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## **Mycorrhizae and Other Root-Associated Fungal Structures of the Plants of a Sandy Grassland on the Great Hungarian Plain**

By

Gábor M. KOVÁCS\*) and Csaba SZIGETVÁRI\*\*)

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### **Summary**

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The results of the studies of the mycorrhizae and other root-associated fungal structures of 87 plant species belonging to 29 different families originated from a strictly protected sandy grassland area of the Great Hungarian Plain are presented. Two-thirds of the studied species formed endomycorrhizae. Quantitative characteristics of the mycorrhizal colonization of their roots have been estimated. Considering the coenological features and the dominance relations of the plant community, the area is dominated by mycorrhizal plants. Only a few plants of the families considered as non-mycorrhizal groups have been colonized by mycorrhizal fungi. The annual species characteristic to the cold-season and to the dry-season subpessimum period are mostly non-mycorrhizal plants, this seems to be some separation in time from the mycorrhizal plants. Endogenous septate fungal hyphae and microsclerotium-like structures have been observed in the roots of the majority of the plants; this phenomenon is probably related to the stressful environment.

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\*) Gábor M. KOVÁCS, University of Szeged, Department of Botany, Hungary; Present address: Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581, Budapest, P.O.Box: 18, Hungary. Fax: +36-1-467-4076; email: [gko-vacs@linux.vMRI.hu](mailto:gko-vacs@linux.vMRI.hu)

\*\*) Csaba SZIGETVÁRI, University of Szeged, Department of Ecology, H-6701, Szeged, P.O.Box: 51, Hungary. E-mail: [szigetva@sol.cc.u-szeged.hu](mailto:szigetva@sol.cc.u-szeged.hu)

### Zusammenfassung

KOVÁCS G. M. & SZIGETVÁRI C. 2002. Mykorrhiza und andere wurzelassoziierte Pilzstrukturen von Pflanzen eines sandigen Graslandes der großen ungarischen Ebene. – *Phyton* (Horn, Austria) 42 (2): 211–223. – Englisch mit deutscher Zusammenfassung.

Es werden hier die Untersuchungsergebnisse von 87 Pflanzenarten aus 29 verschiedenen Familien, die Mykorrhiza und andere wurzelassoziierte Pilzstrukturen betreffen, dargestellt. Die Pflanzen stammen von einer streng geschützten Fläche vom Grasland der großen ungarischen Ebene. Zwei Drittel der untersuchten Arten besitzen Endomykorrhiza. Eine quantitative Abschätzung der Eigenschaften des Mykorrhizabesatzes ihrer Wurzeln wurden durchgeführt. Unter Einbeziehung aller pflanzensoziologischen Faktoren und Dominanz von Pflanzengesellschaften wird die Untersuchungsfläche von Mykorrhizapflanzen beherrscht. Nur ganz wenige Pflanzen aus Familien, die als nicht mykorrhiziert gelten, werden von Mykorrhizapilzen besiedelt. Die für die ungünstige Zeit der Kälte- und Trockenperiode des Jahres charakteristischen Arten, bestehen in der Regel aus nicht mykorrhizierten Pflanzen, was darauf hindeutet, dass eine gewisse zeitliche Trennung zwischen mykorrhizierten und nicht mykorrhizierten Pflanzen besteht. Endogene septierte Pilzhypen und microsclerotium-ähnliche Strukturen wurden in den Wurzeln der meisten Pflanzen beobachtet und diese Beobachtung wird mit den stressreichen Umweltbedingungen in Zusammenhang gebracht.

### Introduction

One of the most characteristic habitat types of the Great Hungarian Plain is the mosaic of steep sand dunes and depressions, where due to the continental climate and poor soil conditions, a special plant community, the open sand grassland with some semi-desert characteristics develops (FEKETE & al. 1995). The vegetation of these special habitats is one of the most studied vegetation types in the region (see FEKETE & TÓTHMÉRÉSZ 1993, KOVÁCS-LÁNG 1993).

In spite of the great botanical interest, the flora of the special habitats of the Great Hungarian Plain has received low attention from the mycorrhizal point of view and only a few mycorrhizal data originating from its grassland communities have been published up to now. A part of them concerns the ectomycorrhizae of two shrub species (JAKUCS & al. 1999, MAGYAR & al. 1999, KOVÁCS & JAKUCS 2001). Some plants of three grassland habitats have been studied regarding their mycorrhization (PARÁDI & al. 1998, BRATEK 2000).

The occurrence and importance of the mycorrhizal colonization, especially by arbuscular mycorrhizal fungi, are generally known in grassland plant communities and in dune areas, and the mycorrhizae of their plants have been well studied (e.g. MEJSTRIK 1972, READ & al. 1976, MOLINA & al. 1978, HOPKINS 1987, MILLER 1987, KOSKE 1988, BLASCHKE 1991, BLASZKOWSKI 1994, DHILLON & al. 1995, PAWLOWSKA & al. 1996, CORDICI & RIN-

CÓN 1997, FONTENLA & al. 2001). Plant species of grassland habitats have been also used in mycorrhizal experiments (ALLEN & ALLEN 1984, FRANCIS & READ 1995, MILLER & al. 1997, VAN DER HEIJDEN & al. 1998a, VAN DER HEIJDEN & al. 1998b).

The main aims of the studies presented here were to obtain data about the mycorrhizal status and other endogenous fungal structures of the plants of a strictly protected sand dune area of the Kiskunság National Park on the Great Hungarian Plain. We also wanted to check whether there is any relationship between the mycorrhizal data of certain plant species and their coenological-ecological characteristics in this grassland community. The study of the endogenous fungal structures of the plants of this area also fits into the project of the investigation of the mycorrhizal status of the flora of the Great Hungarian Plain.

### Material and Methods

#### The sampling area

The studies have been conducted in a sand dune area of the territory IV of Kiskunság National Park near Fülöpháza (46°52–54' N, 19°23–25' E), the altitude is between 100–120 meters. The sand dunes of the area consist of basic wind-blown sand of Danubian origin. The soil of the sand hills is a very poor basic skeleton soil, without expressed soil profile differentiation, clay and colloid fraction is extremely low, and the humus content is less than 1%. The pH measured in distilled water is around or above eight, nutrient concentration is very low (VÁRALLYAI 1993, KOVÁCS-LÁNG & al. 2000). The soils of the depressions have a slightly higher humus fraction and lower pH. The climate of the area is of moderate continental type, the mean annual temperature is 10–11 °C, annual precipitation is about 550 mm with a maximum in June and November. The number of sunny hours is about 2050 per year.

Most of the area is covered by open perennial grassland dominated by xerophilous grasses (like *Festuca vaginata*, *Stipa borysthena*, *Koeleria glauca*) and on the poorest soils by the dwarf-shrub, *Fumana procumbens*. In deeper depressions a meadow-like community is found with denser vegetation formed by *Poa angustifolia*, *Calamagrostis epigeios* and the shrub *Salix repens* ssp. *rosmarinifolia*. Patches of the open annual grassland (dominated by grasses, such as *Secale sylvestre*, *Bromus squarrosus* and *Bromus tectorum*) and weedy species develop where disturbance (e.g. grazing, trampling, drying) destroyed or degraded the original vegetation and nutrients have accumulated in the soil. The sand vegetation types are described in details in MAGYAR 1933, HARGITAI 1940, SOÓ 1957, BIRÓ & MOLNÁR 1998 and BORHIDI & SÁNTA 1999. A considerable proportion of the area, mainly the sites adjacent to dirt roads and those formerly occupied by open perennial grassland, has been invaded by the grass *Cleistogenes serotina* during the last two decades (BAGI 1997, 2000, MOLNÁR 2000). Shrubs and trees are scattered all over the area sometimes forming small patches. As remnants of earlier afforestation efforts, non-indigenous trees also appear in the area (e.g. *Pinus* species, hybrid *Populus* species).

### Root sampling and preparation

Root samples were collected between April and November of 2000. Samples were taken of two or three specimens of each species with the least disturbance from the characteristic habitat of the plant during their flowering period. The roots were put into plastic bags with wet soil and they kept at 4 °C until preparation.

The roots were gently washed in tap water and studied by dissecting microscope. All samples were marked with codes to avoid preconception. To prepare the samples the method used by GRACE & STRIBLEY 1991 was followed with slight modifications. Parts of the root samples were cleared at 60 °C overnight in 10% KOH. If the discolouring was not successful, then the samples were kept at 90 °C for a sufficient time. Afterwards the cleared roots were washed in distilled water for two hours, the water was changed several times. The last washing water was acidified (pH 3-3.5 approximately) with some drops of lactic-acid, and the roots were kept in this solution for 30 minutes. Roots were stained in aniline-blue lactic-acid solution (0.5 g aniline-blue in 30 ml cc. lactic-acid) for 10 minutes. The stained samples were put back into the acidic washing solution to wash out the superfluous stain. Afterwards the root-samples have been ordered on slides and covered with lactic-acid. Remaining roots of the specimens have been put into FEA (formaldehyde : ethanol 70% : acetic-acid; 5:90:5). The voucher materials and the preparations have been deposited into the collection of G. M. KOVÁCS with serial numbers between FH001-FH107.

### Mycorrhizal colonization

The qualitative and quantitative characterization of the colonization were carried out by light microscope. Ectomycorrhizal structures and the typical structures of endomycorrhizae – like vesicles, arbuscules and hyphal coils – were checked. Other fungal structures were also studied. The presence of endogenous septate hyphae was marked. Microsclerotium-like structures resembling e.g. the structures formed by *Microdochium bolleyi*-like fungus (VÅRE & al. 1992) or the *Phialophora* type structures (e.g. READ & HASELWANDTER 1981) were marked. Structures resembling e.g. the sporangia of *Olpidium brassicae* (WOR.) DANG. (VÅRE & al. 1992) were marked as sporangia-like structures. External septate, broad septate hyphae and pigmented, warty hyphae resembling the aerial hyphae of e.g. *Phialocephala fortinii* WANG & WILCOX (WANG & WILCOX 1985) were also marked. The fungal structures described above were used only as qualitative characteristics.

In the case of endomycorrhizal plants the extent and the intensity of the colonization were estimated by a visual method. Five classes were used to characterize the extent of the colonization: class 1: up to 5%; class 2: 6-25%; class 3: 26-50%; class 4: 51-75%; class 5: 76-100% of the root-length colonized (HOPKINS 1987). The intensity of the colonization were characterized by three classes: class A: the colonization at small, widely scattered sites; class B: larger, but separate sites; class C: almost solidly colonized (HOPKINS 1987).

A species was considered as forming endomycorrhizae and the characteristics of the colonization were estimated only if the hyphal coils or arbuscules were observed.



## Results

Root samples of specimens of 89 plant species belonging to 29 different plant families were collected and analysed during the studies (Table 1).

The root samples of 29 species were considered as lacking mycorrhizal structures. Only vesicles, but no arbuscules and hyphal coils were observed in three instances, namely *Minuartia verna* ssp. *collina*, *Silene otites* and *Sedum hillebrandtii*. 62% of the 29 non-mycorrhizal species belong to Brassicaceae, Caryophyllaceae and Chenopodiaceae – 5, 9 and 4 species, respectively (Table 1). The roots of 18 species of the non-mycorrhized plant species contained endogenous septate hyphae and 14 of these also had microsclerotium-like structures; while 6 of them had sporangia-like structures in their roots (Table 1). The roots of some species, like *Alyssum tortuosum*, *Erophila verna* and some species from the Caryophyllaceae, were colonized intensively with these endogenous fungal structures. Exogenous septate hyphae were observed on the roots of 22 species from the non-mycorrhized ones. Broad septate hyphae were observed on 14 species, and in five case pigmented, warty hyphae were found (Table 1).

The roots of 60 plants – 67% of the studied species – contained mycorrhizal structures. The common occurrence of vesicles, arbuscules and hyphal coils in the roots of one species was observed in more than the half of the species (Table 1). The intensity of colonization was quite high, 38 C class, 19 B and just 3 A class were estimated. The extent of the colonization was also quite high, 78% of the mycorrhizal species belong to class 4 and 5, 23 and 21 species respectively (Table 1). Ectomycorrhizal structures were observed on the roots of five species – *Acer negundo*, *Fumana procumbens*, *Helianthemum ovatum*, *Populus alba* and *Salix repens* ssp. *rosmarinifolia* – and all of them had other, endogenous mycorrhizal structures, too (Table 1). The roots of three-quarters of the mycorrhizal plants contained septate, endogenous hyphae besides mycorrhizal structures, 22 of the plants colonized by septate endogenous hyphae had microsclerotium-like structures and only two species had sporangia-like structures in their roots (Table 1). Approximately half of all the species had exogenous septate hyphae extending from their roots, 17 of them had broad septate hyphae, and in the case of five of them, pigmented, warty type of hyphae were also present (Table 1).

Less than half of the studied species have been previously reported in European mycorrhizal status studies (e.g. HARLEY & HARLEY 1987, HARLEY & HARLEY 1990, BLASZKOWSKI 1994, PAWLOWSKA & al. 1996, BARNI & SINISCALCO 2000). Exclusively data on genera were found for approximately one-third of the species (e.g. *Linaria genistifolia*, *Veronica praecox*, *Crepis rhoedifolia*). No data were found for 25% of the species (e.g. *Helichrysum arenarium*, *Cenchrus incertus*, *Alyssum tortuosum*).

Table 1.

Mycorrhizae and other root-associated structures of the plants of the studied sand grassland from the Great Hungarian Plain, ordered by families. (I: intensity of colonization; E: extent of colonization; n: considered as non-mycorrhizal species V: vesicles; A: arbuscules; L: hyphal coils, loops; E: ectomycorrhizae; s: septate endogenous hyphae; ms: microsclerotium-like structures; sp: sporangia-like structures; Ex: exogenous septate hyphae; a: broad, septate hyphae; v: warty, broad, septate hyphae).

Plant	I	E	Structures	Ex.	Plant	I	E	Structures	Ex.
Aceraceae					Dipsacaceae				
<i>Acer negundo</i> L.	C	5	V,A,E+s,ms	es	<i>Scabiosa ochroleuca</i> L.	B	4	V,L+s,ms	es
Apiaceae					Euphorbiaceae				
<i>Pimpinella saxifraga</i> L.	C	4	V,A,I+s,ms		<i>Euphorbia cyparissias</i> L.	n			
Asclepiadaceae					<i>Euphorbia seguieriana</i> Necker	B	4	V,A,L	
<i>Asclepias syriaca</i> L.	C	3	V,A	es	Fabaceae				
Asteraceae					<i>Astragalus onobrichis</i> L.	C	5	V,A,I+s,ms	es
<i>Ambrosia artemisiifolia</i> L.	B	4	V,A,L		<i>Astragalus varius</i> Gmel.	C	5	V,A+s	es
<i>Anthemis ruthenica</i> M. B.	C	4	V,A,I+s	es	<i>Cytisus ratibonensis</i> Schaeff.	A	2	V,A,I+s	
<i>Artemisia campestris</i> L.	C	5	V,A,I+s		<i>Medicago minima</i> (L.) Grufbg.	C	5	V,A,I+s,ms	a,es
<i>Centaurea arenaria</i> M. B. ex Willd.	C	4	V,A,I+s,ms		<i>Ononis spinosa</i> L.	C	5	V,A,I+s	
<i>Chondrilla juncea</i> L.	B	4	A,I+s		Lamiaceae				
<i>Conyza canadensis</i> (L.) Cronqu.	C	4	V,A,I+s		<i>Teucrium chamaedrys</i> L.	C	4	V,A,I+s,ms,sp	a,es
<i>Crepis rheoadifolia</i> M. B.	C	5	V,A,I+s,ms	a,es,v	<i>Thymus odoratissimus</i> Mill.	C	4	V,A+s	es
<i>Echinops ruthenicus</i> (Fisch.) M. B.	C	5	V,L		Liliaceae				
<i>Helichrysum arenarium</i> (L.) Moench	C	4	V,A,I+s		<i>Asparagus officinalis</i> L.	C	5	V,A,L	
<i>Odonites lutea</i> (L.) Clairv.	C	3	L+s	es	<i>Muscari comosum</i> (L.) Mill.	B	3	V,A,I+s,ms	
<i>Senecio vernalis</i> W. et K.	n			a,es	Onagraceae				
<i>Solidago virga-aurea</i> L.	C	4	V,A,I+s	a,es	<i>Oenothera biennis</i> L.	B	3	A+s,ms,sp	
<i>Tragopogon floccosus</i> W. et K.	B	3	V,A,L		Plantaginaceae				
Berberidaceae					<i>Plantago arenaria</i> W. et K.	B	3	V,A,I+s	
<i>Berberis vulgaris</i> L.	C	4	V,A,I+s,ms	es	Poaceae				
Boraginaceae					<i>Boerhaavia ischaemum</i> (L.) Keng	B	3	V,L+s,ms	a,es
<i>Alkanna tinctoria</i> (L.) Tausch	C	5	V,A,L		<i>Bromus squarrosus</i> L.	C	4	V,A+s,ms	es
<i>Buglossoides arvensis</i> (L.) I. M. Johnston.	B	2	A+s	es	<i>Bromus tectorum</i> L.	n			es
<i>Myosotis stricta</i> Link	n			a,es,v	<i>Calamagrostis epigeios</i> (L.) Roth	C	4	V,A,I+s	es,a,v
<i>Onosma arenaria</i> W. et K.	C	5	V,A,I+s		<i>Cenchrus incertus</i> M. A. Curtis	n		s,ms	es



## Discussion

Although several grassland and dune plant communities and some arid areas have been studied regarding the mycorrhizae of their plants up to now, the geographic, climatic and edaphic differences of the presently studied area are considerable. Previous data on grasslands and arid areas mostly originated from other continents (e.g. MILLER 1987, DHILLON & al. 1995, FONTENLA & al. 2001) and the data from sand dunes mainly are from coastal dunes (NICOLSON 1960, KOSKE & HALVORSON 1981, GIOVANETTI & NICOLSON 1983, KOSKE 1988, KOSKE & GEMMA 1990, BLASKOWSKI 1994, CORKIDI & RINCÓN 1997). Although previous mycorrhiza-studies about sandy grasslands in the Hungarian Plain have been published, these have either concentrated on just one plant species (JAKUCS & al. 1999, MAGYAR & al. 1999, KOVÁCS & JAKUCS 2001), or the number of the species studied was quite low and the results have been poorly documented (PARÁDI & al. 1998, BRATEK 2000).

Previous studies on similar plant communities report that nearly all the plants were colonized by arbuscular mycorrhizal fungi (PARÁDI & al. 1998, BRATEK 2000). However, in the present study a relatively high percentage of the plant species was found to be not colonized by endomycorrhizal fungi. Most of them are from families known as non-mycorrhizal, such as Chenopodiaceae, Caryophyllaceae and Brassicaceae. Some species (*Arenaria serpyllifolia*, *Dianthus serotinus*, *Silene borysthenica*, *Silene conica*, *Minuartia verna*, *Salsola kali*, *Bassia sedoides* and *Alyssum montanum*) were studied in the previous works (PARÁDI & al. 1998, BRATEK 2000), and most of them were reported as mycorrhizal.

Most of the annual species characteristic either to the cold-season subpešsumum period (early spring), like *Arenaria serpyllifolia*, *Erophila verna*, *Cerastium semidecandrum*, *Holosteum umbellatum* and *Myosotis stricta*, or to the dry-season subpešsumum period (late summer), like *Bassia laniflora*, *Corispermum nitidum*, *Corispermum canescens*, *Polygonum arenarium* and *Salsola kali*, were found to be non-mycorrhizal. The grouping of grassland species by aspects (KÁRPÁTI & KÁRPÁTI 1954) suggests similar results namely that the non-mycorrhizal plants appear mostly in the early and late aspects. This could be considered as a segregation in time of non-mycorrhizal plants from mycorrhizal ones to avoid a too strong competition, which has been reported previously as a strategy in space (FRANCIS & READ 1995). Considering that most of the non-mycorrhizal plants are non-dominant and this character is probably also true at the rhizosphere – most of these plant species have a weak, shallow root system (SIMON & BATA-NOUNY 1971) – this could be interpreted also as a kind of separation from mycorrhizal plants. As additional relations between the mycorrhizal features and the available coenological data could not be considered, fur-



ther studies of the vegetation are necessary to conclude on a possible correlation between the mycorrhizal features and the community structure.

The dominant plants of the open perennial grassland covered the greatest part of the area are colonized by mycorrhizal fungi. These species are xerophilous grasses, like *Festuca vaginata*, *Stipa borysthénica*, *Koeleria glauca*, and on poorest soils, the dwarf-shrub *Fumana procumbens* (Table 1). The plant species dominating the more dense meadow-like vegetation of the deeper depressions, like *Poa angustifolia*, *Calamagrostis epigeios* and the shrub *Salix repens* ssp. *rosmariniifolia*, are also colonized by mycorrhizae. The most dominant grass species of the open annual grassland (*Secale sylvestre*) is mycorrhizal, just like *Bromus squarrosus*, an other important grass species of this vegetation type, although *Bromus tectorum* has not shown any mycorrhizal colonization (Table 1). Most of the weed species dominating the disturbed areas also form mycorrhizae. The grass *Cleistogenes serotina*, for example, is well colonized by mycorrhizal fungi. This species has invaded considerable part of the studied area since the 1970's, but its role in the grassland community has not been clarified yet (BAGI 1997, 2000, MOLNÁR 2000). Despite of the high percent of the non-colonized species, due to the dominance relations of the studied plant community, the investigated area could be regarded as dominated by mycorrhizal plants.

Most of the aggressive and – considering the scale of the area – invasive species (like *Ailanthus altissima*, *Asclepias syriaca*, *Cleistogenes serotina*, *Ambrosia artemisiifolia* and *Conyza canadensis*) are colonized by endomycorrhizal fungi, just as in other areas of the Great Hungarian Plain (KOVÁCS & BAGI 2001). This result is in accordance with some theories about the endomycorrhizal connections of invasive plant species (RICHARDSON & al. 2000). Although the invasive *Cenchrus incertus* has no mycorrhizal colonization, this species is a pioneer usually successful on open sand surfaces in the studied area (DE LISLE 1963, SZIGETVÁRI 1999).

The mixed colonization of the predominantly ectomycorrhizal species is in accordance with the previously presented data regarding *Populus alba*, *Salix repens* and about the genera *Acer* and *Helianthemum* (HARLEY & HARLEY 1987). The hypothesis about an ontogenetic succession on the roots of *Fumana procumbens* from endo- to ectomycorrhizae (BRATEK 2000) could not be verified in the present study. Both dwarf-shrub species from the *Cistaceae* (*Helianthemum* and *Fumana*) have ecto- and endomycorrhizae, but no relation was found between the ontogenetical state and the type of mycorrhization of them.

Septate endophytic hyphae and more complex structures – usually belonging to the dark-septate group – have been reported from nearly all the climatic areas from a wide range of the host plants (JUMPPONEN & TRAPPE 1998). In Alpine and Sub-Alpine plant-communities the dark-sep-

tate structures have been well studied (HASELWANDTER & READ 1980, BLED-SOE & al. 1990, BLASCHKE 1991, STOYKE & CURRAH 1991, STOYKE & al. 1992, VÁRE & al. 1992), while in the case of grasslands and dunes usually only arbuscular mycorrhizal colonization was reported, although the presence of dark septate hyphae in grassland species is known (NICOLSON 1959, 1960, and see in JUMPPONEN & TRAPPE 1998). A relatively high percentage of plant species examined in the present study were colonized by dark septate hyphal structures. A similarly frequent occurrence of this kind of colonization has been found in another area of the Great Hungarian Plain (KOVÁCS & BAGI 2001). Although neither the taxonomy nor the effect of these fungal endobionts has been completely cleared (JUMPPONEN 2001), it seems that they have a positive rather than a negative effect on the plants, and their importance increases in more stressed environments, as has been suggested as a reasonable explanation of their common and frequent alpine occurrence (VÁRE 1992, READ & HASELWANDTER 1981, HASELWANDTER & READ 1982, STOYKE & al. 1992, GARDES & DAHLBERG 1996, JUMPPONEN & TRAPPE 1998, JUMPPONEN & al. 1998, JUMPPONEN 2001). Their high frequency in the actually studied area and in the plant communities studied previously on the Great Hungarian Plain (KOVÁCS & BAGI 2001) could support the hypothesis about the relations between their occurrence, importance and the level of the stress, particularly caused by drought. One of the main purposes of further mycorrhizal studies in the area should be to try to study further the taxonomical position of these fungi and their possible role in these plant communities.

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Autor(en)/Author(s): Kovacs Gabor, Szigetvari Csaba

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