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Ecology of endomycorrhizae of *Trisetum flavescens* (L.) P. Beauv. and *Alopecurus pratensis* L., and the intensity of soil cultivation

V. MEJSTŘIK

Institute of Environmental Ecology, Czechoslovak Academy of Sciences,
Průhonice, Czechoslovakia

The present study is dedicated to my teacher, Professor Dr T. Dominik, Agricultural College, Szczecin, Poland

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Our study on the dependence of the mode and degree of cultivation of the grassland on the rate of mycorrhizal development in the species *Alopecurus pratensis* and *Trisetum flavescens* suggests that the degree of cultivation, and mainly the amount of available food resources in the soil, influence the development of the endophyte in the roots of the plant hosts.

INTRODUCTION

Studies by Gerdemann (1968) and Mosse (1973) confirmed the generally accepted hypothesis of the importance and advantages of endomycorrhizae to the host plant by modern methods. According to Harley (1969), mycorrhizae influence considerably the uptake of phosphorus and nitrogen by the plant, and play an important role in the natural recirculation of phosphorus in grassland and forest ecosystems (Odum 1963). In view of the fact that these problems have not received necessary attention, they should be treated in more detail. In highly fertilized soils rich in readily assimilable nutrient components the symbiotic relations between the host plant and the fungus are as a rule non existent or of very low intensity. The development of mycorrhizae is influenced by the physical and chemical composition of the soil, the pH, the groundwater level and its changes, the depth of the roots, humus content in the soil, soil temperature, etc. (Mejstřik 1972).

Klečka and Vukolov (1937) were among the first to study the ecology of endomycorrhizae in grass species. Many years later, the present author (Mejstřik 1965, 1972) published several studies concerned with these problems. However, endophytic mycorrhizae still require systematic investigation. Mosse et al. (1973) indicated the perspective of utilization of symbiotic fungal species suitable for formation of mycorrhizae, in order to supply the plant with a sufficient amount of nutrients by natural routes without having to increase the doses of fertilizers and disturb the natural biological balance in the soil.

The intention of this study was to elucidate relationship between the rate of development of endomycorrhizae on the one hand, and the degree of cultivation of the grassland on the other hand. For this purpose, we selected two grass species growing in differently treated localities — semi-cultivated and cultivated meadows — i.e. *Trisetum flavescens* (L.) P. Beauv. and *Alopecurus pratensis* L., in order to study the influence of the growth conditions and the mode of cultivation on the morphology and density of root hairs. I agree with Mosse (1973) in that a managed development of mycorrhizae might result in a higher uptake of phosphorus from the soil, its better utilization by the plant, and an increased participation of phosphorus in its natural circulation. If it were possible to increase naturally the uptake of phosphorus by the plant by as little as 1% of the total consumption of phosphate fertilizers, millions would be saved on a global scale. This indicates clearly the necessity of studies on natural routes of recirculation with the intention to control them to the advantage of a better balance in artificial, man-made ecosystems.

METHODS

Growth conditions: Plant root samples were obtained from meadows of the landscape complex "Broumovsko" in SE Bohemia in a semiproductive agricultural area (Tables 1, 2; Fig. 1). The analyses were performed by the Central Testing and Control Institute of Agriculture.

Exchangeable pH of the soil samples was measured. The degree of cultivation was estimated from the doses of fertilizers applied (mode of application, kind of fertilizer used), the number of harvests, the character of other agrotechnical measures and the contemporary state of the cultures. The permanent grassland was older than five years in all localities under consideration. Vegetation characteristics of the individual grassland localities are shown in Table 3.

Mode of sampling and evaluation of mycorrhizae: We selected four permanent meadows in which agrotechnical measures applied for a number of years could be estimated. From a plot (4×4 m) in each meadow, three soil monoliths (20×20×30 cm) with a tuft of *Tri-*

Table 1

Characteristics of the grassland

Locality	Martin-kovice (A)	Vižňov (B)	Jetřichov (C)	Benešov (D)
Altitude	449	570	440	490
Slope	3-5°	5°	5°	3°
Exposure	SSE	SSE	W	NW
Soils	clay loam	clay loam	loam clay	clay loam
Ground water level (cm)	35	150	100	50
Degree of cultivation	semiextensive	medium extensive	extensive	medium extensive

Table 2

Chemical soil analyses

Locality	Martin-kovice (A)	Vižňov (B)	Jetřichov (C)	Benešov (D)
pH/KCl	4.7	4.8	5.6	5.3
$CaCO_3$ (%)	0.0	0.0	0.0	0.0
Total K mg/l kg soil	55.0	130.0	100.0	60.0
Total Mg mg/l kg soil	92.0	212.0	92.0	200.0
Total P mg/l kg soil	13.0	9.0	6.0	9.0
% of humus	3.98	6.19	3.44	5.45
Total N mg/l kg soil	0.21	0.31	0.12	0.30
C:N	18.95	19.96	28.66	18.16

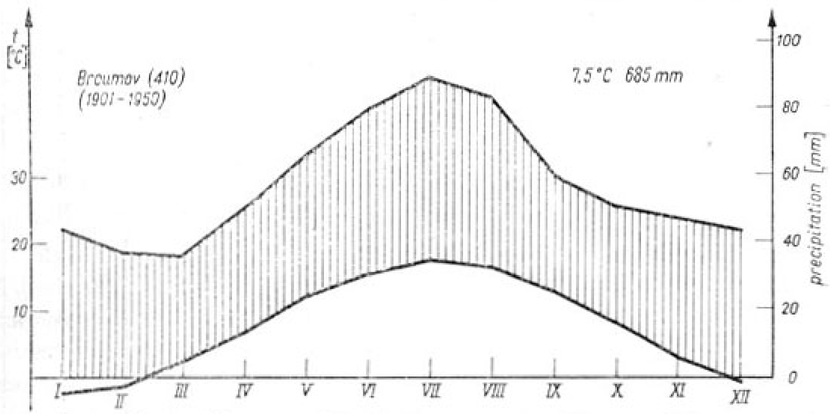


Fig. 1. Climogram of the landscape complex "Broumovsko"

Table 3
Phytosociological record from the localities Martinkovice, Vížňov, Jetřichov and Benešov

Total area	Martinkovice	Vižňov	Jetřichov	Benešov
Analyzed area	300 m²	200 m²	300 m²	200 m²
Total plant cover	20 m²	16 m²	16 m²	20 m²
Mosses	95 %	85 %	85 %	95 %
Slope	0 %	5 %	2 %	5 %
Altitude	3-5°	5°	4°	3°
	449 m	570 m	440 m	490 m
Achillea millefolium	5	3	4	4
Aegopodium podagraria	.	3	.	.
Ajuga reptans	.	.	1	2
Alchemilla monticola	4	.	4	.
Alchemilla sp.	.	.	.	5
Alopecurus pratensis	6	4	5	8
Angelica silvestris	3	4	2	.
Anthoxanthum odoratum	.	5	5	5-6
Arrhenatherum elatius	.	.	2	.
Bellis perennis	.	2	2	.
Briza media	.	.	3	.
Campanula patula	3	2	2	.
Cardamine pratensis	2	1	.	3
Carex panicea	.	.	+	.
Carum carvi	.	1	.	.
Centaurea jacea	.	3	3	.
Centaurea phrygia	3	.	.	4
Cerastium vulgare	.	.	.	3
Cerastium vulgatum	.	4	2	.
Chrysanthemum leucanthemum	2	4	4	2
Cynosurus cristatus	.	.	3	.
Dactylis glomerata	5	3	4	3
Deschampsia caespitosa	4	.	.	3
Equisetum arvense	2	4	.	.
Festuca pratensis	4	.	3	.
Festuca rubra	5	4	5	6
Filipendula ulmaria	1	.	.	.
Fragaria vesca	.	2	.	.
Galium boreale	.	.	.	3
Galium mollugo	6	.	.	4
Galium verum	.	2	.	.
Geranium sylvaticum	.	.	4	.
Heracleum sphondylium	3	.	2	.
Holcus lanatus	3	.	.	3
Hypericum maculatum	3	.	.	.
Hypericum perforatum	.	.	1	.
Knautia arvensis	.	.	1	.
Lathyrus pratensis	.	.	.	5
Leontodon hispidus	4	5	4	.

Lolium perenne	.	.	1	.
Lotus corniculatus	3	4	2	2
Luzula campestris	.	3	.	.
Luzula multiflora	.	.	3	4
Lychnis flos-cuculi	.	.	3	3
Lysimachia nummularia	3	.	.	.
Phleum pratense	.	.	.	3
Pimpinella major	.	4	4	3
Pimpinella saxifraga	.	.	3	.
Plantago lanceolata	.	5	3	3
Plantago major	.	3	2	.
Plantago media	.	.	.	2
Poa pratensis	.	4	4	6
Potentilla erecta	2	.	.	.
Primula elatior	3	.	.	.
Prunella vulgaris	4	.	3	2
Ranunculus acer	3	5	3	4
Ranunculus auricomus	2	4	.	.
Ranunculus repens	4	5	3	.
Rumex acetosa	.	.	.	2
Rumex acetosella	.	.	2	.
Sanguisorba officinalis	7	3	4	6
Succisa pratensis	3	.	.	.
Taraxacum officinale	.	4	2	4
Trifolium dubium	.	3	3	.
Trifolium pratense	.	4	4	4
Trifolium repens	.	.	.	2
Trisetum flavescens	.	7-8	7	6
Veronica chamaedrys	3	4	3	.
Vicia eraca	.	.	3	.

setum flavescens, and three monoliths with an *Alopecurus pratensis* sward were dug up in the second half of September. The blocks were transported to the laboratory and the roots washed in running water. From different sites of the root system of each tuft we took 10 samples of fine roots.

Each sample weighed 5 g, the length of the cut roots was 1 cm. An average sample weighing 5 g obtained from a complex of 40 samples.

The roots were fixed in 70% ethanol, pressed, stained with toluidine blue (Lobanov 1960) and mounted in glycerine-gelatine. We evaluated an average of 180-200 roots for each locality.

Mycorrhizal incidence was estimated by means of a semiquantitative 5-grade scale (Mejstřik 1965). A description is given of all characteristic formations associated with the anatomy and morphology of mycorrhizae. Observations were made on the relationship between the rate of development of the mycorrhizae and the density of root hairs. The latter was evaluated by means of a 5-grade, semiquantitative, scale: 0 denotes 0%

of root surface covered with root hairs; 1 = 1-10% of root surface covered with root hairs; 2 = 11-30% of root surface covered with root hairs; 3 = 31-60% of root surface covered with root hairs; 4 = 61-100% of root surface covered with root hairs.

RESULTS AND DISCUSSION

Grassland consists in the locality Martinkovice of medium cultivated meadows; the groundwater level is relatively high (35 cm). The soil lacks a sufficient supply of the main nutrients, soil pH is relatively low (4.7). Theoretically, these conditions should be optimal for a maximum development of the vesicular-arbuscular type of mycorrhiza.

Trisetum flavescens: mycorrhizae were found on almost all roots collected. The only type present was an endophytic mycorrhiza of the vesicular-arbuscular type (Harley 1969). On the individual roots, the incidence of mycorrhizae varied, the average was 1.8 (Fig. 2) in this locality.

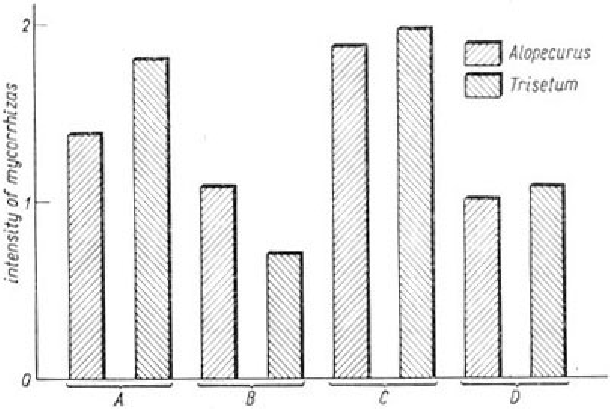
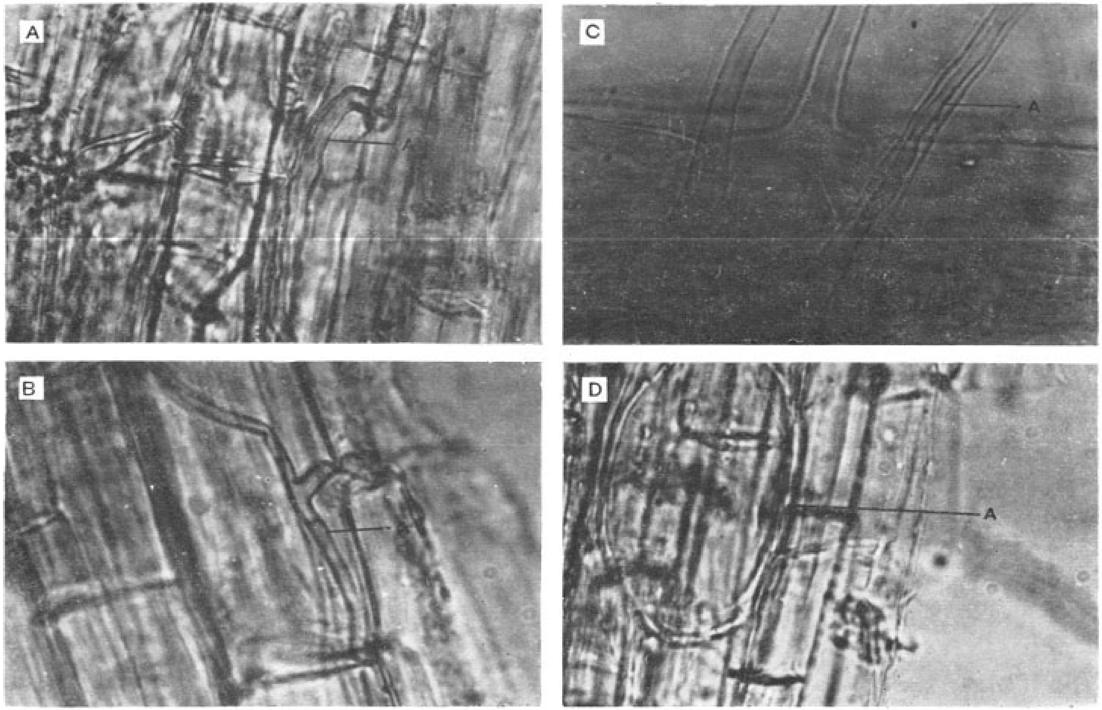


Fig. 2. Mycorrhizal incidence in ***Trisetum flavescens*** and ***Alopecurus pratensis*** in localities cultivated to a different degree
A — Martinkovice, *B* — Vižňov, *C* — Jetřichov, *D* — Benešov

This rate of development indicates medium, but not maximum, values. Mycorrhizal incidence increased in proportion to the decrease in the density of root hairs (Fig. 3-4).

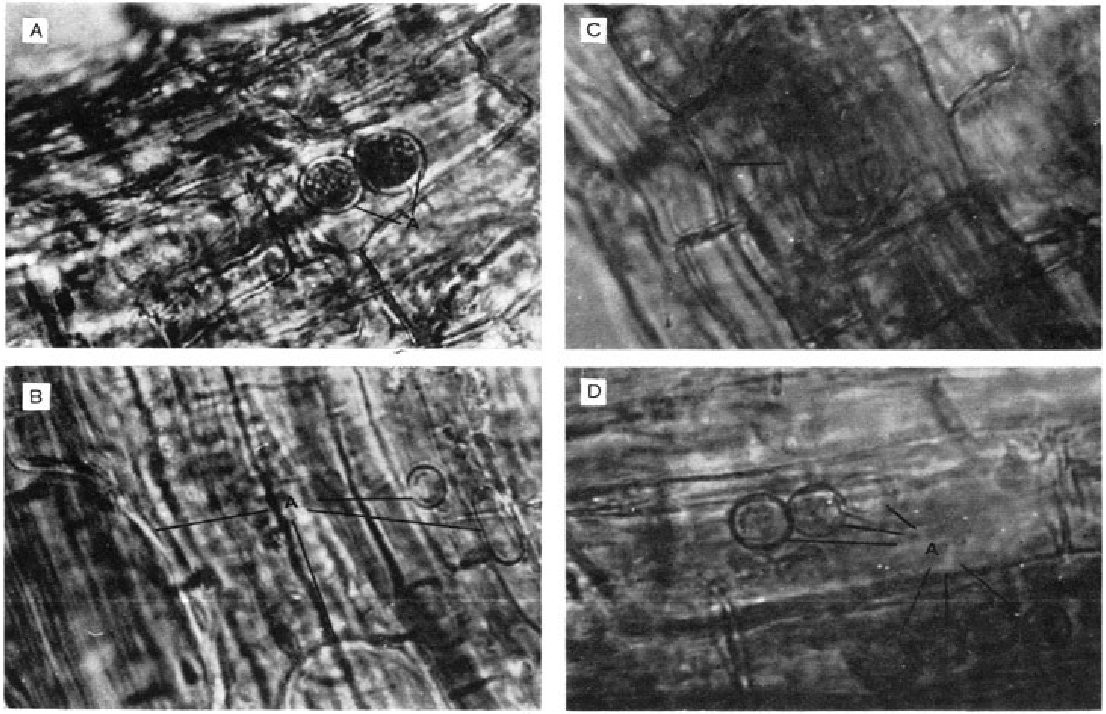
The formations observed on the roots of the host plants were typical of the vesicular-arbuscular type of endophytic mycorrhiza. The parenchymal cells of the root cortex harboured thick, nonseptate or sparsely septate hyphae with frequent swellings (Pl. I.A). In addition we found thin, straight, sparsely branched and poorly staining hyphae (Pl. I.B). Hyphae entered the root tissue through the cells of the epidermis, and occasionally through the root hairs (Pl. I.C). Clustered and distintegrated hyphae, var-

Plate I



A — *Trisetum flavescens* — thickwalled hyphae of the *Endogone* type (*a*) in parenchymal cells (×1000); *B* — *Alopecurus pratensis* — thinwalled, septate hyphae (*a*) in parenchymal cells (×1000); *C* — *Trisetum flavescens* — hyphae penetrating a root hair (*a*) (×500); *D* — *Trisetum flavescens* — large terminal vesicle (*a*) (×1000)

Plate II



A — *Alopecurus pratensis* — thickwalled vesicle with "granular" content (*a*) (×1000); *B* — *Trisetum flavescens* — vesicle of different type (*a*) in parenchymal cells (×1250); *C* — *Alopecurus pratensis* — thickwalled hyphae in pelotonlike formation in the host plant (*a*) (×1000); *D* — *Alopecurus pratensis* — sporelike formations in parenchymal cells (*a*) (×1500) ious pelotons and tubular formations, vesicles and chlamydospores were present in the cells of the root cortex. Digestion of the hyphae by the host plant was also observed.

According to earlier results (M e j s t ř i k 1971), the incidence of arbuscles occurs during the period of flowering and maturing of the host plant. Vesicles are always more numerous in the autumn months; this is associated with the efforts of the symbiotic fungus to survive unfavourable conditions. Vesicles are oval and spherical in shape, terminal and intercalary, and either communicate or not with the hyphae. Vesicles were found in 35% of the roots (Pl. I.D).

Alopecurus pratensis: Hyphae of the symbiotic fungus were present in 83% of roots. The average mycorrhizal incidence attained values of 1.18 indicating a poor development of mycorrhizae (Fig. 2). This value indicates a statistically important difference in the significance level at p 0.05 in contrast to the averages from the locality Jetřichov. The relationship of mycorrhizal frequency and root hair density is illustrated in Fig. 3-4.

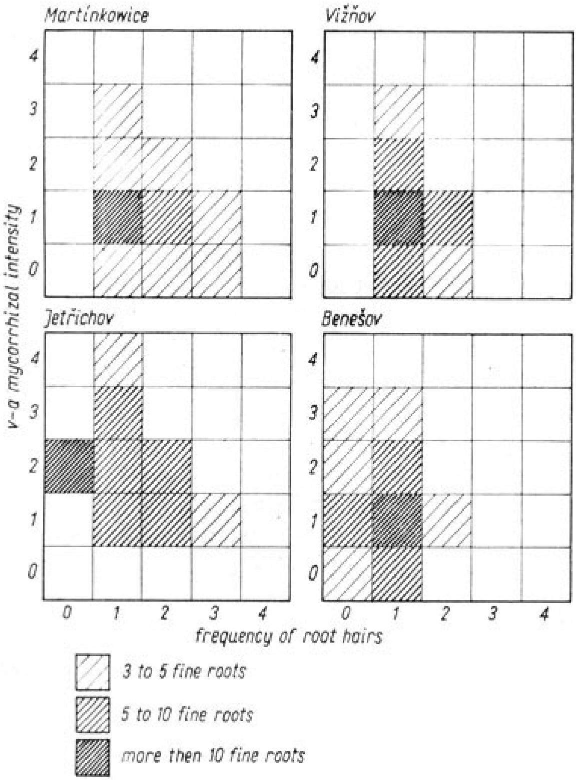


Fig. 3. Dependence of vesicular-arbuscular mycorrhizal intensity on the frequency of root hairs in *Alopecurus pratensis*

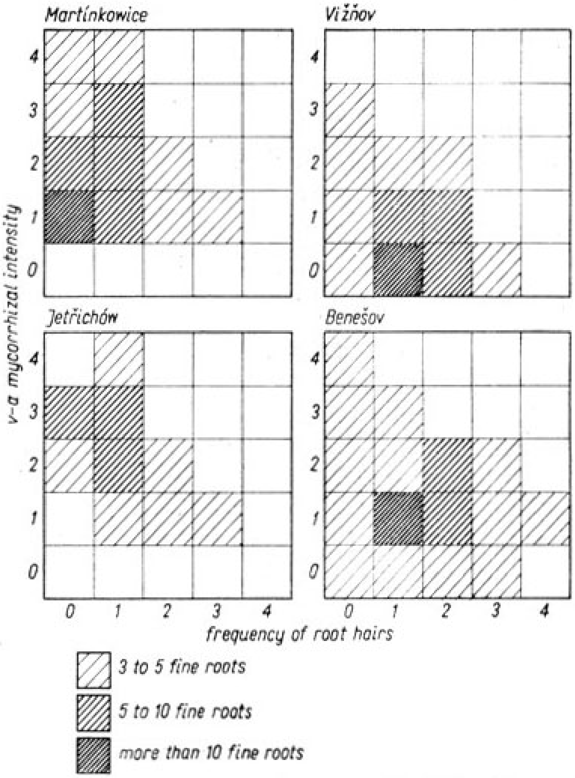


Fig. 4. Dependence of vesicular-arbuscular mycorrhizal intensity on the frequency of root hairs in *Trisetum flavescens*

The anatomy and morphology of the vesicular-arbuscular type of endophytic mycorrhiza did not differ considerably from that of *T. flavescens*. Vesicles were found in 66% of roots; their type was not uniform: thinwalled in 60% thickwalled, filled with a granular substance in 3% of roots, thickwalled contrastingly coloured in 3% of roots (Pl. II.B).

The meadows of the locality Vižňov were cultivated to a medium degree, the level of groundwater occurred at a depth of 80-100 cm. Nutrients (except for phosphorus) indicated medium values. The anticipated poor development of mycorrhizae was confirmed by soil analyses.

Trisetum flavescens: Mycorrhizae were absent in almost one third of the roots examined; average frequency of mycorrhizae was low (0.7) suggesting a very poor development of mycorrhizae (Fig. 2). Mycorrhizal incidence increased in direct proportion to the decreasing density of root hairs (Fig. 3). Hyphae from the surrounding soil entered the roots mainly through the epidermis, by way of the root hairs on rare occasions only.

Distinct appressorium like swellings were seen at the site of entrance of the hyphae through the cellular membrane. Host cells contained disintegrated hyphae, and hyphal digestion was observed. Hyphae did not enter the cells of the endodermis. Tubular and saclike formations were found in 1.7% of roots. Parenchymal cells of the root cortex harboured the two earlier described types of hyphae. Swollen hyphae of *Endogone* type occurred more frequently than straight thin ones. Sporelike formations and vesicles were found mainly close to the endodermis.

Alopecurus pratensis: In comparison with *T. flavescens*, mycorrhizal incidence was higher in *A. pratensis*. Roughly 80% of all roots were infected with the fungus, average values of mycorrhizal incidence were 1.3 (Fig. 2). This average showed statistically significant differences (significance level p 0.05) from that of the plot at Jetřichov, but did not differ significantly from exeperimental plots at Martinkovice and Benešov. Mycorrhizal incidence increased in proportion to a decrease in the density of root hairs (Fig. 3). The mode of infection, the anatomy and morphology of the mycorrhizae were similar to those described for the species *T. flavescens*. Tubular formation on thin, straight, septate hyphae, and saclike formations without communication with the mycelium occurred on 17% of roots. Vesicles were present on 8% of roots. Thickwalled, contrastingly coloured spores occurred on 7% of roots, thickwalled spores folled with a granular substance on 5% of roots (Pl. II.A). Hyphae of *Endogone* type were more frequent in the root tissue than straight, nonseptate hyphae.

The meadows of the locality Jetřichov were badly managed, irregulary harvested and not fertilized. The level of groundwater was 80 cm. Nutritive resources in the soil were poor, the soil acidic, pH 5.8. Hyphae were found in all root samples with a considerable variation in their incidence.

Trisetum flavescens: Average mycorrhizal incidence attained values of 2.0 (Fig. 2), indicating a medium to high degree of mycorrhizal development. Hyphae from the surrounding soil entered the parenchymal cells mainly through cells of the epidermis, with only an occasional entrance through the root hairs. The accepted fact that mycorrhizal incidence depends on the density of root hairs could not be confirmed in this case (Fig. 3). An occasional arbuscle was present. Vesicles of spherical and elliptic shape, terminal and intercalary vesicles occurred with considerable frequency in 27% of root samples (Pl. II.B). They were present mainly in cells situated close to the endodermis. The dominant hyphal type was that of *Endogone*, the incidence of straight, thin, hyphae was sparse.

Alopecurus pratensis: All roots were infected with the fungus, the average incidence of mycorrhizae attained values of 1.88 (Fig. 2). This average was statistically significantly different (p 0.05) from the values assessed in the remaining localities. Mycorrhizal incidence increased in proportion to the decrease in root hair density (Fig. 3). The anatomical structure and the morphology of the mycorrhizae were essentially similar to those described for the species *T. flavescens*. The shape of the vesicles varied in the roots. The presence of vesicles was confirmed in 32% of roots. Noteworthy was the solitary incidence of hyphae of *Endogone* type; the dominant type was straight, thinwalled hyphae.

The meadows of the locality Benešov were cultivated to a medium degree. The soil was acidic (pH 5.3), the amount of nutrients moderate, the level of groundwater at a depth of roughly 60 cm; 80% of the roots were infected with the symbiotic fungus.

Trisetum flavescens: Average mycorrhizal incidence 1.2 (Fig. 2) suggested that the development of mycorrhizae was poorrer. A dependence of mycorrhizal incidence on root hair density could not be confirmed (Fig. 3). Hyphae entered the roots of the symbiont mainly through the cells of the epidermis, rarely through the root hairs. Thin, straight, septate hyphae prevailed the incidence of hyphae of the *Endogone* type was rare. Tubular and saclike formations were present in 10% of roots, arbuscles were absent, vesicles occurred in 3.3% of roots, spores in 6.6% of roots.

Alopecurus pratensis: Hyphae occurred in almost all root samples, but mycorrhizal incidence showed considerable variation. The average mycorrhizal incidence with a value of 1.0 (Fig. 2) and a level of significance p 0.05 was statistically very different from the values assessed for the localities Vižňov and Jetřichov. Hyphae of the *Endogone* type dominated in the parenchymal cells of the root cortex; thinwalled, straight hyphae were scarce. Tubular and saclike formations were present in 7% of the roots, vesicles in 15%, thinwalled spores in 10% of the roots.

General characteristics of the endophyte in both species of host plants: thinwalled, straight, loosely septate hyphae and thin hyphae with poor staining properties measuring 0.9-4.5 μm (2.0 μm in diameter); thickwalled, nonseptate, irregularly swollen hyphae (*Endogone* type) with relatively good staining properties; branching hyphae forming cluster and pelotons inside the cell (Pl. II.C) and appressoria during penetration of the cell membrane. Size of hyphae 2.0-5.4 μm (3.8 μm in diam.). Hyphal size 2.0 to 5.4 μm (3.8 μm in diam.). Vesicles spherical and oval, terminal and intercalary, thickness of membrane 1 μm, size of vesicles 15.8×57.0-6.3×29.9 μm (31.5×18.2 μm in diam.). Arbuscles infrequent; they form typical treelike branched formations inside the cell of the plant host. Host cells contain several types of spores (Pl. II.D).

The common belief that the degree of mycorrhizal incidence depends on the density of root hairs, could not be confirmed unanimously in our experiments as evidenced by Figs. 3, 4. For the species *Alopecurus pratensis*, an indirect dependence of these factors has been found in the localities

Jetřichov and Vižňov; for *Trisetum flavescens*, this was not observed in any of the localities under consideration.

The degree of mycorrhizal incidence was influenced by the mode of cultivation and by the agrotechnical measures applied (Fig. 2). The rate of infection was, evidently, not influenced by the pH of the soil ranging from 4.7-5.6.

Mycorrhizal development in both *Alopecurus pratensis* and *Trisetum flavescens* was highest in the locality Jetřichov, with warst soil cultivation and lowest amount of available nutrients in the soil. Poor mycorrhizal development in both species occurred in the localities Benešov and Vižňov, in which the meadows had received moderate care: phosphorus and nitrogen contents were relatively high, magnesium contents and the amount of humus in the soil extremely high. A higher degree of mycorrhizal development than that anticipated on the basis of considerably high contents of phosporus and nitrogen in the soil was recorded in both species from the locality Martinkovice.

SUMMARY

Our study on the dependence of the mode and degree of cultivation of the grassland on the rate of mycorrhizal development in the species *Alopecurus pratensis* and *Trisetum flavescens* suggests that the degree of cultivation, and mainly the amount of available food resources in the soil influence the development of the endophyte in the roots of the plant hosts. Mycorrhizal development was restricted in more intensively cultivated soils fertilized with high doses of nitrogen and phosphorus. The endophytic mycorrhiza present in both species was of the vesicular-arbuscular type showing vesicle- and arbuscle formation typical of this type. The roots harboured two types of hyphae: those of the *Endogone* type, and thin, straight, septate hyphae.

We have been unable to obtain unequivocal evidence of the accepted fact that the degree of mycorrhizal development depends on the density of the root hairs.

Our results indicate that increased attention should be given to the problem of increased phosphorus uptake by plants infected with mycorrhizae as compared with those not infected with fungi in view of the fact that this natural, biological increase in phosphorus uptake might help to reduce doses of artificial fertilizers and thus help to improve the energetic balance in biocoenoses managed by man.

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Ekologia mikoryzy wewnętrznej u *Trisetum flavescens* i *Alopecurus pratensis* a intensywność uprawy gleby

Streszczenie

Badania nad zależnością rodzaju i stanu uprawy gleby oraz stopnia rozwoju mikoryzy u *Alopecurus pratensis* i *Trisetum flavescens* wskazują, że stan uprawy, a szczególnie ilość przyswajalnych składników pokarmowych w glebie wpływa na rozwój endofitu w korzeniach roślin. Rozwój mikoryzy był ograniczony w glebach intensywniej nawożonych wysokimi dawkami azotu i fosforu. Mikoryza wewnętrzna u obu gatunków należała do typu pęcherzykowato-drzewkowatego. W korzeniach stwierdzono dwa rodzaje strzępek: typowe dla *Endogone* oraz cienkie, proste, septowane.

Nie otrzymano jednoznacznego potwierdzenia przyjętej opinii, że stopień rozwoju mikoryzy zależy od gęstości chwytników. Wyniki badań wskazują na konieczność zwrócenia uwagi na problem wzrostu pobierania fosforu przez rośliny współżyjące z grzybami w porównaniu z roślinami nie wchodzącymi w związki mikoryzowe. Ten naturalny, bilogiczny wzrost pobierania fosforu może pozwolić na zmniejszenie dawek nawozu sztucznego i w ten sposób poprawić bilans energetyczny w biocenozach kierowanych przez człowieka.