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Mycorrhizal Status of Plants in a Mixed Deciduous Forest from the Great Hungarian Plain with Special Emphasis on the Potential Mycorrhizal Partners of *Terfezia terfezioides* (MATT.) TRAPPE (*Pezizales*)

By

Gábor M. KOVÁCS*) and István BAGI*)

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Summary

KOVÁCS G. M. & BAGI I. 2001. Mycorrhizal status of plants in a mixed deciduous forest from the Great Hungarian Plain with special emphasis on the potential mycorrhizal partners of *Terfezia terfezioides* (MATT.) TRAPPE (*Pezizales*). – Phyton (Horn, Austria) 41 (2): 161–168. – English with German summary.

The mycorrhizal status of plants in a mixed deciduous forest from the Great Hungarian Plain was investigated. The sampling area is protected as the red-list *Botrychium virginianum* (*Ophioglossaceae*) lives there; its sporophyte was also studied regarding its mycorrhizal status. Roots of forty-nine plant species were collected and their endogenous fungal structures were described. The rate of the endomycor-

*) G. M. KOVACS, I. BAGI, University of Szeged, Department of Botany, 6701 Szeged, P.O.Box: 657, Hungary. Fax:+36-62-544-236; email: gkovacs@sol.cc.u-szeged.hu

rhizal colonization was estimated and the colonization type was determined. 78% of the plants were mycorrhizal. As the sampling area was a habitat of *Terfezia terfezioides* we looked for characteristic endogenous structures of this fungus. Twelve plant species were supposed to be mycorrhizal partners of *Terfezia terfezioides*.

Zusammenfassung

KOVACS G. M. & BAGI I. 2001. Der Mykorrhizierungsgrad von Pflanzen in einem laubabwerfenden Mischwald der großen ungarischen Tiefebene unter besonderer Berücksichtigung des möglichen Mykorrhizapartners *Terfezia terfezioides* (MATT.) TRAPPE (*Pezizales*). – Phyton (Horn, Austria) 41 (2): 161–168. – Englisch mit deutscher Zusammenfassung.

Der Mykorrhizierungsgrad von Pflanzen wurde in einem laubabwerfenden Mischwald der großen ungarischen Tiefebene untersucht. Beim Probengebiet handelt es sich um ein Schutzgebiet, da hier das in der roten Liste angeführte *Botrychium virginianum* (*Ophioglossaceae*) vorkommt. Am Sporophyten wurde ebenfalls sein Mykorrhizierungszustand untersucht. Wurzeln von 49 Pflanzenarten wurden aufgesammelt und ihre endogenen Pilzstrukturen beschrieben. Der Grad der Besiedelung mit Endomykorrhiza wurde geschätzt und der Besiedelungstyp beschrieben. 78% der Pflanzen waren mykorrhiziert. Da am Probenstandort *Terfezia terfezioides* vorkommt, wurde auf die charakteristischen endogenen Strukturen dieses Pilzes besonders geachtet. 12 Pflanzenarten waren vermutliche Mykorrhizierungspartner von *Terfezia terfezioides*.

Introduction

More than 90% of the vascular plants are mycorrhizal, and the influence of this mutualistic symbiosis on the nutrient uptake of the plants and on the plant community structure is generally known (ALLEN 1992, SMITH & READ 1997, VARMA & HOCK 1998). Although the mycorrhizal status of lots of plants has been investigated, because of the high variability of this character at all organization levels, more information is needed to complete our knowledge on the distribution of the different types of this association (HARLEY & HARLEY 1987, READ & al. 1992).

The vegetation of the Great Hungarian Plain has been well studied, but the mycorrhizal status of the species of the plant communities in this area has been poorly investigated. Some plants of its sandy grasslands have been studied regarding their mycorrhizae (PARÁDI & al. 1998) and ectomycorrhizae have been described from some woody plants of the area (JAKUCS & al. 1999, KOVÁCS & JAKUCS 2000, MAGYAR & al. 1999).

This paper presents the investigations of the mycorrhizal status of plants in a planted deciduous forest of the Great Plain. The area is extremely interesting because the unique Hungarian population of *Botrychium virginianum* (L.) Sw. (*Ophioglossaceae*) lives in this forest; and this stand is one of the largest known populations of this red-list fern in Europe (CSIKY 1997). Therefore plant coenologists have been paid marked attention to this fern for several years (CSIKY 1997, BAGI 1998), and one part of the forest is protected under the framework of a national park. As part of complex ecological investigations of this forest, studies of the mycorrhizae started two years ago with the support and permission of the Kiskunság National Park. The aim of the study of mycorrhizae was to obtain new ecological data about this population of *Botrychium virginianum* and its habitat. This work fits also into studies about the mycorrhizal status of the flora of Great Hungarian Plain.

The other reason to begin comprehensive investigations of mycorrhizae was the occurrence of fruit bodies of *Terfezia terfezioides* (MATT.) TRAPPE (*Terfeziaceae*, *Pezizales*) in large numbers in one part of the forest. Almost nothing is known about the mycorrhizal characteristics of *Terfezia terfezioides,* although other species of the genus *Terfezia* have been well studied, their natural mycorrhizal partners are known and some of them have been used in in vitro mycorrhizal experiments (DEXHEIMER & al. 1985, FORTAS & CHEVALIER 1992, KAGAN-ZUR & al. 1999, TAYLOR & al. 1995). The mycorrhizal connection of this fungus with *Robinia pseudoacacia L.* has been reported previously (BRATEK & al. 1996, KIRÁLY & al. 1992), but it is hardly conceivable that this would be the only mycorrhizal partner of the fungus. The investigation presented here is also a preliminary study of a work in whose framework we would like to find the natural mycorrhizal partners of *Terfezia terfezioides* and obtain data about the mycorrhizal characteristics of the fungus.

Materials and Methods

The studied area

The forest lies on the Great Hungarian Plain next to Kunfehértó (46°21′15″ N, 19°23′54″ E). The mean annual precipitation in the area is 550–600 mm and the mean annual temperature is 10.7 °C. The soil has a variously thick (between 0.5–1 meter), weakly calcareous upper sandy layer. The organic matter content ranges between 8,5 and 3,3% in the upper 30 cm of the soil, the humus-type is highly mineralized moder. The plant community is an oak-ash-elm siverine woodland which is transient to an Anthrisco cerefolii-Robinietum forest.

Samples were collected from one, 150 × 300 meter large square from a more or less homogenous forest. In this square the fruit bodies *Terfezia terfezioides* occured in large numbers.

Root sampling and preparation

Root samples were collected between October, 1999 and October, 2000. As the square was in the protected part of the forest, samples were collected in a manner causing minimal disturbance. Roots from two or three specimens of one species were sampled. The whole plant was collected only if it was unavoidable (e.g. the plant was small). Identification of the roots was carried out by examining their connection with the stems. In some cases, the phenology of the root also determined the plant unambiguosly. The samples were placed into plastic bags together with wet soil and after taking them to the laboratory, they were kept for a maximum of 48 hours at 4 °C before preparation.

The plants were identified according to Simon 2000.

To make preparations from the roots we followed the methods of Grace & Stribley 1991 with small modifications. At first all root samples were studied by a dissecting microscope to look for ectramatrical structures and ectomycorrhizae. After this, parts of the root were put into 10% KOH and left overnight at 60 °C. The remaining root parts were kept in water at 4 °C. If the discoulouring in KOH at 60 °C was not sufficient, the sample was left at 90 °C until it was discoloured. Afterwards, the samples were washed in distilled water for a minimum of 2 hours. Water was changed several times during this procedure. The last washing water was acidified (pH 3.5–3 approximately) with some drops of lactic acid and the roots were left in this solution for a minimum of 30 minutes. Cleared roots were stained with lactic acid-anilin blue (0.5 g anilin blue in 30 ml cc. lactic acid) and were put back into the acidic washing solution to eliminate superfluous stain. The stained root samples were mounted on slides in lactic acid and covered with 24 × 50 mm cover glasses and sealed. If the preparation looked appreciable in a light microscope, the remaining roots were put into FEA (formaldehyde : 70% ethanol : acetic acid, 5v:90v:5v) and deposited into the collection of G. M. Kovács (Table 1) as voucher specimens for further anatomical studies.

Qualification and quantification of mycorrhizal colonization

The intensity and extent of colonization were estimated by a visual method with the help of a light microscope. The entire root preparation was examined with special emphasis on the lateral and fine roots at different magnifications (40–400 ×). Five classes were used to describe 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 length of the root was colonized (Hopkins 1987). The intensity of the colonization was characterized using three types: type A: colonization at small and widely scattered sites; type B: at larger, but separated sites; type C: almost completely colonized; as they were used by Hopkins 1987. Colonization was considered as present when structures of endomycorrhizae (vesicles, arbuscules or hyphal coils) could be detected in the root.

A plant was considered as a potential mycorrhizal partner of *Terfezia terfezioides* if its roots contained typical endogenous structures of *Terfezia terfezioides*, as described from field samples and in vitro experiments (Bratek & al. 1996, Kovács unpublished data), this regards septate hyphae with almost parallel walls forming coils in the cortical cells.

Present septate endophytic fungal structures without mycorrhizal characteristics and microsclerotia-like structures were also studied.

Ectomycorrhizal colonization was detected as a qualitative feature only.

To compare our data with the literature, mostly the check list of Harley & Harley 1987 was used.

Results and Discussion

Root samples of forty-nine plant species were collected and their endogenous fungal structures were described (Table 1).

78% of the collected and investigated plant species were mycorrhizal. Mycorrhizal structures were lacking in the studied root samples of 11 species. Some of them have been reported in previous studies as non-my-

Table 1. Mycorrhizal and other endogenous fungal structures of the plant species (ordered by families) collected in a mixed forest on the Great Plain, Hungary. (No.: the serial number of the root samples in the collection of G. M. KOVÁCS, V: vesicles, A: arbuscules, C: hyphal coils, EC: extent of colonization, CT: type of colonization, E: ectomycorrhizae, S: septate endophytic fungal structures without mycorrhizal characteristics, M: microsclerotia-like structures, a: mycorrhizal structures lacking; the hypothesised partners of *Terfezia terfezioides* are bold, *: the extent and the type could not be estimated).

	V	A	C	EC	CT	E	S	M	No.
Apiaceae									
Anthriscus sylvestris		+	+	4	B		+		HE010
Asteraceae									
Ambrosia artemisiifolia		+	+	3	B		+		HE039
Conyza canadensis	+	+	+	3	B		+	+	HE038
Solidago gigantea	+		+	2	B		+	+	HE036
Taraxacum officinale	+		+	5	B		+		HE019
Berberidaceae									
Berberis vulgaris	+	+	+	3	B				HE048
Brassicaceae									
Alliaria petiolata	a								HE019
Thlaspi perfoliatum	a								HE022
Cannabaceae									
Cannabis sativa	+	+	+	5	C		+	+	HE033
Caprifoliaceae									
Sambucus nigra	+	+	+	4	B		+		HE003
Caryophyllaceae									
Cucubalus baccifer	a								HE042
Silene latifolia ssp. alba	a						+	+	HE017
Saponaria officinalis	a						+	+	HE031
Stellaria media	a						+	+	HE004
Celastraceae									
Euonymus europaeus			+	3	B		+		HE016
Chenopodiaceae									
Chenopodium album	a						+	+	HE034
Crassulaceae									
Sedum telephium ssp. maximum	+								HE009
Fabaceae									
Robinia pseudo-acacia	+	+	+	2	B			+	HE030
Fagaceae									
Quercus robur						+	+	+	HE052
Geraniaceae									
Geranium robertianum	+	+	+	5	C		+	+	HE013
Lamiaceae									
Ballota nigra	+	+		3	B		+	+	HE040
Clinopodium vugare		+	+	3	B		+		HE041
Glechoma hirsuta	+		+	5	C		+	+	HE021
Salvia glutinosa	+		+	3	B		+	+	HE020
Liliaceae									
Muscari racemosum	+	+	+	5	C		+		HE023
Ornithogalum umbellatum	+		+	4	C		+	+	HE026
Liliaceae									
Polygonatum latifolium	+	+	+	4	B		+	+	HE025
Polygonatum odoratum	+	+	+	4	B				HE037
Moraceae									
Morus alba	+	+	+	4	C	+	+	+	HE047
Oleaceae									
Ligustrum vulgare	+			3	B				HE006
Ophioglossaceae									
Botrychium virginianum	+		+	3	B		+		HE014
Papaveraceae									
Chelidonium majus	a						+	+	HE029
Poaceae									
Brachypodium sylvaticum	+	+	+	4	B				HE002
Digitaria sanguinalis	+	+	+	5	C		+		HE044
Setaria pumila		+	+	5	C		+	+	HE045
Polygonaceae									
Fallopia dumetorum	+	+		2	B				HE032
Rhamnaceae									
Rhamnus catharticus	a								HE050
Rosaceae									
Crataegus monogyna	+	+	+	5	B	+	+		HE007
Geum urbanum	+	+		3	B		+		HE028
Prunus padus	+	+	+	4	C		+	+	HE015
Prunus spinosa			+	5	C	+	+	+	HE051
Rubus caesius	+		+	5	C		+		HE011
Rubiaceae									
Galium aparine	+	+	+	4	C		+		HE027
Salicaceae									
*Populus x canescens**	+	+	+			+	+	+	HE049
Scrophulariaceae									
Veronica hederifolia	a						+	+	HE018
Simaroubaceae									
Ailanthus altissima	+	+	+	4	B		+	+	HE043
Ulmacae									
Celtis occidentalis	+	+	+	2	B		+		HE001
Ulmus minor	+	+	+	5	C		+	+	HE046
Urticaceae									
Urtica dioica	a						+	+	HE005
Violaceae									
Viola odorata	+		+	4	C		+	+	HE024

corrhizal such as *Alliaria petiolata* (M. B.) CAVAR & GRANDE, *Saponaria officinalis* L. or *Chelidonium majus* L. (Table 1). In the case of some of them no previous data were found, e.g. *Thlaspi perfoliatum* L. or *Cucubalus baccifer* L., but they belong to genera or families in which the non-mycorrhizal status is common. In other cases, plants which have been reported to be both endomycorrhizal or non-mycorrhizal, were found to be non-mycorrhizal e.g. *Urtica dioica L.* and *Stellaria media* (L.) VILL. (HARLEY & HARLEY 1987).

Whereas the frequency of the non-mycorrhizal plant species seems high, looking just at this, false conclusions could have been drawn about the state of mycorrhization of this plant community. Comparing the mycorrhization data with the results of the coenological studies done in this forest (CSIKY 1997) shows that the non-mycorrhizal plants have a very low coverage and frequency. Even though the relative coverage of these plants in the underwood level could be high in some aspects, this plant community is formed mainly by mycorrhizal plants.

37 species were found to form endomycorrhizae. 11% of them have a class 2 extent of root colonization, 28% have a class 3, 31% have class 4 and class 5 was found in 29%. The type of colonization was mostly B (61%) the remaining were type C, the colonization type A was not found at all in these plants. Vesicles were found in the roots of 86% of endomycorrhizal plants, 70% contained arbuscules and 89% hyphal coils (Table 1).

Some species, previously reported as non-mycorrhizal, e.g. *Anthriscus sylvestris* (L.) HOFFM. or *Ballota nigra* L. had endomycorrhizae. In some cases we have not found previously published data about the mycorrhizal status of the species. Such plants were *Ailanthus altissima* (MILL.) Swingle, *Ulmus minor* MILL., *Veronica hederifolia* L. or *Fallopia dumetorum* (L.) HOLUB (HARLEY & HARLEY 1987).

The sporophytes of *Botrychium virginianum* formed endomycorrhizae as was expected from the results of previous investigations made on other *Botrychium* species and on species from the *Ophioglossaceae* (BURGEFF 1938, HARLEY & HARLEY 1987, SCHMID & OBERWINKLER 1996). The gametophyte of this fern has not been found during this work.

Ectomycorrhizae were detected on the roots of five species, and only one of them (*Quercus robur* L.) was not colonized also by endomycorrhizae, while four species (*Morus alba* L., *Crataegus monogyna* JACQ., *Prunus spinosa* L. and *Populus x canescens* (AIT.) SM.) had both types (Table 1).

Septate endophytic fungal structures without any mycorrhizal characteristics were found in the roots of 80% of the plants, and 52% of the species had microsclerotia-like structures in their roots.

Twelve plant species were considered as the hypothesized mycorrhizal partner of *Terfezia terfezioides* on the base of their endomycorrhizal structures (Table 1). The characteristic structures reported previously by BRATEK & al. 1996 as the mycorrhizae of *Terfezia terfezioides* were found again in the root of *Robinia pseudo-acacia*. The habitats, in which fruit-bodies of *Terfezia terfezioides* have been found up to now in Hungary, were always *Robinia* forests (BABOS 1981, KIRÁLY & al. 1992). However, *Robinia* is not mentioned in the description of the environment of the holotype (see in BABOS 1981). Most of the here presented hypothesized partner species of *Terfezia* are common plants of the Robinietum communities. The herbaceous species, e.g. *Brachypodium sylvaticum* (HUDS.) ROEM. & SCHULT., *Viola odorata* L. or *Glechoma hirsuta* W. et K. are frequent underwood formers not only in the studied area, but in most black locust forests. Some of the woody plants are found usually in *Robinia* forests e.g. *Celtis occidentalis* L., *Crataegus monogyna* JACQ., *Prunus spinosa* L. or *Ulmus* species. *Celtis occidentalis* is mentioned almost in all descriptions of the provenance of *Terfezia terfezioides* (BABOS 1981) and *Crataegus monogyna* was the most frequent shrub found in plant coenological studies together with this fungus (KIRÁLY & al. 1992). There is no reason to compare these possible partners with the plants mycorrhized by other *Terfezia* species because the latters live in a completely different climatic zone, nevertheless some of them form mycorrhizae with herbaceous plants just as with woody ones (e.g. KAGAN-ZUR & al. 1999, TAYLOR & al. 1995).

To identify unequivocally the mycorrhizal partners of *Terfezia terfezioides* further investigations are necessary. The plants supposed to have mycorrhizal connection with *Terfezia terfezioides* are hypothesized partners, as the endomycorrhizae of the fungus could not be surely identified with the methods used in this study. DNA-based molecular studies and in vitro mycorrhizations between the hypothesised plants and sterile strains of *Terfezia terfezioides* are in progress and these might reveal new data that would increase our understanding of the mycorrhizal characteristics of this fungus.

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