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SHORT NOTE

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Red list plants: colonization by arbuscular mycorrhizal fungi and dark septate endophytes

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Abstract Since information concerning the mycorrhization of endangered plants is of major importance for their potential re-establishment, we determined the mycorrhizal status of *Serratula tinctoria* (Asteraceae), *Betonica officinalis* (Lamiaceae), *Drosera intermedia* (Droseraceae) and *Lycopodiella inundata* (Lycopodiaceae), occurring at one of two wetland sites (fen meadow and peat bog), which differed in soil pH and available P levels. Root colonization by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) was quantified. Colonization by AMF appeared to be more frequent in the fen meadow than in the peat bog, and depended on the host plant. Roots of *S. tinctoria* and *B. officinalis* were well colonized by AMF in the fen meadow (35–55% root length) and both arbuscules and vesicles were observed to occur in spring as well as in autumn. In the peat bog, *L. inundata* showed a low level of root colonization in spring, when vesicles were found frequently but no arbuscules. In roots of *D. intermedia* from the peat bog, arbuscules and vesicles were observed, but AMF colonization was lower than in *L. inundata*. In contrast, the amount of AMF spores extracted from soil at the peat bog site was higher than from the fen meadow soil. Spore numbers did not differ between spring and autumn in the fen meadow, but they were higher in spring than in autumn in the peat bog. *Acaulospora laevis* or *A. colossica* and *Glomus etunicatum* were identified amongst the AMF spores extracted from soil at the two sites. *S. tinctoria* and *B. officinalis* roots were also regularly colonized by DSE (18–40% root length), while *L. inundata* was only rarely colonized and *D. intermedia* did not seem to be colonized by DSE at all.

Keywords Arbuscular mycorrhizal fungi · Dark septate endophytes · Red list plants · Colonization intensity · Soil P and spore density

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Introduction

Arbuscular mycorrhiza is frequent in the tropics (Janos 1980; Zhi-Wei 2001) as well as in arctic-alpine habitats (Haselwandter and Read 1980; Haselwandter 1987), and in wet (Rickerl et al. 1994; Ingham and Wilson 1999; Muthukumar and Udaiyan 2000) as well as dry habitats (Barrow et al. 1997; O'Connor et al. 2002). In addition, roots of many plants have been found to be colonized also by dark septate endophytes (DSE) (Sieber 2002; Jumpponen and Trappe 1998) and, in some ecosystems, they can even become the dominant root colonizers (Read and Haselwandter 1981). Since some strains of DSE have been shown to form mutualistic associations (e.g. strains C1 and C2 of Haselwandter and Read 1982), and are thus seen to be mycorrhizal (Jumpponen 2001), it is important to consider these fungi as well as arbuscular mycorrhizal fungi (AMF) in ecosystem studies. Analysis of the small subunit of the nuclear ribosomal RNA gene (18S) placed strain C2 of a DSE in close vicinity to *Phialocephala fortinii* (Jumpponen and Trappe 1998).

Wetland habitats like fen meadows or peat bogs are characterized by a unique flora that includes several endangered species. Nevertheless, these habitats are threatened in various ways. In some areas fen meadows are drained and fertilized for agricultural use. Peat is still cut in some peat bogs, and in addition such ecosystems may be affected by an input of toxic elements and high amounts of nutrients by wet and dry deposition (Colditz 1994). This leads to a disturbance not only of the plant communities, but also of the fungi living in the soil. It has been demonstrated that such disturbances of the soil can

reduce the distribution and abundance of AMF (Brundrett 1991). Information on the mycorrhization of plant species is important for re-establishment programmes. Hence, this project was set up to determine the mycorrhizal status of four endangered plant species occurring in two different wetland habitats.

Materials and methods

The plant species investigated in this project are members of the red list of endangered species of vascular plants of the county of Salzburg (Wittmann et al. 1996) and comprise *Lycopodiella inundata* (L.) Holub (Lycopodiaceae within the Pteridophyta) and the dicotyledonous angiosperms *Drosera intermedia* Hayne (Droseraceae), *Serratula tinctoria* L. (Asteraceae) and *Betonica officinalis* L. (Lamiaceae). In spring and autumn, plants were collected at two different wetland sites, peat bog (47°46′N, 13°00′E; 435 m; dystric histosol) and fen meadow (47°45′N, 13°00′E; 435 m; gleyosol), in or close to the city of Salzburg, Austria. On a scale from 0 (extinct in the wild) to 4 (susceptible), the plant species collected from the peat bog were *L. inundata* (critically endangered) and *D. intermedia* (endangered). In addition, *Molinia caerulea* (L.) Moench (Poaceae) was investigated as a neighboring plant. In the fen meadow, the two plants of interest were *S. tinctoria* (endangered) and *B. officinalis* (locally critically endangered). Here we examined *M. caerulea* and *Carex* sp. (Cyperaceae) as concomitant plants. In general, a minimum of three plant specimens per site were sampled together with the surrounding soil in spring and autumn 1999. Plants were excavated with a spade including about 100 ml soil, transferred to plastic bags and kept in a cooling box for transport. Samples were stored at 12°C before use. Water content of the soil samples was immediately measured by drying a sub-sample at 105°C until the sample retained a constant weight (Öhlinger 1993). The plants were carefully removed from soil (used for chemical analysis and spore extraction) and washed. The roots were stained with trypan blue in lactoglycerol following a standard procedure (Kormanik and McGraw 1982). As the roots of *D. intermedia* are dark-pigmented they were bleached with 30% H_2O_2 before they were stained. For microscopic analysis, slides were prepared by mounting stained roots in 50% glycerol and sealing them with nail polish. The colonization intensity of AMF and DSE was measured by a gridline intersection procedure using a compound microscope (Reichert Zetopan) at 125× magnification (Giovannetti and Mosse 1980). A minimum of 3×20 g rhizosphere soil per plant species was taken for extraction of AMF spores in spring and in autumn. The soil samples were sieved (250 µm, 90 µm and 38 µm), centrifuged twice (in water and 50% sucrose; 2,000 rpm, 5 and 1 min, respectively), and the spores transferred to a filter (0.45 µm, Nalge Company) with gridlines (Daniels and Skipper 1982). Under a dissecting microscope (Nikon SMZ-2B; 50× magnification) the spores were grouped according to their morphological features, counted and identified. For identification of AMF spores, slides were prepared using PVLG (polyvinyl alcohol-lacto-

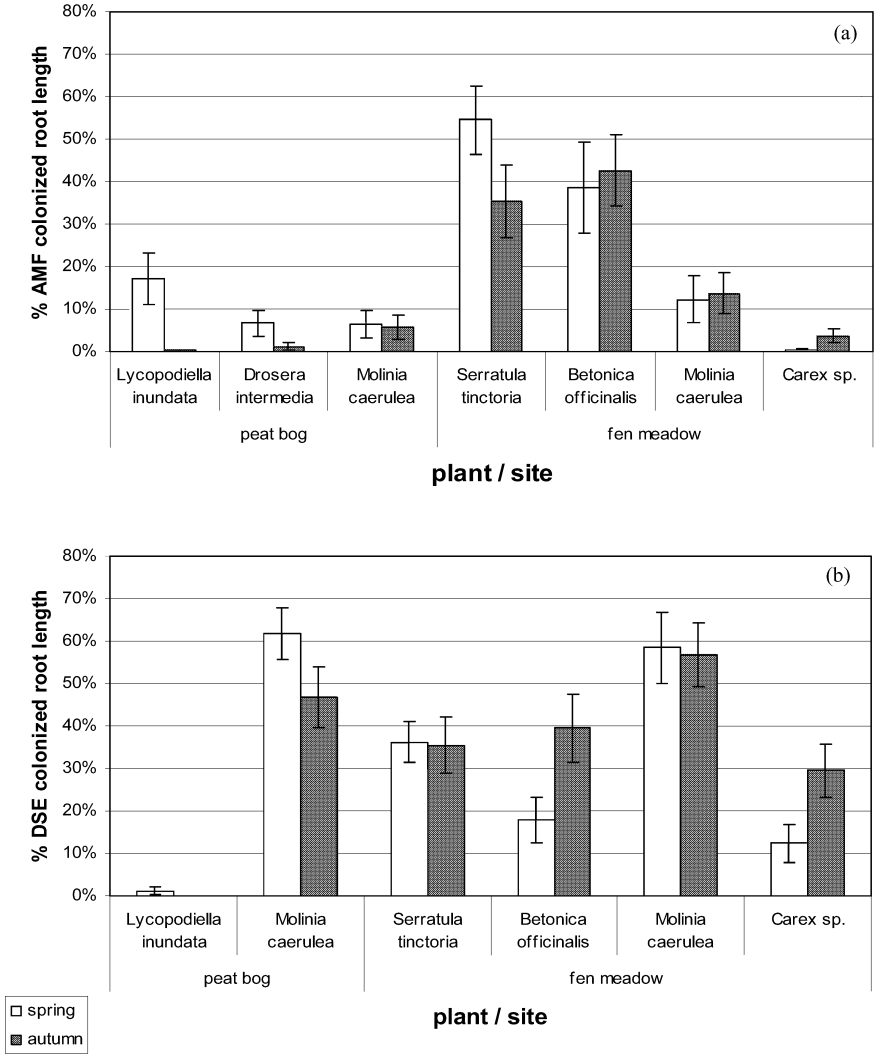

Fig. 1 Arbuscular mycorrhizal fungi (AMF; **a**) and dark septate endophyte (DSE; **b**) colonization intensity (mean ± standard error) of the endangered and concomitant plant species at the beginning and end of the growing season. Intraspecific comparison of the level of colonization between spring and autumn revealed no significant difference

glycerol) or PVLG/Melzers's reagent (mixed 1:1, v/v) as the mounting medium (Brundrett et al. 1996). In addition, the total and plant-available P contents of soils were determined according to methods modified from Jackson (1958) and ÖNORM L 1088 (1989), respectively. Statistical analysis of the data was carried out using the Mann-Whitney U-test of SPSS, version 10.0, at the significance level $\alpha \leq 0.05$. The Pearson correlation coefficient was calculated by using the programming language R (version 2.1.7) at a significance level of $P \leq 0.05$.

Results

In general, plant species collected from the peat bog showed lower AMF colonization than plants sampled in the fen meadow (Fig. 1a). Whereas *L. inundata* was slightly AMF colonized in spring (≤17% root length) in the peat bog, in autumn colonization by AMF was practically non-existent. Although this plant species shows frequent occurrence of vesicles in spring, arbuscules could not be found so far. In the peat bog, *D. intermedia* was also occasionally colonized by AMF (1–7% root length, with occurrence of arbuscules and vesicles), but colonization intensity reached a lower level than in the case of *L. inundata*. In autumn the colonization intensity of both endangered plant species was much lower than in spring, albeit not significantly. *M. caerulea*, which was collected as concomitant plant in the peat bog, was also poorly colonized (6–7% root length) and produced arbuscules and vesicles at both sampling dates. *M. caerulea* is the only plant species investigated in this study in which hyphal coils of the *Paris*-type were detected. All the other plant species formed AM colonization of the *Arum*-type. In spring *L. inundata* was very slightly colonized by DSE (1% root length; Fig. 1b); microsclerotia could not be observed. *D. intermedia* did not show any DSE colonization at all. *M. caerulea* was fairly well colonized by DSE (47–62% root length) with occurrence of microsclerotia.

The fen meadow samples taken in spring and autumn revealed that roots of *S. tinctoria* and *B. officinalis* were regularly colonized by AMF (35–55% root length) at both sampling dates (Fig. 1a). Vesicles were observed frequently, but arbuscules—some of them even degenerated—were less abundant. *M. caerulea* was less colonized by AMF (12–14% root length), with formation of arbuscules and vesicles at both sampling dates. *Carex* sp. was only slightly colonized (≤4% root length) with production of some vesicles, but no arbuscules. Both of the endangered species, *S. tinctoria* and *B. officinalis*, growing in the fen meadow showed a DSE colonization intensity of 18–40% (Fig. 1b) including microsclerotia formation. In the case of the neighboring plants, *M. caerulea* (with 57–58% colonized root length) was more intensely colonized by DSE than *Carex* sp. (12–30% root length); microsclerotia were observed in the roots of these plant species at both sampling dates.

Spore numbers in the peat bog soil were higher than in the soil of the fen meadow. The total number of spores per gram dry weight of soil extracted from the peat bog

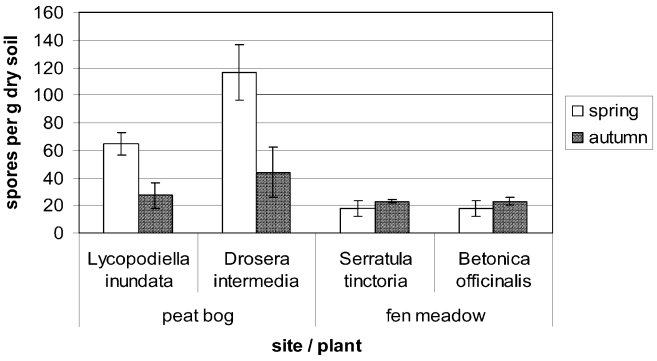

Fig. 2 Comparison of the total number of AMF spores per gram dry soil (mean ± standard error) at the two different locations at the beginning and end of the growing season

exhibited some seasonal fluctuations (Fig. 2). The spore density in rhizosphere soil was measured separately for each plant species. Spore density per gram dry soil of the peat bog was much higher in spring than in autumn, whereas in the fen meadow the total number of spores was at about the same level in spring and in autumn. Some AMF spores extracted from peat bog soil were identified as *Acaulospora colossica* or *A. laevis*. Both *Glomus etunicatum* and *Acaulospora* sp. were abundant in the soil of the fen meadow.

Several soil characteristics varied between the sites. The pH, measured in 0.01 M Ca-lactate, was slightly higher in the fen meadow (pH 5.4) than in the peat bog (pH 3.2). At 88% in the peat bog the soil water content was much higher than in the fen meadow (48%). The plant-available P content was four times higher in the peat bog (0.0101 ±0.0008%) than in the fen meadow (0.0025±0.0004%), whereas total P content varied only slightly between peat bog and fen meadow (0.0773±0.0033% and 0.0660 ±0.0034%, respectively).

Statistical comparison of both AMF and DSE colonization intensities of the plant species did not show a significant difference at $\alpha \leq 0.05$ between spring and autumn. Whereas the Pearson correlation coefficients calculated for AMF and plant-available P, as well as DSE and plant-available P, did not reveal a consistent pattern, for AMF or DSE and pH a pattern seems to exist. For *Carex* sp. growing in the fen meadow a significant negative correlation ($r=-0.821$) exists between AMF colonization and pH, whereas in the peat bog a significant $P \leq 0.05$ positive correlation ($r=0.894$) was found for *D. intermedia*. The correlation between DSE and pH followed the same pattern. In the fen meadow *B. officinalis* and *Carex* sp. showed a negative correlation ($r=-0.921$ and $r=-0.914$, respectively), whereas in the peat bog *M. caerulea* ($r=0.866$) showed a significant positive correlation.

Discussion

Although both the fen meadow and peat bog are rather wet locations, endangered plants collected in these habitats are

all more or less colonized by AMF with occurrence of vesicles and, except in the case of *L. inundata*, also arbuscules. These findings agree with other literature confirming the occurrence of AMF in wetland habitats. Søndergaard and Laegaard (1977) were among the first to prove the existence of AMF even in wetland plants like *Littorella uniflora* and *Callitriche hamulata*. Miller (2000) showed AMF colonization of semi-aquatic grasses along a wide hydrologic dry-to-wet gradient.

Haselwandter (1997) pointed out that a functioning soil flora, including AMF, is a prerequisite for successful re-vegetation projects. Thus, knowledge of the arbuscular mycorrhizal status of endangered plants is needed to be able to prepare a soil environment as natural as possible for plants to be established in restoration projects (Turnau and Haselwandter 2002). Spores extracted from plant-specific habitats provide the basis for single-spore cultures that would serve for identification of the fungi and, together with spores from trap cultures, as inoculum source for re-establishment programmes of endangered wetland plant species.

The results presented above did not reveal any consistent correlation between plant-available P and intensity of colonization. This is in agreement with other literature as in natural ecosystems a correlation between P level and colonization intensity must not necessarily exist (Brundrett 1991) since different AMF species seem to be adapted to different P levels (Land and Schönbeck 1991). The pH seems to have at least some influence on AMF colonization intensity, which can be slightly higher in alkaline versus acid soils (Clark and Zeto 1996). An important parameter that could have influenced fungal colonization is the soil water content, which is considerably higher in the peat bog than in the fen meadow. Miller (2000) pointed out that AMF colonization declines with increasing water level, possibly due to lower oxygen availability and higher solubility of toxic elements (Khan and Belik 1995). Factors like those listed above might explain why Thormann et al. (1999) did not find AMF in any of the herbaceous species they investigated along a peatland gradient.

So far, the role of DSE in the ecosystem is not clearly understood, the relationship between host plants and DSE ranging from mutualistic to parasitic associations. In a growth experiment, Haselwandter and Read (1982) showed an increase in dry weight of roots and shoots and an increase in shoot P content of alpine *Carex* species following inoculation with two strains of DSE. A recent study demonstrated the potential of a typical dark septate root endophyte, *Phialocephala fortinii*, to produce siderophores, which are suggested to play a key role in iron nutrition (Bartholdy et al. 2001). Therefore, it might well be possible that plant nutrition and, in the long run, plant fitness benefit from colonization by DSE in a similar way as is known for AMF. Hence, at least some of the DSE strains isolated so far can be considered to form a true mycorrhizal relationship (Jumpponen 2001). Further information on the functional relationship of endangered plants and DSE is needed, especially as *S. tinctoria* and *B. officinalis* showed a regular colonization by such fungi.

This study provides basic information on the mycorrhizal status of four endangered plant species. Further studies are needed to analyze AMF identities in plant roots by the use of molecular biological methods. Information of this kind is a pre-requisite for comparing the biodiversity of AMF in the plants with that in the soil.

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