Mycorrhiza (2010) 20:445–457
DOI 10.1007/s00572-010-0332-4

REVIEW

The potential role of arbuscular mycorrhizal fungi in protecting endangered plants and habitats

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Received: 24 February 2010 / Accepted: 4 July 2010 / Published online: 21 July 2010

Abstract Ecosystems worldwide are threatened with the extinction of plants and, at the same time, invasion by new species. Plant invasiveness and loss of species can be caused by similar but opposing pressures on the community structures. Arbuscular mycorrhizal fungi (AMF) can have multiple positive effects on plant growth, productivity, health, and stress relief. Many endangered species live in symbiosis with AMF. However, the list of the International Union for Conservation of Nature and Natural Resources (IUCN Red List of Threatened Species) indicates that the mycorrhizal status of most of the threatened species has not been assessed. Rare plants often occur in specialized and also endangered habitats and might utilize specialized or unique AMF. The specificity of any endangered plant to its AMF population has not been investigated. Because most of the current AMF isolates that are available colonize a broad range of plant species, selected inocula could be used to promote growth of endangered plants before the proper and more effective indigenous AMF are characterized. Application of AMF in field sites to protect endangered plants is hardly feasible due to the complexity of plant community structures and the large amount of fungal inocula needed. Endangered plants could, however, be grown as greenhouse cultures together with appropriate fungi, and, at the relevant developmental stage, they could be re-planted into native sites to prevent extinction and to preserve plant community ecology.

Keywords Endangered plants · Plant extinctions · Invasive plants · Arbuscular mycorrhizal fungi · Nature conservation · Symbiosis of plants and fungi

Introduction

We are confronting an episode of species extinction greater than anything the world has experienced for the past 65 million years (P. Raven, see http://www-formal.stanford.edu/jmc/progress/biodiversity.html). Any extinction of a species means a loss of a genetic reservoir which otherwise could have potential for human exploitation and future applications. The International Union for Conservation of Nature and Natural Resources (IUCN Red List of Threatened Species), which is recognized as the most reliable compilation of the world's species classified according to their extinction risk, has produced Red Data Books and Red Lists since 1963 which are annually updated under http://www.redlist.org. The IUCN list is based on the information provided by taxonomists of each country and on existing national lists of endangered plants. The most recent Red List contains 44,838 species of which 12,055 are plants. Of these, 8,457 face extinction (70% of the evaluated species)

Electronic supplementary material The online version of this article (doi:10.1007/s00572-010-0332-4) contains supplementary material, which is available to authorized users.

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while the others are of less concern (IUCN 2008). Species should be classified as endangered only when they have a low specimen number worldwide; however, there are no absolute universally accepted criteria to declare a species as endangered. For example, the Atlantic fern *Phyllitis scolopendrium* is very common in Southern England and can grow even on pavements within the cities, while in Germany, it is considered rare and has, therefore, been put under nature protection. Likewise, *Hedera heli*x is extremely common in Western and Southern Europe where it is found in nearly any forest but is protected in Poland for various reasons. Consequently, these two species are not included in the IUCN list. On the other hand, mycorrhizal plants that are really endangered worldwide such as the species of lady slippers *Cypripedium* or zinc violets (*Viola lutea* ssp. *calaminaria*, *V. lutea* ssp. *westfalica*, Hildebrandt et al. 2006b) are not included in the IUCN list. In fact, of over 12,000 plant species in the Red List (IUCN 2008), less than 1,000 are properly documented, and the IUCN criteria to declare species to be threatened have been recently questioned (Martin 2009; Schatz 2009). The European Commission has started an initiative to assess around 6,000 European species according to the IUCN regional Red Listing guidelines, and a more complete list of endangered vascular plants should be published in early 2011 (http://www.iucnredlist.org.europe).

Mycorrhizal status of endangered plants

Early check-lists on the mycorrhizal status of plants were limited to Great Britain (Harley and Harley 1987; Peat and Fitter 1993). A more recent comprehensive survey of papers on mycorrhizal plant species (Wang and Qiu 2006) contains 3,617 species from 263 families (including those from pteridophytes and bryophytes) where 2,469 species (68%) belonging to 242 plant families (92%) form arbuscular mycorrhiza. The remaining 706 plant species are non-mycorrhizal. For the present study, plants in the IUCN database were examined for their mycorrhizal status by using the list of Wang and Qiu (2006) (electronic supplementary material Table 1, Fig. 1). Only 139 endangered species belonging to 44 plant families of the IUCN database had been screened for their mycorrhizal status. Among them, 54 were assigned to one of the three threatened categories (critically endangered (CR), endangered (EN), or vulnerable (V)), another 13 are categorized as near threatened (NT), and 72 fell into the lower risk least concern (LC) category (electronic supplementary material Table 1). The mycorrhizal colonization is not known for any of the 869 plant species declared extinct or extinct in the wilderness. Among the endangered plants with known mycorrhizal status, 11 were classified as critically endangered, 6 as endangered, and 30 as vulnerable species. To illustrate the lack of information on an outstanding plant beauty being at the front-line of extinction worldwide (Swarts and Dixon 2009), only seven lady slippers (*Cypripedium* sp.), *Cypripedium calceolus, Cypripedium californicum, Cypripedium candidum, Cypripedium fasciculatum, Cypripedium guttatum, Cypripedium montanum*, and *Cypripedium parviflorum*, have been found to be mycorrhizal (Wang and Qiu 2006), whereas the other 11 species of this genus listed in the IUCN list have not yet been examined for their mycorrhizal colonization. Many more examples of this type emphasize the meager data basis about the mycorrhizal status of endangered plants and the need for more research on the status. To date, only one article and a book chapter on arbuscular mycorrhizal fungi (AMF) and endangered plants are available (Fuchs and Haselwandter 2004, 2008).

Causes of plant extinction

The structure of any plant community is complex in almost all cases and undergoes delicate fluctuations within short distances. Often endangered plants are constituents of grasslands or forests where they are threatened by human exploitations, alterations in their natural habitats, or global environmental changes including increasing levels of greenhouse gases or constituents of acid rain. Modifications in soil structure, nutrient availability, and organic matter alter soil microbial diversity and activity both quantitatively and qualitatively, enforcing changes in vegetation cover and diversity. It is predicted that all such threats will become increasingly more important over short time scales (Sala et al. 2000; Tylianakis et al. 2008). To modify community composition in favor of endangered plants is a complicated task, and lessons may be learned by examining the opposite, invasive plants.

In many cases, invasive plants occupy habitats disturbed by human activities. Habitats where plant communities show a lot of disturbance and low species diversity are generally rich in nutrients, particularly in phosphorus and nitrogen. Invasive plants can grow quickly in such habitats so that primary productivity increases while plant biodiversity decreases (Rout and Callaway 2009; Wilsey et al. 2009). In contrast, plants are slow growing in ecosystems with low available resources and have to develop defense processes to protect against animal and fungal attack (Blumenthal 2005). Furthermore, alien species often colonize new habitats in absence of their natural enemies: the combination of “increased resource availability and release from natural enemies” (Blumenthal 2005) allows them to thrive unchecked. A clear example of this is the South African ragwort (*Senecio inaequidens*) introduced to central

Germany some 100 years ago and which in recent years (Werner 2002) has spread abundantly, particularly along railroads, without animal or fungal threat due to its production of toxic pyrrolizidine alkaloids (Scherber et al. 2003). It is only recently that lice (Witte et al. 1990) and beetle (Werner 2002) have adapted to feed on this invader. Another example is black cherry (*Prunus serotina*) from the USA which escaped from its natural above- and below-ground enemies when invading northwest Europe (Reinhart et al. 2003).

Toxin excretion into soils can also help invasive plants to occupy new habitats. An example of such allelopathic behavior (Weir et al. 2004) is *Centaurea maculosa* which displaces native species in its new habitats in Western USA by excreting catechin from its roots (Bais et al. 2003), while plant communities in its site of origin in Romania are remarkably unaffected by this toxin (Thorpe et al. 2009). Invasive plants can cause major shifts in the composition and function of soil microorganisms (Wolfe and Klironomos 2005; Broz et al. 2007), and plant allelochemicals can disrupt the belowground competitive outcome between plants and mycorrhizal fungi (Weir 2007). For example, *Alliaria petiolata* (garlic mustard), a European invader of North American forests, produces anti-fungal compounds and suppresses interactions between native tree seedlings and belowground mycorrhizal fungi in the invaded soils but not in its native European habitat (Stinson et al. 2006; Callaway et al. 2008). It has also been reported that mycorrhizal colonization of maize with *Glomus mosseae* was reduced by allelopathic effects of root extracts of maize (*Zea mays*) and meadow fescue (*Festuca pratensis*). The inhibitory effects of these extracts on maize root growth were, however, not observed in maize colonized beforehand by the same AMF (Džafič et al. 2009). Genetic divergence from native populations is another factor which may favor the adaptation of invasive plants to new environmental conditions (Bossdorf et al. 2008).

In contrast to invasive plants, endangered species do not seem to be so well protected by chemical weapons (toxic compounds); they can be more exposed to animal or fungal enemies than abundant species and risk being overgrown by more productive plants. Some rare plants accumulate pathogens quickly and can only maintain low densities due to species-specific pathogens (Klironomos 2002). On the other hand, endangered plants can sometimes be surprisingly productive in nutrient-rich environments, or they occur in ecological niches with little risk of exposure to more competitive plants. These are, however, generalized traits that might not apply to all cases, and the causes for their rarity are often not obvious. One example is *Wulfenia carinthiaca*, the blue flower of Caranthia, South Austria and a tertiary relict, which only occurs on the northwest slope of the Gartnerkofel mountain and then locally in Albania. At Gartnerkofel, this plant covers stands to almost 100% while no one specimen is found in the neighboring mountains despite their very similar geology.

Arbuscular mycorrhizal fungi in endangered plant ecosystems

The arbuscular mycorrhizal symbiosis is considered to be the primary determinant of plant health and soil fertility in terrestrial ecosystems (Jeffries et al. 2003). The fine hyphae extending out into the soil exploit minerals more efficiently than plant roots alone, and the presence of the fungi consistently reduces soil-borne fungal and nematode attacks on roots (Smith and Read 1997, 2007). AMF can play significant roles in the growth of plants in metal-contaminated soils and in salt marshes (Hildebrandt et al. 2007 and many others). Furthermore, the extensive, dense mycelial network formed by AMF increases the stability of soil particles through the excretion of glomalin, an insoluble and hydrophobic proteinaceous substance, and/or glomalin-related soil proteins, which may prevent disaggregation of water and organic carbon from soil particles, although the issue does not seem to be finally resolved (Purin and Rillig 2007; Hallett et al. 2009; Bedini et al. 2009). These fungi also contribute to relieving drought stress of plants by efficient mobilization of water from soil particles (Wu and Xia 2005; Mena-Violante et al. 2006). They also determine the formation of water stable aggregates in soils (Hallett et al. 2009), in particular in drier areas such as salt marshes (Füzy et al. 2008), although this activity may be plant specific (Querejeta et al. 2003). In addition, AMF colonization can affect vegetative (Miller et al. 1987) and sexual reproduction of plants by impacting on the number of inflorescences, fruit and seed production, and offspring vigor (Shumway and Koide 1994; Nuortila et al. 2004).

These different attributes of AMF will contribute to their role in protecting endangered plants. In the following paragraphs, we focus on some more specific habitats (a) which have been thoroughly studied with respect to AMF, (b) where plants are often threatened by extinction, or (c) which are frequently populated by endangered plants.

Wet stands are dominated by sedges, rushes, sills, and other members of non-mycorrhizal families. However, plants of bogs (Fuchs and Haselwandter 2004) and other wet areas (Wirsel 2004; Wolfe et al. 2006; Likar et al. 2009) can be strongly mycorrhizal, and in wetlands, dicots appear to be more susceptible to AMF than monocots (Weishampel and Bedford 2006). Wetlands are often anoxic (Keddy 2000), and plants survive periods of water-logging by ethanol fermentation (e.g., Smith and ap Rees 1979; Smith et al. 1986). Fermentation by AMF has not been assessed,

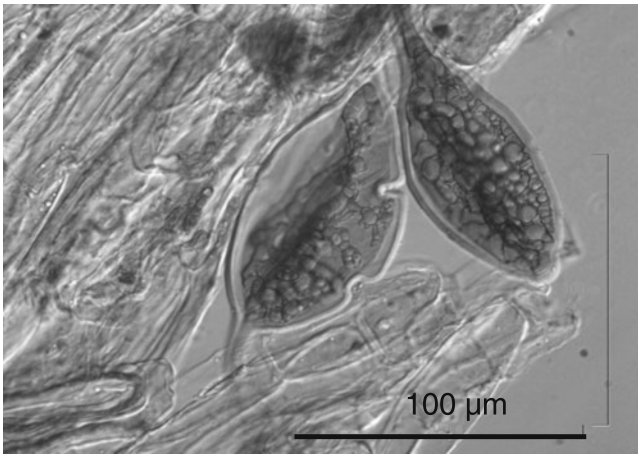

Fig. 1 Spores and hyphae of an arbuscular mycorrhizal fungus filled with a dark septate endophyte within a root of *P. lanceolata*

but greenhouse experiments seem to point to the fact that excess humidity is unfavorable for these fungi so that they may have to meet their energy demand entirely by O_2 respiration. Wetland plants often possess extensive aerenchyma which supplies their roots with sufficient O_2 for respiration to proceed. Strongly AMF-colonized plants of wetlands like the salt aster (Scheloske et al. 2004) and amphibious plants of intermittent aquatic habitats that thrive both submerged and emerged (Sraj-Krzic et al. 2006, 2009) form extensive aerenchyma. Although it remains to be shown whether only plants with aerenchyma are mycorrhizal in wetlands, any correlation between AMF and the ability to form aerenchyma should be considered in strategies to protect endangered plants like *Gratiola officinalis* or *Iris sibirica* in the wetlands of Central Europe.

Dicots dominate in drier habitats and a much higher percentage of plants is colonized by AMF than in wetlands (Muthukumar et al 2004; Weishampel and Bedford 2006; Likar et al 2009). In drier grasslands, like chalk meadows, plants have to cope with large fluctuations in water supply, and AMF may be particularly helpful in mobilizing the residual moisture for survival during drought periods. Dry grasslands are preferred sites for legumes which utilize another symbiotic partner, N_2-fixing rhizobia, to meet their N demand. As revealed using the classical Braun-Blanquet method, the species richness in such dry areas with only a few centimeters of soil above the carbon skeleton is often extreme (Ellenberg 2009). Roots of many plants possess arbuscular mycorrhizal structures. However, the dominant and best growing plants, the so called “upper grasses” such as *Bromus erectus* and *Helictotrichon pubescens*, or the Cyperaceae *C. flacca*, are non-mycorrhizal or at best poorly mycorrhizal (Van der Heijden et al. 1998), indicating that other factors besides mycorrhiza dictate the dominance of plant species on such soils in Europe.

Tree species in forest soils can be ectomycorrhizal or endomycorrhizal (Harley and Harley 1987; Werner 1992; Peat and Fitter 1993); some trees like willows are both ecto- and arbuscular mycorrhizal, and the herbaceous plants below the tree canopy like the common and acid-tolerant *Oxalis acetosella* frequently are colonized by AMF. The large amounts of humic and other acids in forest soils make it particularly difficult to characterize the AMF from herbs. A typical endangered woodland species which is strongly mycorrhizal is *Anemone sylvestris*; this plant is rare on chalky soils in Western Europe but grows at relatively low pH in Eastern European areas. AMF abundantly occur when plants grow on tree stumps or logs under wet conditions, in sites which are often occupied by ferns and *O. acetosella*. The wooden supports absorb water like a sponge which helps the plants to survive during drier periods. Forest soils are not rich in available nutrients such as N, but AMF can transfer such elements to the plants (Turnau et al. 1999; Unrug and Turnau 1999). In addition, such sites are fertilized by insects leaving their excrements. *Glomus tenue* is a dominant fungus in these habitats (Turnau et al. 1999; Unrug and Turnau 1999). The broader hyphae of other AMF can be colonized by dark septate fungi (Fig. 1). Gametophytes and sporophytes of ferns (Turnau et al. 2005) and thalloid liverworts such as *Conocephalum* sp. (Fig. 2) or *Pellia epiphylla* are commonly colonized by AMF in the forest understory (Turnau et al. 1999). Liverworts can grow on wet stones, wooden logs, stumps, mosses, bare soil, or soil covered by dead leaves. They often follow the roots growing at the soil surface, and their rhizoids are interconnected by AMF mycelia to the plant roots. In many cases, their rhizoids remain on the forest litter or mosses and are too short to reach the bare soil. In such cases, the development of mycorrhiza seems to be of utmost importance.

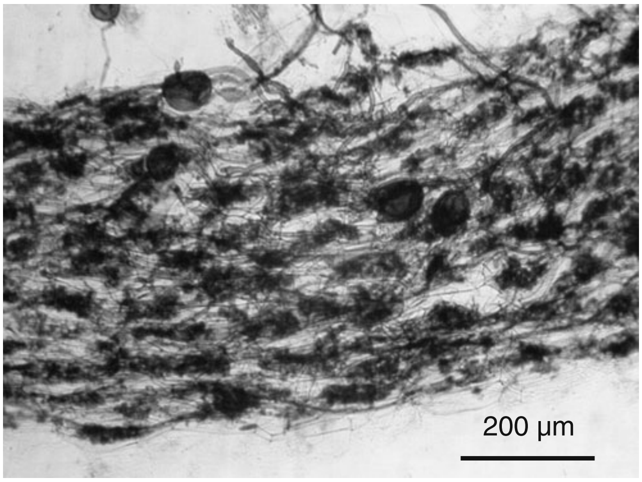

Fig. 2 Middle rib of the liverwort *Conocephalum conicum* with arbuscules occupying almost all plant cells

Salt marshes show a very high biomass productivity worldwide (Keer and Zedler 2002; Perry and Atkinson 2009). Relatively few plants, however, can cope with the high salt load, and so it not surprising that plant community structures of salt marshes are rather similar worldwide. Early botanists (Stocker 1928) already noted that the vegetation is essentially the same irrespective of the salt type in the marsh: NaCl, Na_2SO_4, Na_2CO_3, K_2CO_3, or KCl. These salts are dissociated in soils and tightly bind water causing drought stress to plants; salt marshes should therefore be hostile environments to plants. Salt has been reported to inhibit AMF spore germination, hyphal growth, as well as root colonization (Juniper and Abbott 1993; Juniper and Abbott 2006). Surprisingly, some plants from saline habitats, such as the salt aster (*Aster tripolium*) or the sea plantain (*Plantago maritima*), are highly mycorrhizal with almost all the root system containing fungal structures (Hildebrandt et al. 2001; Neto et al. 2006). Also, saline habitats that have been studied in Europe can contain a fairly high number of AMF spores with up to 80% belonging to one single species, *Glomus geosporum* (Hildebrandt et al. 2001; Carvalho et al. 2001; Landwehr et al. 2002; Grzybowska 2004). This fungus, which occurs also in non-saline soils, is apparently forced to sporulate heavily under the harsh sodic soil conditions (Wilde et al. 2009). From these observations, it is tempting to assume that *G. geosporum* can confer salt tolerance to plants, but all our attempts to demonstrate this have failed in greenhouse experiments (Füzy et al. 2008).

Molecular analysis of AMF occurring in two different salt marshes (a NaCl marsh on the Dutch coast and a potash inland saline habitat; Wilde et al. 2009) showed that *G. geosporum* sequences was, indeed, retrieved from roots of halophytes. The PCR products from roots, however, came mainly from DNA of *Glomus intraradices*-related species, of which most were recalcitrant to culture and were available only as sequences in the databanks. Temporal temperature gradient gel electrophoresis (TTGE) patterns of samples collected from the abandoned part of Sečovlje salterns in southern Slovenia indicated that at least six different AMF ribotypes colonize 12 halophytic plant species in the site (Sonjak et al. 2009a, b). AMF have repeatedly been discussed to confer salt tolerance to host plants (Carvalho et al 2003; Ruiz-Lozano 2003; Caravaca et al. 2005; Evelin et al 2009). About 7% of the global land surface is dominated by salt and is therefore non-amenable to agriculture. Therefore, cultures of AMF retrieved from roots of halophytes could have an enormous potential application if they were to confer salt resistance to plants (e.g., Tian et al. 2004). In addition, inland salt marshes frequently have a rather restricted size, but offer a fascinating biology which is often endangered due to human exploitations or alterations of the landscape.

Soils contaminated with heavy metals raise similar intriguing questions on the interaction between plants and AMF (Bothe et al. 2009). Some plants are particularly adapted to heavy metals and can thus endure high levels of different metals that are toxic to other plants. Altogether, metal-tolerant plants, metalophytes, are relatively rare and occur in plant families of totally unrelated taxonomy. Most metalophytes can only thrive on heavy metal soils due to their lack of competitiveness on non-polluted areas. Because metal-contaminated sites are infrequent, the metalophytes thriving there are endangered. Some are close to extinction such as *Thlaspi cepaeifolium* ssp. *cepaeifolium* and *Alyssum wulfenianum* in the South-Eastern Alps (Schmeil and Fitschen 2009), or the zinc violets, *Viola lutea* ssp. *westfalica* of the lead ditch at Blankenrode, Westfalia and *V. lutea* ssp. *calaminaria* at the three-country corner of Belgium, Germany, and The Netherlands (Hildebrandt et al. 2006b). The mechanisms that enable metalophytes to cope with the heavy metal toxicity vary from one plant taxon to another. Mycorrhizal fungi can alleviate heavy metal stress of plants and different mechanisms have been proposed: binding of metals to fungal cell walls, sequestration in vacuoles, or other processes (Bothe et al. 2009). Specifically adapted heavy-metal-tolerant AMF isolates exist that are particularly active in conferring metal tolerance to plants (e.g., Hildebrandt et al. 1999; Tonin et al. 2001). Some metalophytes can utilize AMF to grow on heavy metal soils; this is the case of the Asteraceae *Berkheya coddii* from Ni-ultramafic soils of South Africa (Turnau and Mesjasz-Przybylowicz 2003). Several *Thlaspi* species (*Thlaspi goesingense*, *Thlaspi caerulescens*, *Thlaspi calaminare*, *Thlaspi cepaeifolium*) of the Brassicaceae are particularly adapted to heavy metal-contaminated soils, and some have been reported to harbor AMF under field and experimental conditions, though the degree of colonization is generally low (Regvar et al. 2003; Regvar and Vogel-Mikuš 2008; Pongrac et al. 2009; Sonjak et al. 2009a).

The zinc violets are clear-cut examples of the exploitation of AMF by plants for heavy metal tolerance (Hildebrandt et al. 1999; Tonin et al. 2001). Their roots are intensely colonized (Fig. 3a) and have a yellow color associated with the synthesis of mycorradicin subsequent to mycorrhiza development (Klingner et al. 1995). Estimations of percentage root colonization indicate a positive correlation with heavy metal content in the soils (Hildebrandt et al. 1999). However, contrary to earlier claims (Nauenburg, 1986), growth of the zinc violets is not confined to heavy metal soils. Both the blue and yellow forms have been grown in several non-polluted gardens, including a vegetable garden where the roots of blue violet were highly mycorrhizal despite the fact that the soil was fertile due to river sediments (unpublished data). This plant is not obligatorily dependent on AMF since seeds have

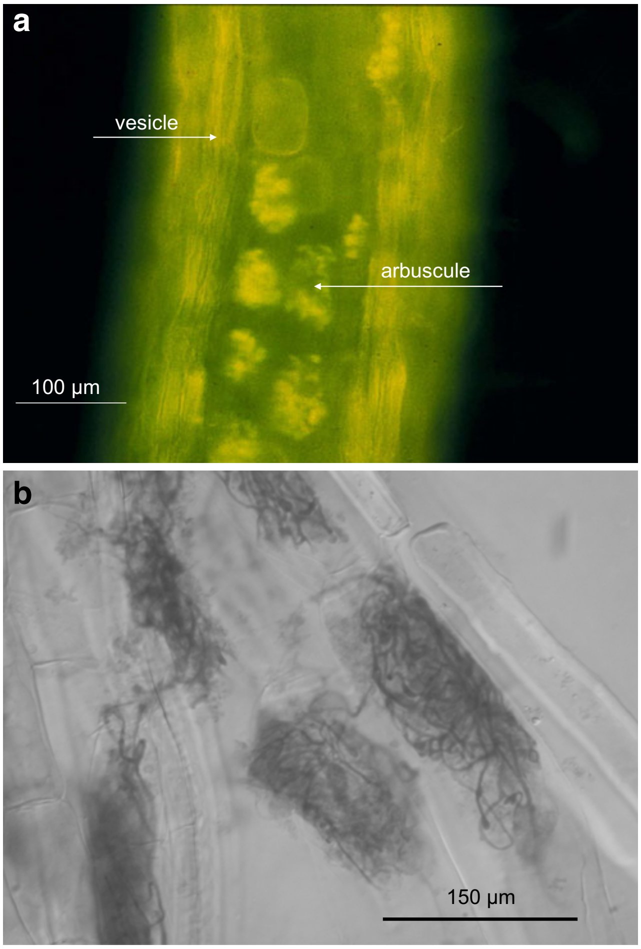

Fig. 3 Mycorrhizal colonization of two critically endangered plants. **a** Roots of the yellow zinc violet (*Viola calaminaria* ssp. *calaminaria*) from the heavy metal soil Breinigerberg at Stolberg near Aachen, Germany, are strongly colonized by arbuscular mycorrhizal fungi. The fluorescence image shows arbuscules and vesicles in a root. Photo taken by Ulrich Hildebrandt, Cologne. **b** *Soldanella carpatica* colonized by the arbuscular mycorrhizal fungus *G. tenue* with a typical fine mycelium, small vesicles, and arbuscules densely filling the root cells. Photo by S. Zubek, Kraków

been germinated in sterilized garden soil and inoculated with *G. intraradices* with no effect on plant growth (unpublished data). Shoots of the zinc violet accumulate only low levels of heavy metal when grown in contaminated soils (Ernst 1982). However, recent particle-induced X-ray emission (micro-PIXE) analyses have indicated that heavy metal concentrations are higher in the violet grown in a heavy metal soil than in a non-polluted soil (Vogel-Mikuš et al 2009). AMF apparently cannot prevent heavy metal accumulation in the roots of the zinc violet, but they can limit it so that the threshold value for metal toxicity is not exceeded.

Soils in forest areas that are polluted by industry can become more N rich which often results in dominance by a single or few plant species. An example of this is the non-mycorrhizal European *Carex brizoides* that out-competes AMF plants (Turnau 1991). Changes in plant community composition have also been observed in forests in Poland in response to industrial dusts from electrofilters (Grodziński et al. 1984, Greszta 1988). In soils exposed to such dusts, which contain heavy metals together with Al, Ca, and Mg, most AMF-colonized plants were the first to disappear, and those remaining were poorly colonized by AMF. Mycorrhiza-forming plants were replaced by non-mycorrhizal species such as *C. brizoides* and *Urtica dioica* (Vierheilig et al. 2006) in plots treated with Al dust (Turnau 1991). In contrast, dust with the highest concentrations of Cd and Zn did not prevent AMF colonization of *O. acetosella* (Turnau et al. 1996). A general feature appears to be that soil conditions of low pH values accompanied by a high content of heavy metals mostly exclude AMF. Such polluted sites are overtaken by ericoid and ectomycorrhizal plants (Turnau 1991; Martino et al. 2000; Lacourt et al. 2000). There are several well-known places with such features, among which the Cu mines in Parys Mountain, North Wales, or the pyrite mines in South-Eastern Portugal are dominated by endemic *Erica andevalensis* (Turnau et al. 2007). For severely Cd, Zn, and Pb polluted sites in Northern Slovenia, TTGE banding patterns indicated shifts in the root fungal community from less metal-tolerant basidiomycetes to more tolerant ascomycetes at the plots with the highest metal concentrations (Regvar et al. 2006), and in particular, dark septate endophytes were enriched at most of the polluted locations (Likar and Regvar 2009).

Arbuscular mycorrhizal fungi as determinants of plant community structure in endangered habitats

Aboveground components direct the composition and functioning of belowground microorganisms and, vice versa, belowground organisms determine plant community structure and productivity (Wardle et al. 2004). As previously pointed out, rare plants have been described as hosts to pathogens that affected their growth (Klironomos 2002), whereas abundant and invasive species can be more resistant to pathogens (Reinhart et al. 2003; Wardle et al. 2004). Van der Heijden et al. (1998) showed that both the diversity and the productivity can be dependent on the species composition of the AMF. In their greenhouse experiments, *Carex flacca*, as the only non-mycorrhizal plant had the highest biomass in the non-inoculated controls, whereas other plants tested benefited to different extents from different AMF taxa. Furthermore, AMF have been shown to greatly affect plant productivity either by stimulating (*Glomus etunicatum*) or reducing (*G. intraradices*) plant biomass (Klironomos et al. 2000).

In wetlands, the distribution of AMF is patchy over small spatial scales (Wolfe et al. 2007), and these fungi may be important drivers of plant community composition also in such soils (Wolfe et al. 2006). Thus AMF might have big impacts on ecosystem performance, particularly in nutrient poor environments (van der Hejden et al. 2008). They can cause a range of effects on plant growth from positive through neutral to negative, depending on plant and fungal species, genotypes, and local environmental conditions (Jones and Smith 2004; Wolfe et al. 2006; Van der Hejden et al. 2008), and the effect of AMF on an individual plant can vary with time (Hart and Reader 2002). All findings that AMF determine plant community structures are based on microcosm (Klironomos 2002), mesocosm (Wolfe et al. 2006), or macrocosm (Klironomos et al. 2000) experiments but not on field studies. Since protozoa, collemboles, nematodes, pathogenic, and saprophytic fungi can interact with AMF and plant roots (Bonkowski et al. 2009), it appears to be extremely difficult to manipulate natural ecosystems over larger spatial scales by applying distinct AMF with specific beneficial properties.

Factors affecting AMF colonization of roots and relation to efficacy of the interaction

Extant AMF may operate in a dynamic continuum between parasitism and mutualism (Johnson et al. 1997) where a finely tuned balance keeps mycorrhizal interaction in a stable state wherein neither partner conveys disadvantage to the other (Kogel et al. 2006). This has been illustrated in another type of beneficial fungal–plant interaction where mutation of one single gene, *noxA* encoding the reactive oxygen species generating NADPH oxidase, in *Epichloë festucae* caused a switch from mutualism to parasitism in *Lolium perenne* (Tanada et al. 2006). In nature, plants often show signs of mycorrhizal colonization, even when belonging to classical non-mycorrhizal families such as Brassiceae (DeMars and Boerner 1996) or Chenopodiaceae (Smith and Read 1997). In molecular terms, this is difficult to understand because an effective plant–mycorrhizal interaction requires the expression of a program of plant symbiosis genes (Parniske 2008). Of these, the *SYMRK* and *CCaMK* genes, essential for the interaction of plants with either AMF are absent from *Arabidopsis* and their inactivation results in incompatible effects on AMF (Seddas et al. 2009). Specific plant and fungal protein expression is associated with mycorrhizal colonization, of which phosphate transporters are the best known examples (Bucher 2007). Perhaps another route independent of the above-mentioned gene cascade (Parniske 2008) exists, the inactivation of which could result in an ineffective symbiosis or even in a pathogenic interaction.

Root samples of plants belonging to non-mycorrhizal taxa collected from natural habitats can occasionally be colonized by AMF, but are more often not. The genera *Biscutella* (Orłowska et al. 2002) and *Thlaspi* (Regvar et al. 2003) in the Brassicaceae can be weakly associated with AMF, with arbuscule formation at the flowering state. In pennycress (*Thlaspi* sp.), which has been studied in more detail, species of this genus are heavy metal tolerant (Regvar and Vogel-Mikuš 2008). AMF colonization can have a protective role for plants on metal-polluted sites even when present in very low percentages, as described for *Thlaspi praecox* by the Slovenian partner laboratory (Pongrac et al. 2007). In salt marshes, the salt glasswort (*Salicornia europaea*, Chenopodiaceae) has been found to be colonized by AMF, but only at few sites and not throughout the year (Hildebrandt et al. 2001; Landwehr et al. 2002). The salt marsh grass, both on the European sea coast (*Puccinellia maritima*) and in inland marshes (*Puccinellia distans*), can exhibit AMF colonization even with arbuscule formation, but scores are nil with most samples taken from different saline habitats (Hildebrandt et al. 2001; Landwehr et al. 2002). Colonization of *Puccinellia* species by AMF may be further affected by a simultaneous interaction with the endophyte, *Epichloe typhina*, which colonizes aboveground parts of the grass (Olejniczak and Lembicz 2007). In the case of *P. distans*, this type of endophyte can have a negative effect on mycorrhizal development (K. Turnau, unpublished data; Guo et al. 1992), but in other cases, they can have a positive effect (Novas et al. 2009). These fungi cause toxicosis, and animals were shown to select individual plants that remained non-infected (Belesky and Bacon 2009) which might in some cases decrease the number of mycorrhizal plants.

The weak AMF colonization of *Puccinellia* roots could also indicate a non-beneficial interaction in this plant. A pathogenic interaction is unlikely since plants in salt marshes do not show impaired growth compared with non-colonized individuals (H. Bothe, unpublished observation). However, as for other plants, a symbiosis involving effective AMF can be considered a prerequisite to promote growth of endangered plants.

The many estimates that have been reported for mycorrhizal colonization of environmental samples vary greatly (between 0 and 100%). The threshold value for the degree of mycorrhizal colonization which will determine an effective symbiosis is not clear. The efficacy of AMF symbiosis for a plant is difficult to assess in the natural environment, and the data currently available principally refer to estimates of mycorrhizal colonization for which several non-comparable methods exist (e.g., Giovanetti and Mosse 1980; Trouvelot et al. 1986; Feldmann et al. 2008). Feldmann et al. (2009) stated that root colonization by

AMF should be of at least 20–30% for the symbiosis to be effective in terms of plant growth and that otherwise the plant will not or rarely benefit from AMF. Higher values than 30% determined by the gridline intersect method (Giovanetti and Mosse 1980) do not make the symbiosis more efficient (Feldmann et al. 2009). Obviously, all this will also depend on the AMF involved. An investigator therefore needs to take a large number of samples in order to state that an endangered plant is mycorrhizal or not, which is not necessarily coherent with the red-list status of the plants.

Do specifically adapted AMF exist that could be particularly effective in promoting the growth of endangered plants?

Plant diversity and nutrient acquisition have been reported to be enhanced with increasing fungal diversity (van der Heijden et al. 1998). In heavy metal soils, the frequency of mycorrhizal fungi (determined by spore counts in soil or AMF colonization of roots) is generally lower than in non-polluted soils (Hildebrandt et al. 1999; Tonin et al. 2001), but specifically adapted AMF fungi exist there that are apparently more effective in conferring heavy metal tolerance to plants than conventional isolates from culture collections (Weissenhorn and Leyval 1993; Leyval et al. 1997; Kaldorf et al. 1999). Maherali and Klironomos (2007) suggested, from experiments with *Plantago lanceolata*, that members of the Glomeraceae may be more effective in protecting plants against soil pathogens because they are better at colonizing roots than other AMF. In contrast, isolates of the Gigasporaceae efficiently mobilized P from soil due to their extensive formation of extraradical mycelia, while members of the Acaulosporaceae with lower root colonization levels and extraradical hyphae formation did not contribute significantly to either process (Maherali and Klironomos 2007). Habitat specificities also known to exist: members of the Glomeraceae tend to occur predominantly in clay soils whereas AMF of the Gigasporaceae tend to be more frequent in sandy soils (Lekberg et al. 2007). The development and activity of a single AMF can be affected by its coexistence of another AMF in one root system (Janouskova et al. 2009) or of specific bacteria (Hildebrandt et al. 2006a). The importance of mycorrhizal specialization has recently been evoked in the case of conservation attempts of the highly threatened hammer (*Drakaea*) and spider (*Caladenia*) orchids (Swarts and Dixon 2009). The search for AMF partners within the roots of endangered plants can yield a new fungal taxon, as recently shown for *Otospora bareai* isolated from rare and endemic plants near Granada, Spain (Ferrol et al. 2008). However, the symbiotic status of such fungi needs to be confirmed as well their eventual preference for the plants from which they were isolated. Less than 200 different AMF have been properly characterized to date, and others exist only as sequence deposits in world databases. Several studies have reported that the fungal sequences obtained from spores in soils and from roots can overlap but that they frequently do not match. Recent examples come from studies of salt marsh (Wilde et al. 2009) and grassland (Hempel et al. 2007) ecosystems. Repeated attempts to isolate and grow AMF described only from sequences consistently failed (e.g., Wilde et al. 2009), so that to find the optimal fungal combination that is most effective in supporting growth of a targeted endangered plant is a challenge for the future.

Can mycorrhizal fungi be exploited to prevent extinction of endangered plants?

Fungi such as *G. intraradices* and *G. mosseae* which are commonly used as symbionts for producing mycorrhizal plants, or a mixture of several fungi, could be used in any application to promote growth of endangered plants. As already mentioned, endangered as well as invasive plants can be colonized by AMF and they could, consequently, exploit indigenous AMF in their natural habitats (Fumanal et al. 2006; Zubek et al. 2008; Shah et al. 2009; Sharma et al. 2009; Pringle et al. 2009; see Fig. 3a, b). Inocula developed from native AMF isolated from the natural habitat of a plant are often found to be superior in promoting growth compared with laboratory cultures

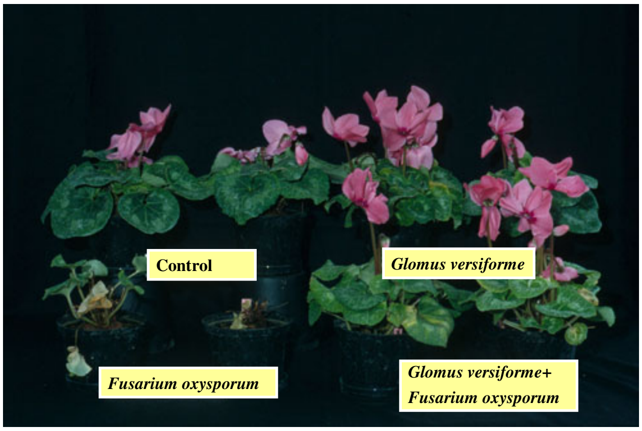

Fig. 4 Growth promotion by the arbuscular mycorrhizal fungus *Glomus versiforme* and the relief of the attack by the pathogenic *Fusarium oxysporum*. Two cyclamen plants for each application. **a** Control: cyclamen alone in sterilized soil. **b** *G. versiforme*: the plants supplemented with this arbuscular mycorrhizal fingus. **c** *F. oxysporum*: the plants supplemented with this pathogen. **d** *G. versiforme*+*F. oxysporum*: cyclamen supplemented with both fungi. Experiment and courtesy of Dr. H. Baltruschat, The University of Giessen, Germany

(Vosátka and Dodd 2002; Orłowska et al. 2005; Zubek et al. 2009). In a case study with *Arnica montana*, a plant endangered due to uncontrolled exploitation for medicinal use, colonization levels and production of secondary metabolites differed between plants inoculated with different AMF strains, although there was no significant differences in plant growth (Jurkiewicz et al. 2010). Here, however, laboratory strains were more effective at enhancing plant photosynthetic performance than crude inocula obtained from the natural stands (Jurkiewicz et al. 2010). While the addition of helper bacteria may enhance the beneficial effects of AMF on plant growth (e.g., Pivato et al. 2009), attempts to apply beneficial microorganisms (either bacteria or fungi) at field sites are often faced with the problem that they are out-competed by indigenous microbes for unknown reasons. Besides AMF, other fungi such as dark septate endophytes colonize plants in different ecosystems and may possibly also support plant growth, as pointed out by Fuchs and Haselwandter (2008).

As Hodge (2009) expressed, it is troublesome to predict how plants will respond to environmental changes due to their responsiveness and plasticity. For example, roots are very effective in responding to various nutrient patches. It might therefore be difficult, if not impossible, to improve growth of endangered plants in their *in situ* habitats. Any targeted application of AMF in habitats like grasslands, forest, or wetlands might not be feasible due to the complexity of indigenous communities and their delicate interactions influencing above- and belowground plant organs. Such an application may eventually be possible with really endangered plants that thrive in very specialized ecological niches, such as the Southern Alpine tertiary relicts *Saxifraga arachnoidea* or *Aquilegia thalictricifolia* that survive in caves with almost 100% water humidity under chalk rocks. Also, successful propagation of native plants for any habitat restoration would require an understanding of the dependency of these plants on mycorrhizal fungi and the biology of the AMF that grow there (Jayahandran and Fisher 2008).

An additional consideration is that large-scale applications would require the production of large amounts of inocula (Jeffries et al. 2003). Small companies nowadays sell AMF inocula (propagules in peat, expanded clay, or other carriers) for the production of ornamental plants in greenhouses or in private buildings. In horticulture, small amounts of AMF inocula can be supplied to plants in a relatively small volume of substrate and over a relatively limited surface area (Jeffries et al. 2003). Such an application can lead not only to enhanced growth of plants but also to earlier flowering and reduced damage by phytopathogenic fungi, as illustrated for cyclamen in Fig. 4. Cyclamen is a horticultural plant, but all related wild species in this genus occurring worldwide are in the critically endangered category. Thus, endangered plants could be multiplied and inoculated with appropriate AMF in the greenhouse and once at the appropriate developmental stage, they could be re-introduced into their natural habitats. The use of indigenous AMF and soils with comparable properties to those of natural sites would contribute to reducing significant disturbance of the community structure in endangered plants' natural sites.

Conclusions

Most published data on AMF in endangered plants are restricted to simple assessments of root colonization and identification of the AMF taxa involved by morphological or molecular criteria is rarely done. However, such approaches can lead to the characterization of new fungi, as shown by the study of endangered plants in the Spanish Sierra Nevada (Ferrol et al. 2008), and open the possibility of identifying AMF that supports optimal growth of an endangered plant species. The potential therefore exists to use AMF to promote growth and fitness of endangered plants and prevent their extinction. It is expected that research on this subject will be a center of interest in coming years.

Acknowledgements The authors are indebted to Dr. M. Geoffrey Yates, Lewes, Sussex, Professor Douglas Zook, Boston University, USA, and reviewers for helpful comments and for improving the English of the manuscript. MR acknowledges the support of the Slovenian research program ARRS P1-0212 “Biology of Plants”. KT was supported by project 197/N-COST/2008/0 financed by the Polish Ministry of Science and Higher Education. Meetings of several COST Actions: 838, 856, 859, and 870 gave valuable inputs for this review.

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