ACTA MYCOLOGICA
Vol. 30 (2): 213-222
1995

The influence of pre-crop plants on the occurrence of arbuscular mycorrhizal fungi (*Glomales*) and *Phialophora graminicola* associated with roots of winter *XTriticosecale*

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B ł a s z k o w s k i J.: *The influence of pre-crop plants on the occurrence of arbuscular mycorrhizal fungi (Glomales) and Phialophora graminicola associated with roots of winter XTriticosecale.* Acta Mycol. 30 (2): 213-222, 1995.

The influence of four pre-crop plant species on the occurrence of arbuscular mycorrhizal fungal (AMF; *Glomales, Zygomycetes*) spores, mycorrhizae, and *Phialophora graminicola* (Deacon) Walker associated with roots of field-cultivated *XTriticosecale* Wittmack cv. Malno was investigated. The pre-crop plant species were *Hordeum vulgare* L., *Lupinus luteus* L., *Pisum sativum* L., and *Vicia faba* v. *major* Harz. Most spores and species of AMF were found when *XTriticosecale* was cultivated following *P. sativum*. Prior cropping with *L. luteus* caused the occurrence of the lowest number of spores among *XTriticosecale* roots. Mycorrhizal colonization of *XTriticosecale* was highest when planted after *P. sativum* and lowest when grown after *L. luteus*.

Key words: mycorrhizal fungi, *Phialophora graminicola*

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are associated with most cultivated plants, including *Secale cereale* L. and *Triticum aestivum* L. (B ł a s z k o w s k i, 1993). *XTriticosecale* Wittmack is an artificially produced amphiploid of a hybrid between *Secale* sp. and *Triticum* sp. (T a r k o w s k i, 1989). The latter is a poorer host of AMF than the former species (C z a j k o w s k a - S t r z e m s k a, 1988). Both the mycotrophic status of *XTriticosecale* and its response to AMF is poorly known (B ł a s z k o w s k i, 1991 a).

Many investigators indicate that AMF may increase plant growth (B ł a s z k o w s k i, 1991 a). The main causes of such an influence on plants are the increased root absorptive surface offered by extramatrical hyphae (R h o d e s, G e r d e m a n n, 1975), the use of soil mineral resources being poorly or unavailable for roots of autotrophic plants (H a y m a n, 1983), and the increased tolerance to physical (A l l e n, C u n n i n g h a m, 1983; S i e v e r d i n g, T o r o, 1988), chemical (G a r c i a - - R o m e r a, O c a m p o, 1988; G r i f f i o e n, E r n s t, 1989), and biological (R o s s, 1972; S c h ö n b e c k, 1978) stresses.

In short season crops, a significant effect of AMF on plant growth must depend on early infection (H a r i n i k u m a r, B a g y a r a j, 1988). Thus, this is related to inoculum density, which can be increased either through inoculation or through judicial (reasonable) manipulation of agronomic practices (S i e v e r d i n g, 1986).

Prior cropping of soils with non-mycorrizal plants can inhibit mycorriza formation in subsequent mycotrophic plants (H a r i n i k u m a r, B a g y a r a j, 1988). In contrast, crops preceded by highly mycotrophic plants usually harbour both greater mycorrhizal infections and more numerous populations of AMF (D o d d et al., 1990; H a r i n i k u m a r, B a g y a r a j, 1988).

Phialophora graminicola (Deacon) Walker is a non-pathogenic fungus (H o l d e n, 1976) which may restrict root colonization by *Gaeumannomyces graminis* (Sacc.) Arx et Olivier var. *tritici* Walker (D e a c o n, 1973 b; B ł a s z k o w s k i, 1990). Like AMF, it probably improves plant nutrition (C o w a n, 1979). *Phialophora graminicola* has been found to occur commonly in roots of graminaceous plants growing in Poland (B ł a s z k o w s k i, 1991 b) and other regions of the world (B a l i s, 1970; D e a c o n, 1973 a; R e a d, H a s e l w a n d t e r, 1981).

Phialophora graminicola has highly increased its occurrence following irrigation, but has not shown any marked response to increasing soil nitrogen concentrations (B ł a s z k o w s k i, K o s z a ń s k i, K a c z m a r c z y k, 1993). However, there is a lack of data concerning the effect of crop rotation on this species.

The aim of this study was to determine the influence of four pre-crop plants on the occurrence of AMF, mycorrhizae, and *P. graminicola* associated with roots of *XTriticosecale* cultivated subsequently.

Acknowledgement: This research was in part supported by The Committee of Scientific Research (grant no. 6.P205.043.05).

MATERIALS AND METHODS

In 1990, a field experiment at the Agricultural Experiment Station Lipki near Stargard Szczeciński was conducted. The following conditions were set up:

— soil – a good rye complex; pH 6.3; 1.3-1.5 % of humus; and 13-15 mg $100g^{-1}$ of P_2O_5,
— pre-crop plants – barley (*Hoderum vulgare*) cv. Bielik; yellow lupine (*Lupinus luteus*) cv. Rada; garden pea (*Pisum sativum*) cv. Ramir; broad bean (*Vicia faba* var. *major*) cv. Grot,
— host plant – *XTriticosecale* cv. Malno,
— experimental design – split block with four replicates,
— fertilization (kg/ha) – N as NH_4NO_3 at 75 broadcast in random order to the plots; P at 50 as superphosphate; K at 50 as KCl.

Mycorrhizal colonization was determined based on 25 randomly selected plants with roots collected in the milky rape of seeds (stage 11.2-3, after L a r g e, 1954) separately from each plot. In the laboratory, roots were washed first in a tap water

for 5 min. Five-centimetre-long root fragments were subsequently cut off from a depth of 5-10 cm and then divided into 1-cm-long segments. Fiftly 1-cm-long root segments coming from a particular plot were stained according to the P h i l l i p s and H a y m a n (1970) method to determine mycorrhizal colonization. Additionally, in the same root segements, the proportion of *P. graminicola* was determined. This fungus was identified based on the criteria described and illustrated by D e a c o n (1973 a).

Spores of AMF were recovered from rhizosphere soils by wet sieving and decanting (G e r d e m a n n, N i c o l s o n, 1963). Soils were collected at the milky ripe stage of plants. Arbuscular fungi were recognized based on their original descriptions, specimens deposited in the collection of the author of this paper, and information obtained from Drs R. E. Koske (University of Rhode Island, U.S.A.), J. B. Morton (West Virginia University, U.S.A.), and C. Walker (U.K. Forestry Commission). Data were processed by a one way analysis of variance. Spore and species density values were log transformed [log (x+1)] before statistical analysis. The statistical significance of differences between means was determined using the least significant difference at 0.05 calculated from the Tukey test. The species compositions of spore populations isolated from the root zone of *XTriticosecale* preceded by four pre-crop plant species were subjected to cluster analysis. Each species was considered a character, and each character had two possible states of presence (coded as 1) and absence (coded as 0). The distance coefficients were used in cluster analysis by computing Euclidean distances with a single amalgamation rule and raw data based on the computer program STATISTICA for Windows, release 4.5. Scientific names are according to W a l k e r and T r a p p e (1993). Specimens have been deposited at the Department of Plant Pathology, University of Agriculture, Szczecin.

RESULTS

From the root zone of *XTriticosecale* cultivated in four pre-crop treatment, a total of 442 spores representing 12 species in four genera of AMF, were isolated (Tab. 1). Most spores were recovered from under *XTriticosecale* growing after garden pea (175 in dry soil), followed by cultivation of board bean (164) and barley plants (95). Only eight spores in 100 g of dry soil were associated with *XTriticosecale* roots when the plant in the previous growing season was yellow lupine.

The fungi dominating in the *XTriticosecale* rhizosphere soils were species of the genus *Glomus* (Tab. 1). The proportion of spores of this genus in the overall number of spores of all the AMF recovered was 87.3 %. The proportion of *Glomus* spp. was highest when *XTriticosecale* was preceded by garden pea (80.6 %).

The dendrogram analysis of the species composition of the AMF revealed (Fig. 1) that populations most closely related were those of garden pea and board bean with a linkage distance value of 1.73, followed by those representing barley and broad bean (2.0) and barley and garden pea (2.24). The least significant correlations were between yellow lupine and garden pea (3.32) and between barley and yellow lupine (2.45).

Table 1

Spore numbers of arbuscular mycorrhizal fungal species isolated from the root zone of *XTriticosecale* preceded by four plant species

Fungal species	*Hordeum vulgare*	*Lupinus luteus*	*Pisum sativum*	*Vicia faba* var. *major*
Acaulospora "61"	0^a	0^a	15^b	11^b
Entrophospora infrequents (Hall) Ames et Schneider	0^a	0^a	4^a	0^a
Glomus caledonium (Nicol et Gerd.) Trappe et Gerd.	17^a	0^a	25^b	31^b
Glomus constrictum Trappe	9^a	8^a	23^b	30^b
Glomus dominikii Błaszkowski	11^b	0^a	17^b	12^b
Glomus etunicatum Becker et Gerd.	27^b	0^a	45^c	43^c
Glomus fasciculatum (Thaxter) Gerd. et Trappe emend. Walker et Koske	0^a	0^a	6^a	10^a
Glomus macrocarpum Tul. et Tul.	0^a	0^a	5^a	0^a
Glomus microcarpum Tul et Tul.	18^b	0^a	11^b	16^b
Glomus occultum Walker	9^a	0^a	9^a	4^a
Scutellospora dipurpurescens Morton et Koske	4^a	0^a	11^a	7^a
Scutellospora pellucida (Nicol. et Schenck) Walker et Sanders	0^a	0^a	4^a	0^a
Total	95^b	8^a	175^c	164^c

Means followed by the same letters are not statistically different by the Tukey test at 0.05 level

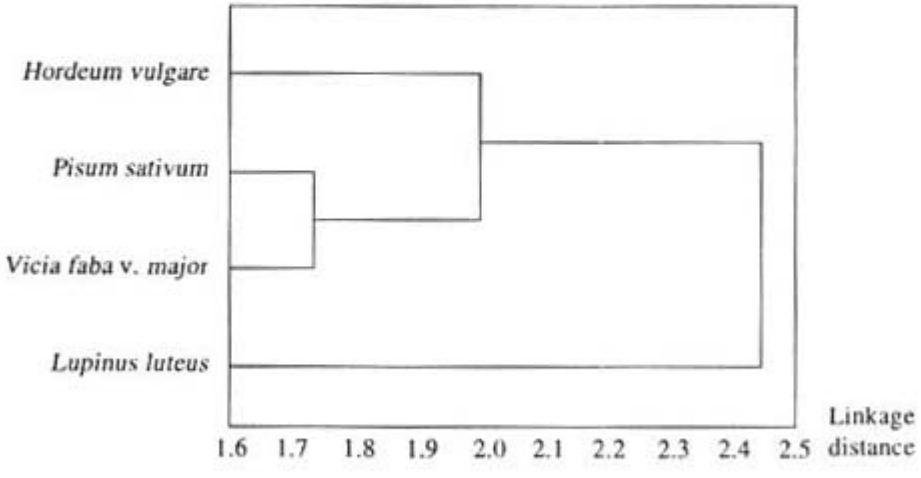

Fig. 1. Dendrogram illustrating the linkage distance between species compositions of arbuscular fungal populations recovered from the root zone of *XTriticosecale* preceded by four plant species

Considering jointly all pre-crop treatments, the AM fungi species dominating in the root zone of *XTriticosecale* were: *G. etunicatum*, followed by *G. caledonium*, *G. constrictum*, *G. microcarpum*, and *G. dominikii* (Tab. 1). One unidentified species, i.e., *Acaulospora* "61", formed abundant spore populations in the root zone of garden pea and board bean. This fungus produces spores singly in the soil, laterally on the neck of a sporiferous saccule. Spores are pale yellow (3A3) to orange (5B8) (K o r n e r u p, W a n s c h e r, 1983); globose to subglobose; (98-) 116 (-140) µm diam; rarely ovoid; 100 x 130 µm, attached to the saccule by a slightly raised collar 8.1-10.5 µm wide x 3.7-5.0 µm long surrounding a hole 7.5-9.8 µm diam. Spore contents at maturity are occluded by a septum formed by continuation of spore-wall growth. Spore wall structure of this fungus consists of seven walls (1-7) which are aggregated in three groups (A, B, C). Group A is composed of three adherent walls (walls 1-3). Wall 1 is evanescent, hyaline, (0.8-) 1.0 (-1.3) µm thick, usually completely sloughed in mature spores. Wall 2 is laminated, pale yellow (3A3) to orange (5B8), (2.2-) 2.8 (-3.9) µm thick. Wall 3 is unit, hyaline, (0.5-) 0.7 (-0.8) µm thick, separable from wall 2. Group B consists of two hyaline, thightly adherent, unit walls (walls 4, 5), each 0.4-0.6 µm thick. Group C has two adherent hyaline walls (walls 6, 7). Wall 7 is amorphous, 10.0-12.5 µm thick in PVLG, (0.8-) 1.1 (-1.2) µm thick and beetroot purple (13D8) in Melzer's reagent. Spore contents are of hyaline oil droplets. Sporiferous saccule is hyaline; globose to subglobose; 90-130 µm diam; neck is 50-80 µm long, tapering from 17-24 µm diam at the saccule to 15-20 µm diam at the point of spore attachment. Saccule wall is composed of a hyaline, smooth, 0.8-1.2 µm thick layer. Saccule collapses at maturity and is usually detached among mature spores.

Acaulospora "61" is most closely related to *A. dilatata* Morton, *A. longula* Spain et Schenck, *A. mellea* Spain et Schenck, and *A. morrowiae* Spain et Schenck due to the similarity in spore colour and the presence of a beaded membranous wall adherent to an amorphous wall in the innermost wall group C (M o r t o n, 1994). The features separating *Acaulospora* "61" from the species mentioned above are spore size and the number of walls in groups A and B. *Acaulospora mellea* and *A. morrowiae* have a 3-walled structure of group A with walls of the same types as in *Acaulospora* "61". However, the middle group B of spores of the first two species is represented only by one semi-rigid wall, whereas group B in *A.mellea* has two such walls tightly adherent to each other. Group B of *A. longula* spores also consists of a single wall. Two semi-rigid walls forming wall group B occur in *A. dilatata* spores, but, like in *A. longula*, their structural wall is composed of only one laminated wall (vs. three walls in *Acaulospora* "61").

Of the species dominating (with spores density at and above 40 in 100 g dry soil), significantly more spores of *G. caledonium*, *G. constrictum*, *G. etunicatum*, and *G. microcarpum* were associated with *XTriticosecale* when grown after garden pea and broad bean than following the cultivation of barley (Tab. 1). *The XTriticosecale* after yellow lupine treatment was represented only by *G. constrictum*

spores. Most spores of *G. dominikii* were isolated when the pre-crop was garden pea followed by board bean and barley, but these differences were not statistically significant.

Pisum sativum compared with the other pre-crop plants examined caused in *XTriticosecale* a significant enhancement of both the degree of mycorrhizal infections and the number of root fragments with vesicles, arbuscules, intramatrical hyphae, and *G. tenue* infections (Tab. 2). *XTriticosecale* grown on the pea plots also had the greatest percentage of roots with extramatrical hyphae, altough not differing significantly from that regarding the *XTriticosecale* after bean treatment.

Although the pre-crop plant species did not have any significant effect on the proportion of *XTriticosecale* roots colonized by *P. graminicola*, this fungus most frequently occurred when *XTriticosecale* succeeded barley (Tab. 2).

There were significant ($P < 0.05$) correlations between the spore densities recovered from the root zone of the plant species examined and their degree of mycorrhizal infection ($r = 0.97$), percentage of roots with vesicles ($r = 0.96$), and intramatrical ($r = 0.96$) and extramatrical hyphae ($r = 0.97$). Additionally, the degree of infection was significantly correlated with the proportion of roots with intramatrical ($r = 0.99$, $P < 0.05$) and extramatrical hyphae ($r = 1.00$, $P < 0.01$).

Table 2

Effect of pre-crop plants on the occurrence of arbuscular mycorrhize, *Glomus tenue*, and *Phialophora graminicola* in *XTriticosecale* roots

Specification	*Hordeum vulgare*	*Lupinus luteus*	*Pisum sativum*	*Vicia faba* var. *major*
Infection degree*	0.71^{b}	0.43^{a}	1.21^{d}	0.98^{c}
Vesicles (%)	34^{ab}	16^{a}	44^{b}	37^{ab}
Arbuscules (%)	11^{a}	12^{a}	21^{b}	14^{ab}
Intramatrical hyphae (%)	9^{a}	31^{a}	98^{c}	76^{bc}
Extramatrical hyphae (%)	62^{b}	11^{a}	75^{c}	58^{c}
Glomus tenue (%)	32^{b}	34^{a}	91^{c}	68^{b}
Phialophora graminicola (%)	88^{bc}	8^{a}	6^{a}	14^{a}

* 0 = no infection; 1 = low infection areas; highly scattered; 2 = greater infection areas, more uniform; 3 = roots uniformly infected

Means followed by the same letters are not statistically different by the Tukey test at 0.05 level

DISCUSSION AND CONCLUSIONS

The presence of numerous spore populations representing four of the six genera of AMF known supports many reports of the common occurrence of these fungi in agricultural soils (B ł a s z k o w s k i, 1993).

In the present study, spores of the genera *Entrophospora, Gigaspora*, and *Sclerocystis* were lacking. *Entrophospora infrequens* (Hall) Ames at Schneider and *G. gigantea* (Nicol. et Gerd.) Gerd. et Trappe had been revealed in soils of both the Agricultural Experiment Station Lipki and other cultivated sites in Poland, altough in low densities (Błaszkowski, 1993). In Poland *Sclerocystis rubiformis* Gerd. et Trappe has been found so far to be associated only with wild plants (Błaszkowski, 1993). *Entrophospora infreguens, G. gigantea*, and *S. rubiformis* have been reported from cultivated sites of other regions of the world (An et al., 1993; Bentivenga, Hetrick, 1992; Hamel et al., 1994; Johnson et al., 1991; Schenck, Kinloch, 1980). The lack of fungi mentioned above in soils sampled by the author of this paper may result from their, among others, susceptibility to stresses caused by agricultural practices (Dodd, Jeffries, 1989; Jasper et al., 1989; Schenck, Siqueira, Oliveira, 1989), adaptation to other soil conditions (Day, Sylvia, Collins, 1987), seasonality of sporulation (Gemma, Koske, Carreiro, 1989; Hayman, 1970), and omitting due to excessively low number of collected soil samples (St. John, Koske, 1988). Franke and Morton (1984), Gazey et al. (1992), and Jasper et al. (1993) showed that sporulation is dependent on a threshold level of mycorrhizal colonization. Thus, the absence of sporulation does not indicate the absence of fungal organism. The absence of spores does indicate, however, that mycorrhizal biomass of detected organisms has reached critical mass for sporulation, and the quantity of spores may provide some measure of fungal fitness.

This and other investigations (Błaszkowski, 1993; Czajkowska-Strzemska, 1988) provide evidence that garden pea and board bean are good host plants for the rapid build-up of infective propagules of AMF. Most leguminous plants are highly mycotrophic (Harley, Harley, 1990; Harley, Smith, 1983).

The distinctive low number of spores associated with *XTriticosecale* preceded by yellow lupine supports the results of other investigators that this plant is one of few exceptions among the members of the *Leguminosae*, being either an autotrophic or rarely forming arbuscular mycorrhiza plant species (Czajkowska-Strzemska, 1988; Harley, Smith, 1983).

The predominance of fungi of the genus *Glomus* in the isolated spore populations corresponds with the results of other investigations conducted in both agricultural soils and sites with natural vegetation (Ferrer et al., 1989; Gianinazzi-Pearson et al., 1980; Scheck, 1981; Nemec et al., 1981). This suggests that these fungi are best adapted to a wide range of environmental conditions. A similar conclusion has been drawn by, e.g., Błaszkowski (1993), Gerdemann and Trappe (1974), and Mosse, Stribley and LeTacon (1981).

The dominating species in the AM fungi populations recovered were: *G. etunicatum*, followed by *G. caledonium, G. constrictum, G. microcarpum*, and *G. dominikii*. Except for *G. dominikii*, the other fungi species are known to occur in agricultural soils of different regions of the world (Błaszkowski, 1993). *Glomus caledonium* and *G. constrictum* were previously found among the species

dominating in cultivated soils of Poland (Błaszkowski, 1993). *Glomus dominikii* is a stable colonizer of different plants cultivated at the Agricultural Experiment Station Lipki. However, its incidence in other agricultural sites of Poland is rare (Błaszkowski, 1993). Recently, this species has been recovered from maritime dune soils of the Słowiński National Park (Błaszkowski, unpubl.).

The higher mycorrhizal colonization of *XTriticosecale* preceded by the garden pea and board bean treatment compared with the other two plant species tested correspond with the result discussed above regarding the influence of leguminous plants on sporulation of AMF.

Pisum sativum and *Vicia faba* var. *major* significantly increased the proportion of *XTriticosecale* roots with arbuscules, intra- and extramatrical hyphae, and *G. tenue* (Greenhall) Hall infections. This suggests that these plants may function as biofertilizers for succeeding crops. Arbuscules are the sites of nutrient exchange (Bonfante-Fasolo, 1984). Extramatrical hyphae play a crucial role in the uptake and translocations of nutrients to the host plant (Cooper, Tinker, 1978) and can act as a source of inoculum (Sylvia, 1992) as well as improve the water relations of the plant (Hardie, Leyton, 1981), and increase soil aggregation (Miller, Jastow, 1990). *Glomus tenue* has been shown to effectively absorb soil phosphorus (Powell, 1979).

No significant effect of the pre-crop plant species investigated on the occurrence of *P. graminicola* in *XTriticosecale* roots was found. This fungus is a non-pathogenic parasite inhibiting the development of *Gaeumannomyces graminis* var. *tritici* (Deacon, 1973 b) and improving the nutrient status of plants in a manner similar to that known in AMF (Cowan, 1979). *Phialophora graminicola* has been found to be commonly associated with wheat and many other grass species growing in Poland (Błaszkowski, 1991 b, Błaszkowski, unpubl.). The higher incidence of *P. graminicola* following the cultivation of barley may indicate some preference of this fungus to members of the *Gramineae*.

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