)


Biomass of aboveground (g)
Population: CS1
Population: CK1
0
1
2
3
4
5
Gi
Gm
Soil: CS1
Soil: CK1
Fungi
Non-Outlier Max
Non-Outlier Min
75%
25%
Median

c)



d)

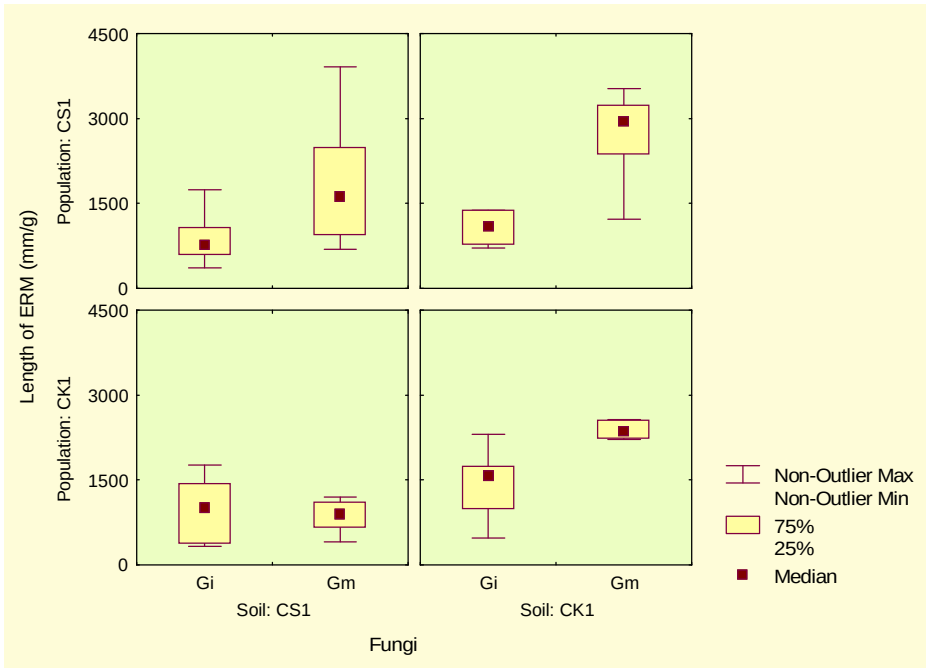
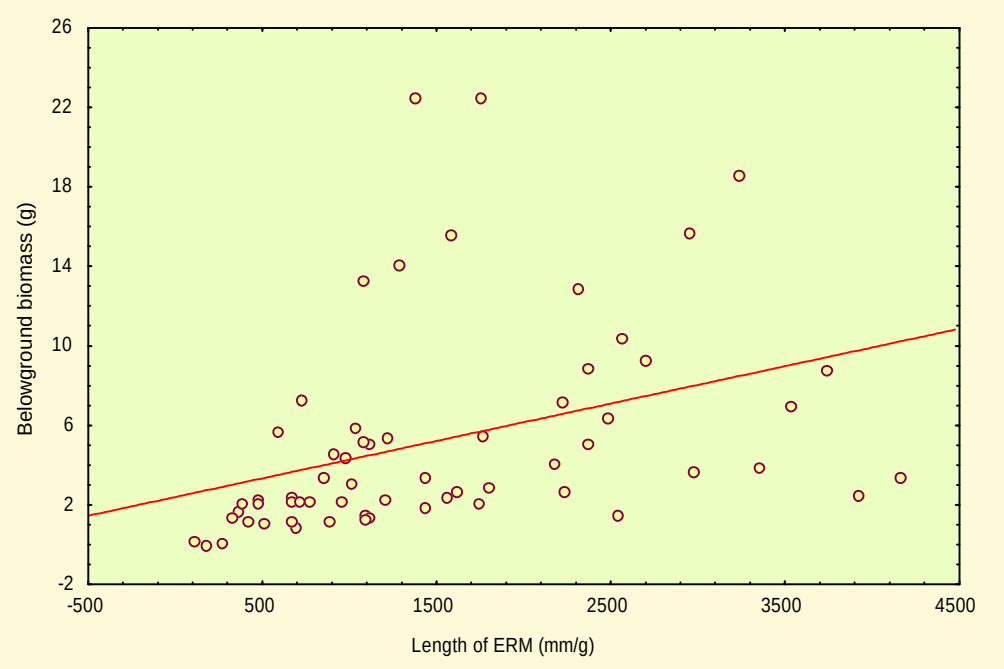


Fig. 15. Effect of soil, fungal isolate and plant population on aboveground biomass (a), belowground biomass (b), root colonization (c), and length of ERM (d) in et the end of the pot experiment. CS1 – České Středohoří - Malíč, CK1 – Český Kras - Koda; Gi – *Glomus intraradices*, Gm – Glomus mosseae. There were significant differences between soils (a, b) and fungal isolates. Table 6.

There was significant positive correlation of ERM length and aboveground ($p<0.01$, $R^2=32.1\%$, Fig 16a) and belowground ($p<0.01$, $R^2=14.9\%$, Fig.16b) plant biomass. There was no correlation between concentration of phosphorus and aboveground biomass ($p=0.32$), root colonization ($p=0.73$) and length of the ERM ($p=0.93$).

a)



b)

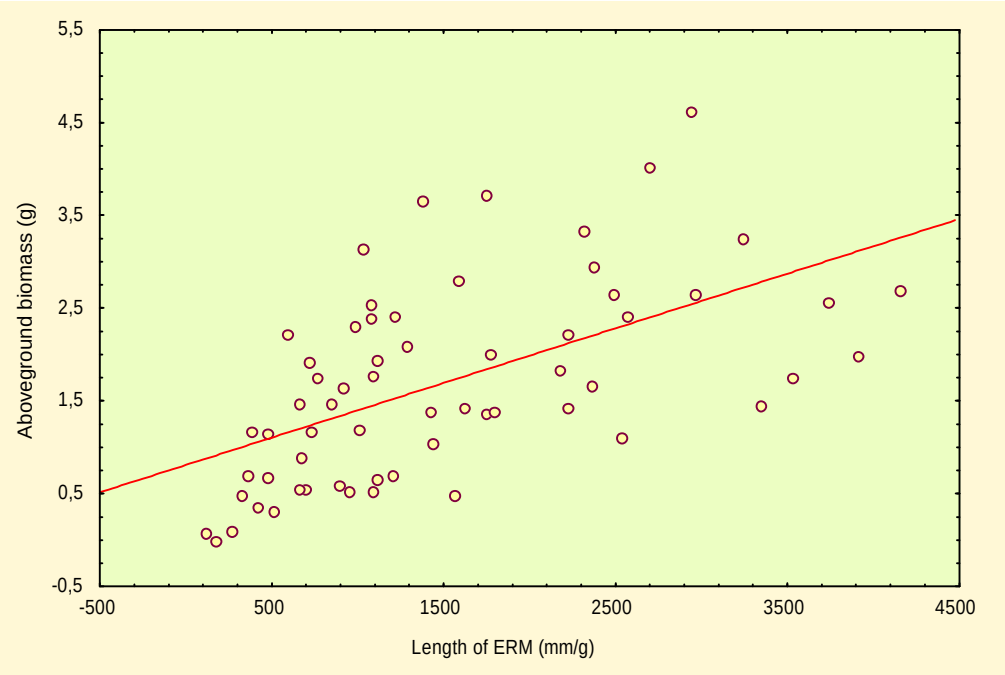


Figure 16. Dependency of belowground (a) and aboveground (b) biomass on the length of ERM at the end of the pot experiment. Both dependency were significant.

3.5. "Big" experiment

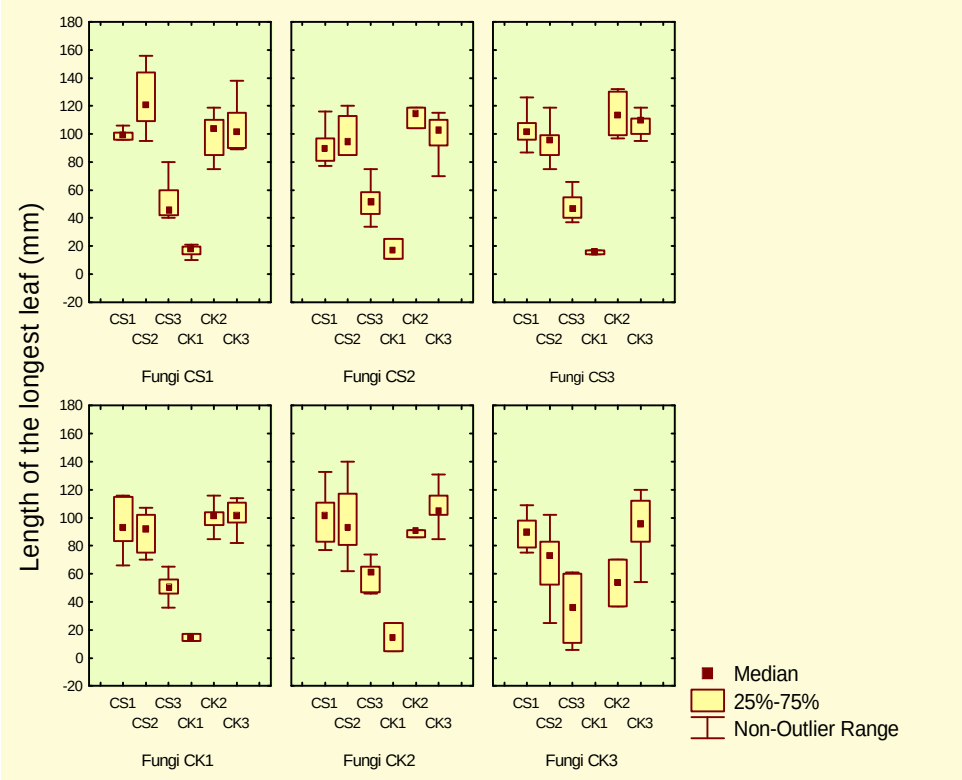
After 1 month of growth, inoculated plants were significantly larger than non-inoculated plants (Fig.17b).

In the first comparison, I determined mycorrhizal responsiveness of plants from each of the six local populations when grown in their original soils and inoculated either with the original AM isolate or isolates originating from the other localities. Our results showed significant effects of fungi, time and complex population and soil on length of the longest leaf. There was also significant interaction between fungal isolates and complex population-soil. There were no differences between regions (Tab. 10, Fig. 17 a,b)

	Df	Df Error	F	p
Region of PS	1	4	0,08	0,79
Region of fungi	1	6	0,21	0,66
Fungi	6	29	8,27	**<0.001**
PS	4	29	35,63	**<0.001**
Time	1	1482	3301,02	**<0.001**
Region of PS × Region of fungi	1	29	0,01	0,90
Fungi × PS	29	24	6,17	**<0.001**
Time × Region of PS	1	4	0,01	0,91
Time × Region of fungi	1	4	1,08	0,36
Time × Fungi	4	24	3,20	**0,03**
Time × PS	4	24	121,40	**<0.001**
Time × Region of PS × Region of fungi	1	24	0,06	0,81
Time × Fungi × PS	24	1482	1,13	0,30

Table 10. Effect of region, fungi, time, complex population-soil (PS) and their interaction on the length of the longest leaf during the pot experiment (repeated measurements ANOVA with hiererchical design). Significant values are in bold.

a)



b)

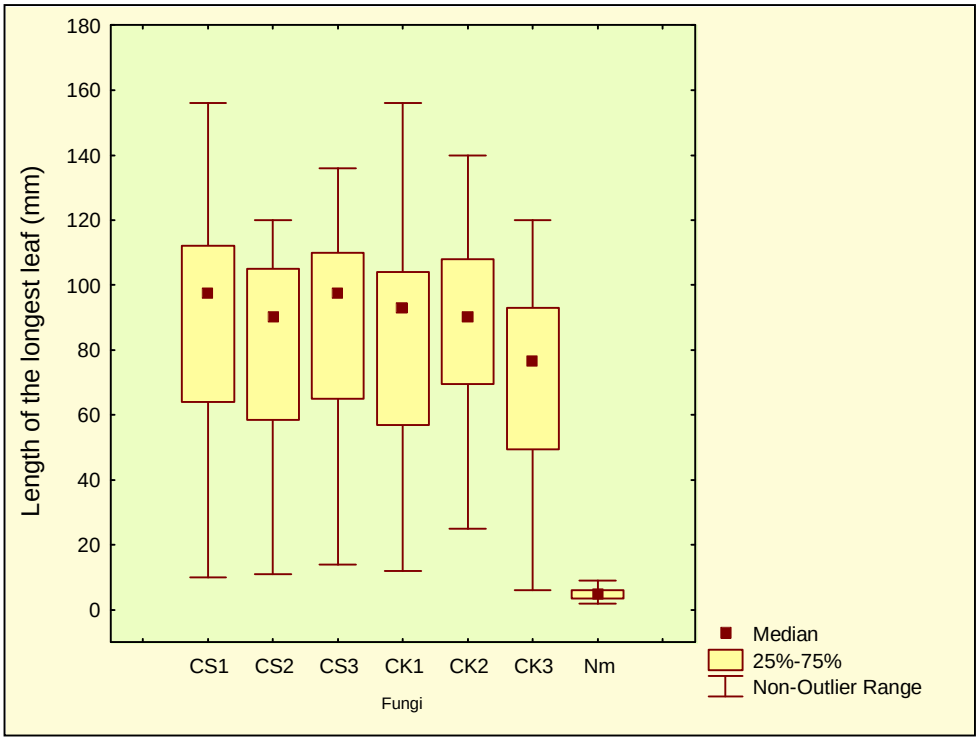


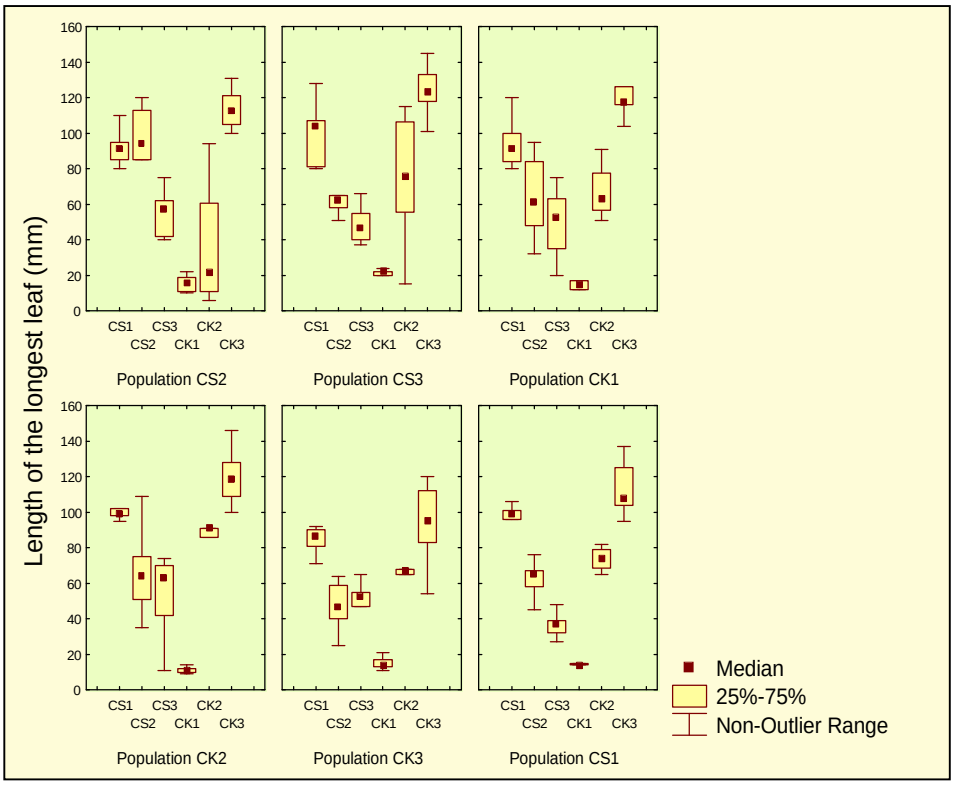
Figure 17. Effect of AMF origin on the length of the longest leaf for each complex population-soil (a) and for all complexes population-soil confided (b). Data after 5 months of growth. CS= Region České Středohoří (1=Malíč, 2=Holý Vrch, 3=Encovany), CK=Region Český Kras (1=Koda, 2=Karlík, 3=Lochkov)

To study the response of plant populations to different complex of AM isolates plus soil I tested plant population inoculated with each isolate in the AM isolate's original soil (complex soil-fungi). The results showed significant differences in length of the longest leaf between complex soil-fungi (SF), and time. There was also significant interaction between population and SF, SF and time, SF and population and time (Tab.11 and Fig. 18 a, b).

	Df	Df error	F	p
Region of SF	1	4	0,07	0,81
Region of population	1	5	0,01	0,92
Population	5	23	1,72	0,17
SF	4	23	14,84	**<0.001**
Time	1	1319	986,85	**<0.001**
Region of SF × Region of population	1	23	3,19	0,09
Population × SF	23	23	2,62	**0,01**
Time × Region of SF	1	4	0,03	0,88
Time×	1	5	0,09	0,78
Population × Time	5	23	1,05	0,41
SF × Time	4	23	8,54	**<0.001**
Region of SF × Region of population × Time	1	23	2,05	0,17
Population × SF ×Time	23	1319	12,16	**<0.001**

Table 11. Effect of region, time, complex soil-fungi (SF), population and their interaction on the length of the longest leaf during the pot experiment (repeated measurements ANOVA with hierarchical design). Significant values are in bold.

a)



b)

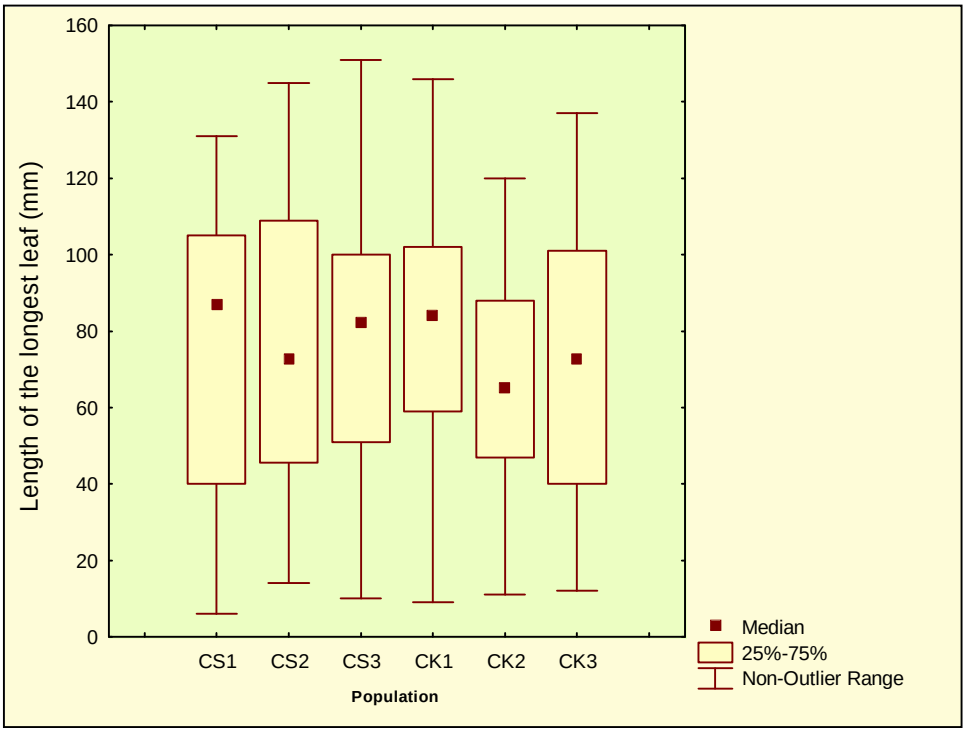


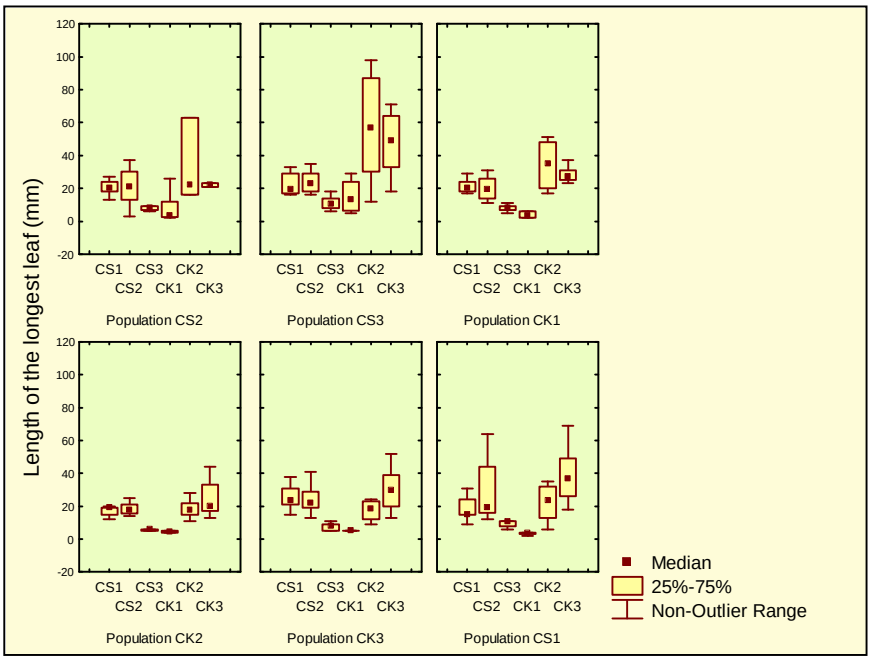
Figure 18. Effect of population on the length of the longest leaf compared between complexes soil-fungi (a) and compared over all fungi and soils (b). Data after 5 months of growth. CS= Region České Středohoří (1=Malíč, 2=Holý Vrch, 3=Encovany), CK=Region Český Kras (1=Koda, 2=Karlík, 3=Lochkov)

Further I tested whether the response of plant populations to inoculation with their original AM isolate is related to soil conditions. The results showed that there was significant effect of complex population-fungi (PF), time and soil. There were also significant interactions between soil and PF, soil and time. Plants in the soil CK1 grew very slowly independent on fungal or populations origin. Similar results were also observed in soil CS3 (Tab.12, Fig. 19 a, b).

	Df	Df Error	F	p
Region of soil	1	5	0,10	0,76
Region of PF	1	5	0,11	0,76
Soil	5	24	46,97	**<0.001**
PF	5	24	4,16	**0,01**
Time	1	1248	1242,57	**<0.001**
Region of soil x Region of PF	1	24	0,09	0,76
Soil x PF	24	19	2,36	**0,03**
Time x Region of soil	1	4	0,21	0,67
Time x Region of PF	1	5	5,02	0,08
Soil x Time	4	19	39,57	**<0.001**
PF x Time	5	19	0,92	0,49
Time x Region of soil x Region of PF	1	19	1,57	0,23
Soil x PF x Time	19	1248	1,54	0,06

Table 12. Effect of region, time, complex population-fungi (PF), soil and their interaction on the length of the longest leaf during the pot experiment (repeated measurements ANOVA with hierarchical design). Significant values are in bold.

a)



b)

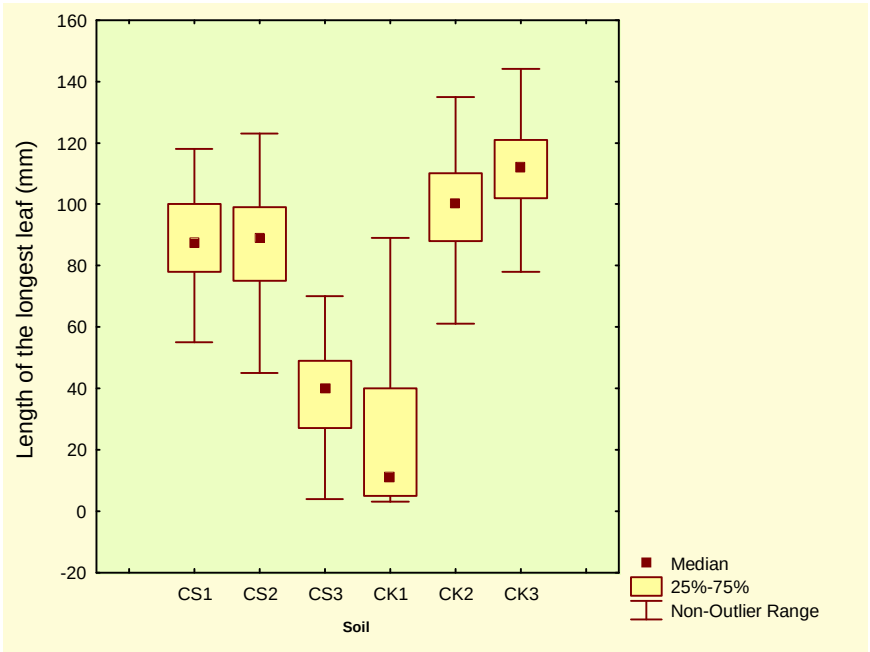


Figure 19. Effect of soil on the length of the longest leaf compared between complexes population-fungi (a) and compared over all fungi and populations (b). Data after 5 months of growth. CS= Region České Středohoří (1=Malíč, 2=Holý Vrch, 3=Encovany), CK=Region Český Kras (1=Koda, 2=Karlík, 3=Lochkov)

3.6. Experiment in non-sterile soil

Results from experiment with non-sterile soils show significant effect of soil. All population grew very slowly when grown in soil from Region CS. This result was observed also in the firs pot experiment and particularly in the big experiment (Tab. 13, Fig. 20).

	Df	Df Error	F	p
Region of soil	1,00	4	25,53	**0,01**
Region of population	1,00	5	5,48	0,07
Soil	4,00	20	15,67	**<0.001**
Population	5,00	20	2,15	0,10
Region of soil x Region of population	1,00	20	2,88	0,11
Soil x Population	20,00	147	1,06	0,40

Table 13. Effect of region, soil and population on length of the longest leaf during the pot experiment (repeated measurements ANOVA with hierarchical design). Significant values are in bold.

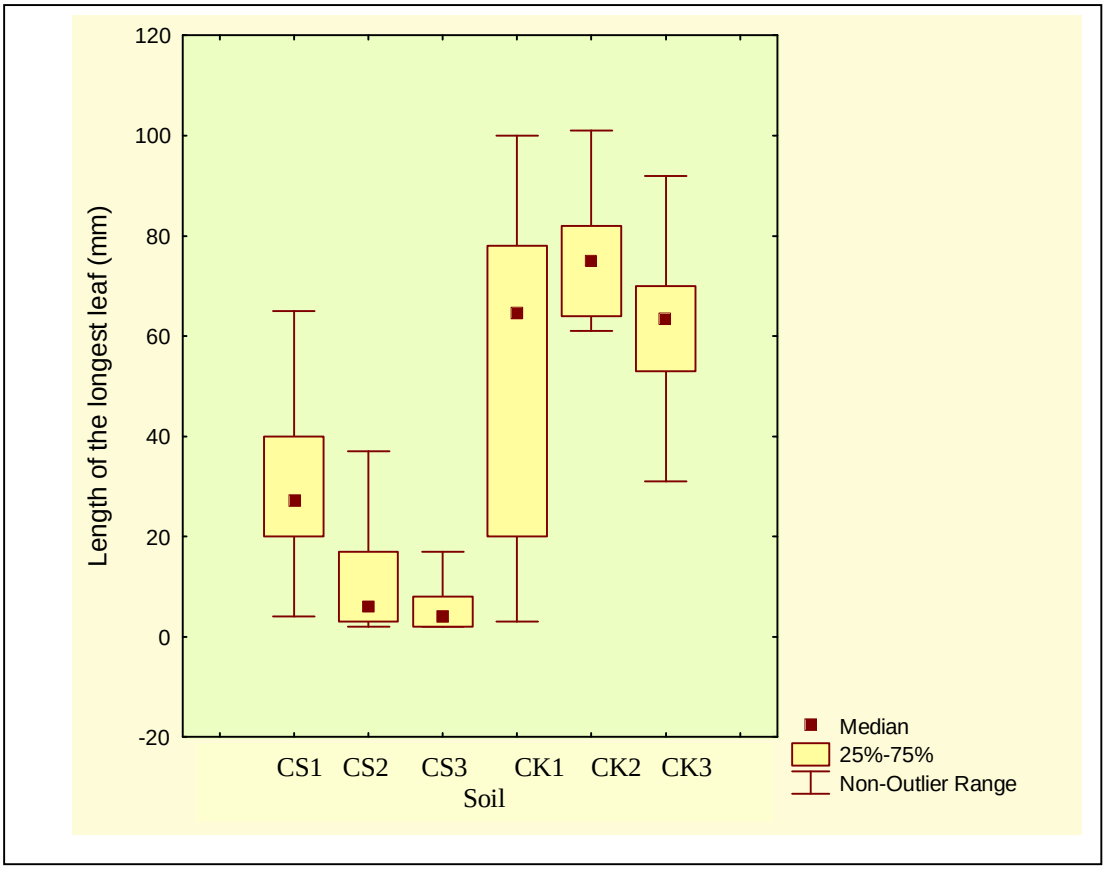


Figure 20. Effect of soil on the length of the longest leaf. Data after 2 months of growth. CS= Region České Středohoří (1=Malíč, 2=Holý Vrch, 3=Encovany), CK=Region Český Kras (1=Koda, 2=Karlík, 3=Lochkov)

4. Discussion

The results of my study show that populations of *Aster amellus* are adapted to different levels of mycorrhizal colonization. This contrasts with conclusions of Schulz et al. (2001), who did not find significant effect of plant population on root colonization. The study of Schulz et al. (2001) is the only study looking on adaptation of plants to soil conditions mediated by AM fungi. However, they did not assess plant colonization in the field. Furthermore, they studied a species growing in soils with very wide range in content of phosphorus. Their results thus represent a very extreme case using a species with a very wide range of habitats. Our study thus documents for the first time existence of local adaptation of plant population to AM colonization.

Results of our first pot experiment suggest that *Aster amellus* is an obligate mycotrophic plant species with high dependency upon mycorrhiza as all plants in non-inoculated treatment died within a few months. Similar result was found only in few studies. Janos (1980) postulated that some highly mycotrophic plants could not grow without mycorrhiza. For example, *Anthyllis cytisoides* did not grow without mycorrhizal associations (Diaz et al. 1996) and *Dicornia guianensis* was unable to absorb phosphorus in the absence of mycorrhizal association (de Grandcourt et al. 2004). Dry biomass of *Aristida longiseta* growing in sterilized soil was reduced by 93 % and plant mortality dramatically increased compared to non-sterilized treatments (van Auken and Brown 1998).

The conclusion that *Aster amellus* is an obligate mycotrophic plant species apparently does not correspond with our observation of low colonization (5 %) of CK1 plants in the field. Obviously such colonization is sufficient to establish effective mycorrhizal association. Positive effects of low colonization on plants growth were recognized by van der Heijden (2001) on *Salix repens* (colonization lower than 5%) or by Fay (1996) on *Hordeum vulgare*. Some other *Asteraceae* plants (e.g. *Artemisia ludoviciana, Aster ericoide, A. laevis, A. sericeus, Solidago canadensis*), which are very dependent on mycorrhizal symbiosis, have root colonization about 30 % (Wilson 1998).

Our results on root colonization in the field showed strong differences between plants from different regions. One of the examples showing similar results is that of Van Aarle et al. (2003) who compared root colonization of *Plantago lanceolata* growing on acid soil and on limestone and found significantly lower root colonization (5 – 20 %) in the acid soil as compared to limestone (30 – 60 %). A possible explanation of differences in root colonization between plants from different regions could be differences in mycorrhizal inoculation potential of the soils. However, mycorrhizal inoculation potential was very high for all soils and there was no significant relationship between this potential and root colonization in the field. Thus MIP was not probably a limiting factor for development of mycorrhizal symbiosis at the localities with observed low root colonization.

Difference in root colonization could be also due to difference in nutrient soil content. Soils from Český Kras were more acid and had higher content of nutrients. They also supported better development of ERM. More ERM may mean better acquisition of nutrients for the plants. Thus plants from Český Kras seemed to have better conditions for more effective growth (Laing et al. 2000) and could form less mycorrhiza. Better growth conditions in soil from Český Kras were confirmed also in the pot experiment: plants grown in soil from CK1 (Český Kras) had significantly larger aboveground and belowground biomass as compared to CS1 (České Středohoří). Better growth conditions in soils from Region 2 (CK) are also supported by my preliminary results from experiment with non-sterile soils and particularly from big experiment. In the big experiment plants grown in CK1 soil were, however, very small. This lower growth may be due to changes of physical properties of the soil during long-term storage.

To decide, if observed difference in root colonization in the field could are a result of plastic response of plants to the environment or a result of their local adaptations (Briggs and Walters 1997) I performed the first pot experiment. Results of the experiment confirmed significant effect of population on root colonization. There was also a significant interaction between population and soil. Plants from population CS1 grown in the soil with higher content of nutrients (soil CK1) did not decrease their mycorrhizal colonization and the root colonization was thus much higher than that of plants from CK1 in the same soil. In such a case, I would suspect that the AMF might behave as parasites in CS1 population and have thus negative effect on plant growth. Surprisingly, there were no differences in plant size between

the two populations in this soil. One could speculate that this might be due to balance of negative and positive effect of AMF on plant growth.

Agreement between root colonization in the field with the result of the pot experiment suggest that differences between populations are not caused only by plastic response of plants to soil conditions of the localities but also by real adaptations of plants from different populations to different level of mycorrhizal colonization.

One of the weak points of the first experiment is that I did not use native fungal isolates but rather species from a laboratory collection. While I recognize that it would be better to use the native fungal species, obtaining the native fungal cultures is very time consuming and I were not able to get them for the first experiment. Using non-native fungi could be problematic mainly if inoculation with different fungal species resulted in different colonization of the roots, and if there were different dominant fungal species on the different localities. In that case colonization pattern observed in the field could differ from the patterns observed in the greenhouse not due to the absence of adaptations but rather due to the differences in fungal identity between field and the experiment. The same could happen if there was the same species at all localities but different from that used in the experiment, and there was an interaction between population and fungal species. The fact that we found correspondence between colonization patterns in the field and in the greenhouse and did not find any strong differences between the two fungal isolates studied, however, suggests that this is not a major issue of our dataset.

To tested possibilities of local adaptation of plant population to AMF, I repeated the pot experiment with all 3 populations from each region, their soils and indigenous fungal isolates. Due to the long process of fungal isolation, the experiment was started in the spring 2006, so the results are not complete yet. Preliminary results indicate that there are differences in growth between different fungi, soil and populations. There is, however, no agreement in conclusion between populations within regions.

Conclusions

Our results show significant differences in the degree of mycorrhizal colonization between plant populations of the same species from different regions. The AMF proved to be decisive for successful growth of the studied species; none of

the tested populations was able to grow without AMF. In the pot experiment, a significant effect of plant population and soil on mycorrhizal colonization was found. The correspondence between mycorrhizal colonization of plants in the field and in both soils in the pot experiment demonstrates that the observed differences in root colonization are really genetically based and the plants are thus adapted to a certain level of root colonization.

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