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Arbuscular mycorrhizal symbiosis of herbaceous invasive neophytes in the Czech Republic

Arbuskulární mykorhizní symbióza bylinných invazních neofytů České republiky

Kateřina Štajerová[*], Marie Šmilauerová & Petr Šmilauer

Faculty of Science, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic, e-mail: s.dracek@seznam.cz, majka@prf.jcu.cz, petrsm@jcu.cz
[*]*Present address: Department of Invasion Ecology, Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 43 Průhonice, Czech Republic*

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Arbuscular mycorrhizal symbiosis is the most frequent and ancestral type of mycorrhizal symbiosis. It is estimated that at least 80% of terrestrial plant species are able to form a mutualistic relation with fungi. Consequently in the context of successful plant invasions, arbuscular mycorrhizal fungi may have a favourable if not a crucial role. The mycorrhizal status of 23 invasive species is reported here for the first time. This study also tested whether the intensity of mycorrhizal colonization of the roots of invasive species is related to that of the dominant species of invaded plant community. This is partly supported by our results when total percentages of mycorrhizal colonization were compared. In addition, the effect of habitat and community characteristics on the intensity of colonization of the roots of invasive species by arbuscular mycorrhizal fungi was tested and several significant correlations were revealed. At the among-species level, the total mycorrhizal colonization decreases and the relative arbuscular colonization increases in the roots of invasive species with increasing nitrogen availability in the habitat. Both these relations are significant after phylogenetic correction, which suggests this is an evolutionary adaptation. There are also negative correlations between the relative arbuscular colonization of invading species and the light and temperature demands of the species present in the community, and a positive correlation between the relative arbuscular colonization of the invaders and soil wetness. That all these relations are revealed at the within-species level possibly reflects differences among the habitats studied.

Keywords: alien, arbuscular mycorrhiza, dominant species, habitat, light, moisture, nitrogen, non-native, temperature

Introduction

With the growing movement of world biota, many plant species establish themselves far outside their native ranges. In some cases, these species become invasive, crucially affecting native species and their communities (Drake et al. 1989, di Castri et al. 1990, Pyšek et al. 1995, Chytrý et al. 2005), often resulting in economic losses and a reduction in biodiversity (Mooney & Hobbs 2000, Pyšek et al. 2006, Richardson & Pyšek 2006, Stohlgren et al. 2006). The seriousness of this problem demands a thorough understanding of the factors that influence the process of invasion.

Much of the work focuses on the aboveground rather than belowground level despite these two subsystems being closely interlinked (Wardle et al. 2004). However, several recent studies have demonstrated that the role of belowground biota in invasions by exotic plants cannot be overlooked, in particular, the role of arbuscular mycorrhizal fungi, hereinafter referred to as AMF (Richardson et al. 2000a, Stampe & Daehler 2003, Callaway et al. 2004b, Wolfe & Klironomos 2005, Koske & Gemma 2006, MacKay & Kotanen 2008).

Arbuscular mycorrhiza (hereinafter AM) is the most ancestral and the commonest type of mycorrhizal symbiosis (Brundrett 2002), in which the fungal hyphae penetrate the cortical cell wall of the host plant root. It is characterized by the arbuscules and vesicles formed by the aseptate, obligately symbiotic fungi of the phylum *Glomeromycota* (Schüßler et al. 2001). The nature of interaction between the plant and these fungi can vary from mutualistic to parasitic (Johnson et al. 1997) and depends on the genotype of both partners (Klironomos 2003). A considerable number of studies indicate that the intensity of colonization by AMF can be influenced by various environmental factors such as light availability, temperature, moisture, soil pH and availability of nitrogen (Smith & Read 1997, Jakobsen et al. 2002, Rillig et al. 2002, Apple et al. 2005).

AMF help plants to acquire nutrients such as phosphorus, nitrogen, potassium and zinc from the soil in exchange for photosynthates (organic carbon) supplied by the host plant (Smith & Read 1997). AMF also protect their host plants against attack by soil-borne pathogens (Azcón-Aguilar & Barea 1996) and have a direct effect (positive or negative) on the growth and biotic interactions of plant species (van der Heijden et al. 1998).

It is estimated that more than 80% of terrestrial plant species, including invasive ones, have a symbiotic relation with mycorrhizal fungi (Wang & Qiu 2006). Moreover, the ubiquity, cosmopolitism and low host-plant specificity of many species of AMF provide a good opportunity for invasive plants to form a mycorrhiza in their new ranges (Richardson et al. 2000a). Therefore, arbuscular mycorrhiza can be expected to have a favourable effect on the process of plant invasion. On the other hand, such an effect is probably not so crucial or ubiquitous. Pyšek (1998) records that of the six plant families to which most of the invasive plants belong four are non-mycorrhizal families: *Amaranthaceae*, *Brassicaceae*, *Chenopodiaceae* and *Polygonaceae*.

Invasive mycorrhizal plants do not have a competitive advantage over native species, unless they utilize the mycorrhizal symbiosis in an unusual way (Richardson et al. 2000a). Such a mechanism can determine their success and affect the resident plant community and ecosystem functioning (Callaway et al. 2004a). For instance, some invasive plant species are capable of influencing the network of hyphae of the AMF in the soil with which they are associated and those of native species, through earlier root activity or greater provision of carbon (Hawkes et al. 2006).

While mycorrhizal species might be prominent among invasive plants, absence of a dependence on mycorrhiza seems to be advantageous in particular cases. The African fynbos is an example of a habitat invaded by non-mycorrhizal alien species. Its extremely poor soils are unsuitable for most introduced plants that form associations with AMF (Allsopp & Stock 1993, Allsopp & Holmes 2001). In the temperate zone most non-mycorrhizal species are expected to prefer disturbed sites, where there is a low density of mycorrhizal propagules, e.g. ruderal sites or dumps (Gange et al. 1990). On the other hand, few non-mycorrhizal invasive species are able to establish themselves in later successional stages. Such a species is the garlic mustard, *Alliaria petiolata*, which has successful invaded mature temperate forest in North America. This species seems to profit from its ability to decrease the abundance and function of AMF communities in the soil by means of exudations (Roberts & Anderson 2001, Stinson et al. 2006, Burke 2008).

Based on empirical and experimental data, Urcelay & Díaz (2003) present a simple conceptual model for predicting the response of diversity of natural plant communities (with different degrees of mycorrhizal dependence and positions in the dominance hierarchy) to AMF. They hypothesize that this response is determined by an interaction between the mycorrhizal dependence of subordinate and that of the dominant species. This model inspired us to predict a positive relationship between the intensity of colonization by AMF of native dominants of plant communities and that of invaders, because invading plant species, at least at the beginning of the invasion process, are subordinate species in these plant communities.

The aims of the study were (i) to provide information about the mycorrhizal status of 44 invasive species of herbaceous plants in Central Europe, (ii) to compare the intensity of the mycorrhizal colonization of the roots of invasive species with that of the dominant species in invaded plant communities, and (iii) to assess which habitat characteristics correlate with the intensity of colonization by AMF of the roots of the invasive species.

Materials and methods

Target species and site selection

This study focused on neophyte species (species introduced after 1500 A. D., see Richardson et al. 2000b, Pyšek et al. 2004 for definitions), which have already invaded a substantial range of habitats in the Czech Republic (based on Pyšek et al. 2002). Forty four invasive species of herbaceous neophytes were selected, including two lianas, but excluding post-invasive species, which used to be invasive but have now ceased spreading, and water macrophytes. The roots of the selected species were collected from all over the Czech Republic in 2007 and 2008 (Fig. 1).

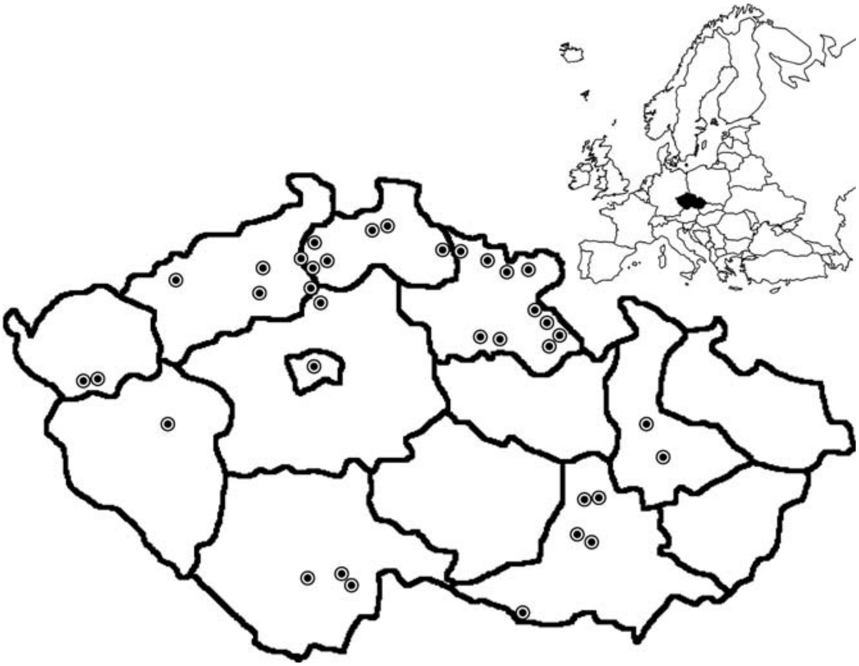

Fig. 1. – Map of the Czech Republic with the sampling sites indicated.

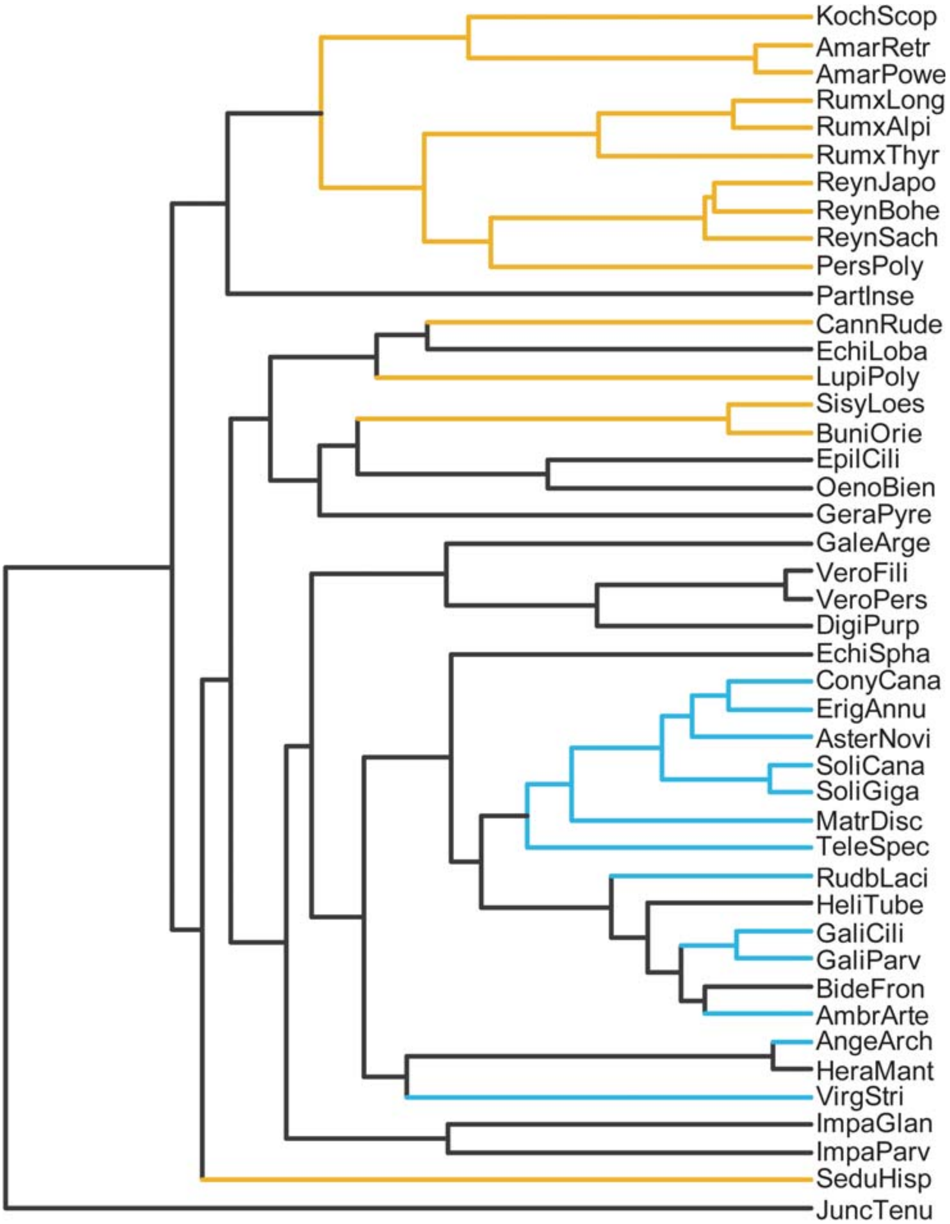

Fig. 2. – The phylogenetic tree of the invasive neophytes studied based on data in the BiolFlor database (Klotz et al. 2002). Abbreviations of the species names are based on the first four letters of both the genus and species (see Table 2 for full species names). Colours: yellow, average total colonization of the species by AMF not higher than 1%; black, average between 1 and 20%; blue, average at least 20%.

Common species were sampled mainly in the vicinity of large cities and on route to the sites of less abundant species. Localities of less abundant species (e.g., *Cannabis ruderalis*) were found using Hejný & Slavík (1988, 1990, 1992), Slavík (1995, 1997, 2000), Slavík & Štěpánková (2004), Mlíkovský & Stýblo (2006) and information supplied by local botanists. Characteristics of these sites, such as sampling date, locality, altitude, geographic location and habitat type are summarized in Electronic Appendix 1.

Sample collection and processing

At each site, root samples of three individuals of both the invasive neophyte and the dominant native species were collected for assessing their colonization by AMF. The total number of samples processed in this study was 798. As far as possible the roots sampled were from adult plants at the flowering stage, because AMF development in host roots depends on the host species phenology (Šmilauer 2001). The six plants sampled at each locality were chosen at random.

In addition, a phytosociological relevé was made at each locality, using a plot size of 16 m^2. Abundance and dominance of each species were estimated visually, using a percentage scale (1–100%).

Plant species nomenclature follows Kubát et al. (2002). The taxon labelled as *Aster novi-belgii* agg. in this study includes all invasive *Aster* species occuring in the Czech Republic (Pyšek et al. 2002), namely *A. lanceolatus*, *A. novi-belgii*, *A.* ×*salignus* and *A. versicolor*.

Community composition recorded in the phytosociological relevés was used to estimate site characteristics (based on the Ellenberg indicator values characterizing ecological preferences of individual species, Ellenberg 1992), community diversity (Shannon-Wiener index) and richness, using the software Turboveg (Hennekens & Schaminée 2001, Hennekens 2008) and JUICE (Tichý 2002, Tichý & Holt 2006).

Ellenberg indicator values (Ellenberg et al. 1992) are designed to characterize the habitat demands of Central European vascular plants and are used here to describe the habitat conditions at the sampled sites. For a summary of the Ellenberg indicator values used in the present study, see Table 1.

Root samples were washed free of soil and at least 10 fine roots were stained with Chlorazol Black E following the standard method described by Vierheilig et al. (2005), with the following modifications: clearing 16 hours in 10% KOH at room temperature, neutralized in 3.5% HCl for 2 minutes, stained using 0.03% w/v of Chlorazol Black E in lactoglycerol (14:1:1 lactic acid, glycerol and deionized water) in a 90°C water bath for an hour and destained in lactoglycerol for several days in a refrigerator.

For each sample, about 10 stained root fragments, each approximately 1.5 cm long, were randomly selected and mounted on slides in destaining solution (lactoglycerol). These semi-permanent slides of stained roots were sealed with nail polish and examined later.

They were examined at magnifications of 200× and 400× for the presence of arbuscules in order to distinguish AMF from other, especially non-mycorrhizal fungal assemblages present in the root. The intensity of colonization by AMF was then assessed at a magnification of 100× by estimating the percentage of root length with AMF structures (namely arbuscules, vesicles and hyphae) within each visual field.

The percentage of the root length colonized was estimated for the whole root sample as a weighted average $P = \sum_j (P_j \cdot L_j) / \sum_j L_j$, where P_j is the estimated intensity of colonization of the j-th root segment by AMF , where L_j is its length, expressed as the number of visual fields that the j-th root spans when observed at a magnification of 100×. This formula was used to estimate colonization intensity by all mycorrhizal structures: arbuscules, vesicles

Table 1. – Ellenberg indicator values used in the statistical analyses. Abbreviations: EIV, Ellenberg indicator value; Observed range, range of EIV recorded at the localities studied; Characteristics of EIV, brief characterization of EIV.

EIV	Observed range	Characteristics of EIV
Light (EL)	4.5–7.8	From deep shade to full light conditions
Temperature (ET)	4.8–6.6	From very low to very high temperatures
Moisture (EM)	4.0–6.6	From extreme dryness to permanently or almost constantly under water
Reaction (ER)	5.0–8.0	From extreme acidity to high-pH soils
Nitrogen (EN)	4.6–8.0	From extremely infertile to extremely nutrient-rich sites

and total mycorrhizal colonization (including arbuscules, vesicles and hyphae). The term "relative arbuscular colonization" means the relative proportion of segments with arbuscules in the total mycorrhizal colonization.

When comparing the colonization by AMF of the invasive species with that of the native dominant species, the total mycorrhizal colonization of both species and the relative arbuscular colonization of the invasive species with the total mycorrhizal colonization of the native dominant species were compared. When testing the effects of environmental conditions on the AM symbiosis of invasive species, total mycorrhizal and relative arbuscular colonization were used, with the latter in our dataset representing the best approximation of the extent of symbiotic exchange between the host plants and AM fungi.

Species were classified as mycorrhizal if the arbuscules were in its roots. These structures are the key feature defining an AM association (Brundrett 2004). Our records were then checked against the lists of mycorrhizal associations published by Harley & Harley (1987) and Wang & Qiu (2006).

Statistical analyses

To reliably asses the presence and extent of AM symbiosis and compare it both at the among-species and within-species levels at different sites, each species must be represented by multiple populations. This condition was fulfilled only for the 17 invasive species (with samples from at least four different localities/sites) highlighted in bold in Table 2, which were the species used in statistical analyses.

The sampling sites were treated as independent observations in all the statistical analyses. Consequently the information obtained from the three specimens of each invasive plant or the three specimens of the corresponding native dominant species was averaged (directly or by including the random effect of site into the model).

Hypotheses about the relation of mycorrhizal colonization of the invasive species with the colonization of the most dominant native species and site conditions (estimated using Ellenberg indicator values) were tested using mixed-effect linear models with the populations (sites) treated as a random effect. Therefore, effects of explanatory variables could be studied at two hierarchical levels: within-species (among-population) level, representing the response of invasive species to differences in site conditions, and among-species level representing differences between individual invasive species.

Where appropriate, phylogenetic correction was also performed, using the method of Desdevises et al. (2003) and a phylogenetic tree (Fig. 2) based on data from the BiolFlor

Table 2. – Summary of the AM status of the invasive neophytes studied and recorded in the literature. Abbreviations: H&H, Harley & Harley (1987); W&Q, Wang & Qiu (2006); PS, present study; NO, non-mycorrhizal species; YES, mycorrhizal species; YES/NO, both mycorrhizal and non-mycorrhizal status recorded; ?, no information about AM status; **bold type**, invasive neophytes included in the statistical analyses (with at least four populations sampled).

Invasive species	Family	H&H	W&Q	PS
Amaranthus powellii	*Amaranthaceae*	?	?	NO
Amaranthus retroflexus	*Amaranthaceae*	NO	NO	NO
Ambrosia artemisiifolia	*Asteraceae*	?	YES	YES
Angelica archangelica	*Apiaceae*	YES	YES	YES
Aster novi-belgii agg.	*Asteraceae*	YES	YES	YES
Bidens frondosa	*Asteraceae*	?	YES	YES
Bunias orientalis	*Brassicaceae*	?	?	NO
Cannabis ruderalis	*Cannabaceae*	?	?	YES
Conyza canadensis	*Asteraceae*	?	YES	YES
Digitalis purpurea	*Scrophulariaceae*	YES/NO	YES/NO	YES
Echinocystis lobata	*Cucurbitaceae*	?	?	YES
Echinops sphaerocephalus	*Asteraceae*	?	?	YES
Epilobium ciliatum	*Onagraceae*	YES	YES	YES
Erigeron annuus	*Asteraceae*	YES	YES	YES
Galeobdolon argentatum	*Lamiaceae*	?	?	YES
Galinsoga ciliata	*Asteraceae*	YES	YES	YES
Galinsoga parviflora	*Asteraceae*	YES	YES	YES
Geranium pyrenaicum	*Geraniaceae*	?	?	YES
Helianthus tuberosus	*Asteraceae*	?	?	YES
Heracleum mantegazzianum	*Apiaceae*	?	?	YES
Impatiens glandulifera	*Balsaminaceae*	YES/NO	YES/NO	YES
Impatiens parviflora	*Balsaminaceae*	YES/NO	YES/NO	YES
Juncus tenuis	*Juncaceae*	?	?	YES
Kochia scoparia subsp. *scoparia*	*Chenopodiaceae*	?	?	NO
Lupinus polyphyllus	*Fabaceae*	?	?	NO
Matricaria discoidea	*Asteraceae*	?	?	YES
Oenothera biennis	*Onagraceae*	YES	YES	YES
Parthenocissus inserta	*Vitaceae*	?	?	YES
Persicaria polystachya	*Polygonaceae*	?	?	NO
Reynoutria ×bohemica	*Polygonaceae*	?	?	NO
Reynoutria japonica	*Polygonaceae*	?	?	NO
Reynoutria sachalinensis	*Polygonaceae*	?	?	NO
Rudbeckia laciniata	*Asteraceae*	?	?	YES
Rumex alpinus	*Polygonaceae*	YES/NO	YES/NO	YES
Rumex longifolius	*Polygonaceae*	?	YES	NO
Rumex thyrsiflorus	*Polygonaceae*	?	?	NO
Sedum hispanicum	*Crassulaceae*	?	?	NO
Sisymbrium loeselii	*Brassicaceae*	NO	NO	NO
Solidago canadensis	*Asteraceae*	?	YES	YES
Solidago gigantea	*Asteraceae*	?	YES	YES
Telekia speciosa	*Asteraceae*	?	?	YES
Veronica filiformis	*Scrophulariaceae*	YES	YES	YES
Veronica persica	*Scrophulariaceae*	YES	YES	YES
Virga strigosa	*Dipsacaceae*	?	?	YES
Total		15	21	44

database (Klotz et al. 2002). Phylogenetic correction was not applied in the test of the correlation between mycorrhizal colonization of the invasive species and the dominant native species, as the choice of composition of the recently invaded communities cannot be subject to evolutionary inertia.

To estimate the variation in total mycorrhizal colonization attributable to individual levels of the hierarchical sampling design, variance components were estimated using the method described in Venables & Ripley (2002).

All estimates of mycorrhizal colonization (both for invasive and dominant species) were transformed by arcsin-transformation to decrease heterogeneity of variances. Pearson's correlation coefficient was used to measure the direction and strength of the relationship between all of the Ellenberg indicator values used in the statistical analyses. Statistical analyses were performed using the R program, version 2.8 (R Development Core Team 2008).

Results

This study provides new records of the mycorrhizal status of 23 invasive neophyte species not cited by Wang & Qiu (2006) and Harley & Harley (1987), see Table 2. In addition, the mycorrhizal status of four invasive neophytes (*Digitalis purpurea*, *Impatiens glandulifera*, *I. parviflora* and *Rumex alpinus*) was clarified. However, the results of this study do not accord with the positive AM status attributed to *Rumex longifolius* by Wang & Qiu (2006), citing Eriksen et al. (2002).

Our results indicate that in the field about 70% of the invasive species examined are mycorrhizal. The majority of them belong to the *Asteraceae*. On the other hand, most of the invasive species of the family *Polygonaceae* (second most numerous plant family in the data set) studied are non-mycorrhizal.

The results support the prediction of a positive correlation between the extent of mycorrhizal colonization of invading plant species and that of the native species dominating the invaded community, but only if the total mycorrhizal colonization percentages are compared at the among-species level ($F_{1,15} = 4.81$; $P = 0.045$).

The analyses that focused on the relation between habitat and community characteristics, and the intensity of AMF colonization of the roots of invasive species revealed several significant correlations. At the among-species level, the total mycorrhizal colonization decreases and the relative arbuscular colonization of the roots of invasive species increases with nitrogen availability in the habitat (Table 3, last row) and both these relations are significant after phylogenetic correction.

At the within-species level, there are negative correlations between relative arbuscular colonization of invading species and the light and temperature demands of the species present in the community, and a positive correlation between relative arbuscular colonization of the invaders and soil wetness (Table 3).

Decomposition of the variation in total mycorrhizal colonization into variance components revealed the highest differences among species (0.045), with smaller and similar differences among habitats (0.031) and individual plants (0.032).

Table 3. – Effects of habitat and community characteristics on the relative arbuscular colonization and total mycorrhizal colonization of the roots of invasive neophyte species. Abbreviations: Richness, species richness of the community; Diversity, species diversity of the community (Shannon-Wiener index); EL, Ellenberg indicator value for light conditions; ET, Ellenberg indicator value for the temperature demands; EM, Ellenberg indicator value for moisture; ER, Ellenberg indicator value for soil reaction; EN, Ellenberg indicator value for nitrogen availability; InvArb/InvTot, relative arbuscular colonization of invasive neophyte species; InvTot, total mycorrhizal colonization of invasive neophyte species; InvSpec, relation at the among-species level; InvSpec/FC, ditto, after phylogenetic correction; Within, relation at the within-species level; ▲, positive correlation; ▼, negative correlation. The number of residual dfs is 13 at the among-species level, 12 ditto, after phylogenetic correction, and 55 at the within-species level.

	InvArb/InvTot			InvTot		
	InvSpec	InvSpec/FC	Within	InvSpec	InvSpec/FC	Within
Richness	F = 0.37 (n.s.)	F = 0.29 (n.s.)	F = 0.02 (n.s.)	F = 0.01 (n.s.)	F = 0.08 (n.s.)	F = 0.09 (n.s.)
Diversity	F = 0.15 (n.s.)	F = 0.05 (n.s.)	F = 0.16 (n.s.)	F = 2.33 (n.s.)	F = 0.03 (n.s.)	F = 0.005 (n.s.)
EL	F = 0.06 (n.s.)	F = 0.78 (n.s.)	**F = 7.65 ($<10^{-2}$)▼**	F = 2.00 (n.s.)	F = 0.88 (n.s.)	F = 0.01 (n.s.)
ET	F = 0.02 (n.s.)	F = 1.14 (n.s.)	**F = 9.88 ($<10^{-2}$)▼**	F = 0.06 (n.s.)	F = 0.11 (n.s.)	F = 0.05 (n.s.)
EM	F = 1.28 (n.s.)	F = 0.17 (n.s.)	**F = 5.54 (0.02) ▲**	F = 0.55 (n.s.)	F = 2.31 (n.s.)	F = 0.59 (n.s.)
ER	F = 0.11 (n.s.)	F = 0.33 (n.s.)	F = 0.30 (n.s.)	F = 3.40 (n.s.)	F = 2.11 (n.s.)	F = 0.23 (n.s.)
EN	**F = 18.75 ($<10^{-3}$)▲**	**F = 10.98 ($<10^{-2}$)▲**	F = 0.43 (n.s.)	**F = 8.26 (0.01)▼**	**F = 4.76 (0.05)▼**	F = 0.23 (n.s.)

Discussion

Mycorrhizal status of the invasive neophyte species

Mycorrhizal dependence of plant species is traditionally determined by comparing plant growth (under experimental conditions) with and without AMF symbionts (Fitter & Nichols 1988, Gange et al. 1990, Merryweather & Fitter 1996). Therefore, field sampling can be used only to identify species that are not dependent on AMF, but in particular cases can enter into a symbiotic relation with them. This study focused on many plant species sampled over a large geographical area and used the presence and extent of an AM symbiosis in the field as an indication of an AM dependency. Therefore, due caution needs to be exercised when comparing these results with those based on experiments.

The AM status of invasive neophytes revealed by this study accord with that presented in two compilation studies, Harley & Harley (1987) and Wang & Qiu (2006), with a few exceptions. The AM status of *Digitalis purpurea*, *Impatiens glandulifera*, *I. parviflora* and *Rumex alpinus* recorded in an earlier publication and cited by Wang & Qiu (2006) was elucidated. No AM structures were observed in the roots of *Rumex longifolius* (*Polygonaceae*) although this species is recorded as AM dependent in Wang & Qiu (2006), referring to Eriksen et al. (2002). This latter study indicates a weak or no colonization by AMF (AM structures were present only in very few specimens, less than 1%). The lack of this species by AMF accords with the fact that the species of *Polygonaceae* are often considered non-mycorrhizal (Smith & Read 1997).

However, colonization by AMF still depends on many other factors, e.g. host plant species phenology (Sanders & Fitter 1992a, 1992b, Šmilauer 2001, Ruotsalainen et al. 2002), AMF diversity and species composition within different habitats and communities (Gange et al. 1990), or seasonal and ontogenetic variations (Jakobsen et al. 2002). Therefore, the intensity of colonization by AMF of roots can be influenced by specific habitat conditions.

The finding that most of the mycorrhizal invasive species in our study belong to the family *Asteraceae* is partly influenced by the fact that 14 of the 44 invasive species studied belong to this family. It also accords with Pyšek (1998) who places *Asteraceae* among the four families contributing most alien species to local floras.

Relation to the colonization by AMF of native dominant plant species

Our prediction, based on the hypothesis of Urcelay & Díaz (2003), that the AM status of the invasive species would be correlated with that of native dominants, was partly supported by the results. There is a positive relation between the total mycorrhizal colonization of the invasive neophytes and that of the dominant species. On the other hand, the same analysis using relative arbuscular colonization instead of total mycorrhizal colonization is not significant, possibly because of the considerable effect on the analysis of the results for situations where both the invasive species and dominant plant were non-mycorrhizal. This is supported by the frequent occurrence of non-mycorrhizal invasive neophytes with non-mycorrhizal dominants, (Electronic Appendix 2).

It is also important to note that while the non-significant effect for relative arbuscular colonization was reasonably strong (with its F statistic close to the value obtained for the significant effect of total AMF colonization), the results for within-species effects suggest an unimportant relation. This suggests that the level of the colonization of invasive species by AMF is not associated with the conditions prevailing in the invaded community.

Before these novel results can be reliably explained many questions need to be answered by further research. Particularly useful would be a better understanding of how the colonization of dominant species by AMF changes during the invasion of a plant community by alien plant species. This relation is best tested by a field experiment combining invasive species with varying degrees of dependence on AM with plant communities of different mycorrhizal status.

Relations to habitat and community characteristics

Use of Ellenberg indicator values, instead of environmental parameters measured at each site, could be seen as an important limitation of the present study, but circular reasoning was avoided by excluding the invasive species when calculating the environmental scores for the sites. On the other hand, use of these indirect measures has the advantage that they represent the average site conditions, integrated over long periods of time, unlike a limited number of measurements one could achieve using the direct approach.

Nitrogen is one of the most important elements limiting plant growth in terrestrial ecosystems (Schulze et al. 2002). Invasive plant species occurring in habitats with an indicated high availability of nitrogen had a lower total mycorrhizal colonization and higher relative arbuscular colonization of their roots (Table 3). The effect of the availability of nitrogen is revealed at the among-species level, even after phylogenetic correction, which suggests this is an evolutionary adaptation.

According to Rillig et al. (2002), a high availability of nitrogen (as ammonium or nitrate) could have various effects (none, positive or negative) on the intensity of colonization by AMF of plant roots, with noticeable differences among different localities. Other studies, focusing on separate and the combined effects of N and P, point to the N:P ratio as the most important factor determining the intensity of colonization by AMF (Corkidiki et

Table 4. – Correlations between Ellenberg indicator value descriptors used in the statistical analyses (Pearson's correlation coefficient). Correlation estimates in the upper triangle and corresponding significance estimates for test of H0: $\rho = 0$, in the lower triangle. Abbreviations of the various Ellenberg indicator values: EL, light conditions; ET, temperature demands; EM, moisture; ER, soil pH; EN, availability of nitrogen.

	EL	ET	EM	ER	EN
EL	x	0.4968	–0.4861	0.0276	–0.3748
ET	$9.90 \cdot 10^{-5}$	x	–0.6595	0.0985	–0.1970
EM	$1.80 \cdot 10^{-6}$	$3.77 \cdot 10^{-12}$	x	–0.0194	0.4501
ER	n.s.	n.s.	n.s.	x	0.2982
EN	$3.48 \cdot 10^{-4}$	$6.74 \cdot 10^{-2}$	$1.22 \cdot 10^{-5}$	$5.03 \cdot 10^{-3}$	x

al. 2002, Treseder & Allen 2002, Johnson et al. 2003, Blanke et al. 2005). Their findings can also explain seemingly conflicting results of Rillig et al. (2002). Some authors also interpret the Ellenberg indicator values for nitrogen as an indicator of general fertility rather than of the availability of nitrogen (Hill & Carey 1997, Ertsen et al. 1998).

Generally, eutrophic habitats often host a few fast-growing nitrophilous species (e.g., *Aegopodium podagraria* and *Urtica dioica*), which soon become dominant. It is likely that the relatively high percentage of arbuscules in the roots of mycorrhizal invasive species occuring at such sites may be a reaction to increasing competition from fast-growing nitrophilous species, especially in terms of their ability to utilize the phosphorus. This ability is supposedly achieved both by root characteristics (e.g., density and length of root hairs) and the presence of AMF in the roots of the plants (Richardson et al. 2009).

The intensity of colonization of plant roots by AMF is similarly influenced by other factors, such as light, temperature and availability of water (Smith & Read 1997). The results of our analyses of these habitat characteristics indicate that roots of invasive neophyte species have less arbuscules in habitats with high light and temperature values and low soil moisture values (Table 3). As all these effects were revealed at the within-species level it is most likely they reflect differences among the habitats studied. The coincidence of these results can be explained by the negative correlation between moisture on one hand and temperature and light on the other (see Table 4).

The positive effect of soil moisture on the colonization of roots by AMF accords with the results of Apple et al. (2005), who studied the seasonal dynamics of AMF in the roots of two species growing in the Mojave desert. They found significant changes in the percentage of all AMF structures (arbuscules, vesicles and also hyphae) associated with soil moisture. For the effect of light and temperature there are few published and quite conflicting results that can be compared with our results. Many studies focus on the effect of global warming (i.e., temperature increase) on AMF and the shifts in the composition and function of their communities (e.g., Fitter et al. 2000, Rillig et al. 2002). In general, there is an increase in the colonization of roots by AMF with increasing temperature (Rillig et al. 2002) up to about 30 °C (Smith & Read 1997).

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Souhrn

Arbuskulární mykorhizní symbióza je dnes považována za nejčastěji se vyskytující a zároveň nejstarší typ mutualistického vztahu mezi rostlinou a houbou. Je odhadováno, že se vyskytuje nejméně u 80% všech druhů vyšších rostlin. Proto i v souvislosti s rostlinnými invazemi nemůže být její vliv opomenut, ačkoliv nepatří k těm nejdůležitějším. Ve srovnání s dosud publikovanými údaji tato práce přináší nové informace o mykorhizním statutu pro 23 invazních neofytů. Těmito druhy jsou *Amaranthus powellii*, *Bunias orientalis*, *Cannabis ruderalis*, *Echinocystis lobata*, *Echinops sphaerocephalus*, *Galeobdolon argentatum*, *Geranium pyrenaicum*, *Helianthus tuberosus*, *Heracleum mantegazzianum*, *Juncus tenuis*, *Kochia scoparia* subsp. *scoparia*, *Lupinus polyphyllus*, *Matricaria discoidea*, *Parthenocissus inserta*, *Persicaria polystachya*, *Reynoutria ×bohemica*, *R. japonica*, *R. sachalinensis*, *Rudbeckia laciniata*, *Rumex thyrsiflorus*, *Sedum hispanicum*, *Telekia speciosa* a *Virga strigosa*. V rámci této studie jsme také testovali, zda je intenzita mykorhizní kolonizace invazního druhu korelována s její intenzitou v kořenech dominantního druhu invadovaného společenstva. Tento vztah byl průkazný, ale pouze při použití celkové mykorhizní kolonizace. Dále jsme testovali vliv vlastností rostlinného společenstva a charakteristik biotopu na míru kolonizace arbuskulárními houbami v kořenech invazních neofytů a našli jsme několik průkazných vztahů. Na mezidruhové úrovni se zvyšující se dostupností dusíku celková mykorhizní kolonizace invazních druhů klesá, zatímco relativní podíl arbuskul se zvyšuje. Oba tyto vztahy zůstaly průkazné i po fylogenetické korekci, což naznačuje možnou evoluční adaptaci. Dvě negativní korelace jsme našli při srovnání relativního podílu arbuskul s Ellenbergovými hodnotami pro světlo a teplotu. Při porovnání se zvyšující se vlhkostí stanoviště byl vztah pozitivní. Všechny tyto korelace se projevovaly na vnitrodruhové úrovni a proto spíše odráží jednotlivé stanovištní rozdíly v rámci všech studovaných lokalit.

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