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THE EFFECT OF MYCORRHIZATION AND MULCHING OF APPLE TREES ‘GOLD MILENIUM’ AND BLACKCURRANT BUSHES ‘TIBEN’ ON THE OCCURRENCE OF ARBUSCULAR MYCORRHIZAL FUNGI

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A B S T R A C T

The study was conducted in the Pomological Orchard and a greenhouse complex of the Research Institute of Horticulture in Skierniewice (RIH) in 2006-2008. Its aim was to identify arbuscular mycorrhizal fungi (AMF) present in the trap cultures containing rhizosphere soil and to determine mycorrhizal frequency (colonization) of AMF in the roots of apple trees ‘Gold Milenium’ and blackcurrant bushes ‘Tiben’. The apple trees and blackcurrant bushes were mulched (with a peat substrate, bark, sawdust, manure, compost, or straw) and inoculated (the AMF inoculum was produced by MYKOFLOR).

Samples of the soil and roots were collected from under the apple trees and blackcurrant bushes in experimental combinations and in the control. In order to identify the spores of arbuscular mycorrhizal fungi, trap cultures (with rhizosphere soil and sand) were set up with plantain (*Plantago lanceolata* L.). The spores were isolated from the trap cultures and microscopic specimens were prepared to identify the species of AMF, which were distinguished on the basis of their morphological features. Mycorrhizal frequency was determined in the specimens of apple and blackcurrant roots dyed with aniline blue.

In total, eight species of AMF were identified in the trap cultures established with the soil samples taken from the root zone (containing rhizosphere soil) of apple trees ‘Gold Milenium’ and blackcurrant bushes ‘Tiben’: *Glomus aggregatum*, *G. caledonium*, *G. claroideum G. constrictum*, *G. intraradices*, *G. macrocarpum*, *G. mosseae* and *Gigaspora margarita*. In the trap cultures with the rhizosphere soil of apple, the greatest number of species was found in the combinations with manure and the mycorrhizal inoculum (5 species), and compost (4 species). In the case of blackcurrant, the use of the mycorrhizal substrate and straw resulted in the largest number of AMF species (5 and 4, respectively).

In the 2008 season, the highest mycorrhizal frequency in apple was obtained in the mycorrhized roots (44.4%), lower in the combinations with compost, sawdust, manure, bark, straw, and peat, and the lowest in the roots of NPK control plants (5.56%). The highest mycorrhizal frequency in the roots of blackcurrant bushes cv. 'Tiben' was recorded following the application of the mycorrhizal inoculum (12.22%), lower in the combinations with sawdust, compost, straw, peat, manure, and bark, and the lowest in the NPK control (1.67%).

Key words: arbuscular mycorrhizal fungi (AMF), mulching, trap culture, AMF species, mycorrhizal frequency in roots

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) of the phylum *Glomeromycota* are a very important component of the rhizosphere. They play a significant role in the nutrition of plants, their protection against pathogens, and in improving soil texture (Allen, 1991; Azcón-Aquilar and Barea, 1992; Smith and Read, 1997; Wright and Upadhyaya, 1998; Sas Paszt and Głuszek, 2007; Głuszek et al., 2008). In that respect, identification and characterization of the species of fungi that naturally colonize the roots of fruit crop plants are very important aspects of scientific research.

Arbuscular mycorrhizal fungi most often colonize the roots of wild species of apple trees, e.g. *Malus communis* L. and *Malus sylvestris* Mill., which possess arbuscular mycorrhizal symbionts (Harley and Harley, 1987). The roots of wild species of blackcurrant are also colonized by AMF, such as those of *Ribes nigrum* L., *R. rubrum* L., *R. spicatum* Robson, *R. uva-crispa* L., and by ectomycorrhizal fungi, e.g. *Ribes alpinum* L. (Harley and Harley, 1987).

There are numerous reports in the literature on the effects of inoculation with AMF on the assimilation and accumulation of phosphorus and other nutrients by the roots of apple trees (Covey et al., 1981; Miller et al., 1985; Gnekow and Marschner, 1989; Schubert and Lubraco, 2000; Cavallazzi et al., 2007).

There have been many studies on the use of mycorrhizal fungi to inoculate apple plants grown in *in vitro* cultures, which help in the acclimatization of these plants to field conditions (Fortuna et al., 1996; Schubert and Lubraco, 2000; Meikle and Amaranthus, 2008). There have also been many reports on the use of symbiotic fungi, such as AMF, on soils affected by apple replant disease (Kandula et al., 2006; Ridgway et al., 2008). However, there are no reports on AMF inoculation of blackcurrant bushes. There have been very few studies on the occurrence of native symbiotic AMF in fruit plants treated with organic mulches in conventional and organic orchards (Purin et al., 2006).

Application of mycorrhizal inocula and organic mulches can increase the diversity of AMF species in the rhizosphere. Maintaining this diversity in the environment of an ecological orchard has a beneficial effect on the growth and health status of the cultivated fruit plants.

The aim of the study was to assess the effect of mycorrhization and mulching of apple trees cv. 'Gold Milenium' and blackcurrant bushes cv. 'Tiben' on the number of AMF species and mycorrhizal frequency in plant roots.

MATERIAL AND METHODS

Experimental design

The work was carried out in the RIH Pomological Orchard in 2006-2008.

The field experiment was of a two-factorial design with the following factors:

Factor A – two species of fruit plants

1. Apple cv. 'Gold Milenium'.
2. Blackcurrant cv. 'Tiben'.

Factor B – soil mulching

1. Control (unmulched plants, fertilized with NPK only).
2. Peat substrate (pH 6.5).
3. Bark (coniferous trees).
4. Sawdust (coniferous trees).
5. Manure (granulated, bovine).
6. Compost (plant fragments).
7. Mycorrhizal inoculum (produced by MYKOFLOR, Końskowola, Poland, containing 3 species of mycorrhizal fungi: *Glomus intraradices, Glomus mosseae, Glomus etunicatum*).
8. Straw (rye).

Apple trees cv. 'Gold Milenium' (one-year-old maidens) were planted in August 2003 in one row, 1.2 m apart, in a random block design in 4 replications. A replication (plot) consisted of 3 trees; there was a 1 m wide isolation strip between the plots. All the experimental combinations were fertilized with standard NPK fertilizers (11.7 g ammonium nitrate, 7.7 g potassium sulphate, and 19.8 g superphosphate per tree).

Blackcurrant bushes cv. 'Tiben' were planted in the autumn of 2003 in one row, 0.75 m apart, in a random block design in 3 replications. A replication (plot) consisted of 3 bushes; there was a 1 m wide isolation strip between the plots. All the experimental combinations were fertilized with standard NPK fertilizers (15.7 g ammonium nitrate, 13.8 g potassium sulphate, and 17.0 g superphosphate per bush).

Mulch was applied in the amount of 25 l per each apple and blackcurrant plot (in the spring of every year). The MYKOFLOR mycorrhizal inoculum was applied at a rate of 200 g per apple tree and 150 g per blackcurrant bush. The AMF inoculum was applied in the spring of every year of the experiment.

The trees and bushes were watered by means of a computer-controlled irrigation system. Plant protection treatments were carried out in accordance with the recommendations for commercial plantations.

Sample collection

Soil and root samples were taken from the root zone of the apple trees and blackcurrant bushes in August 2006 and April 2008. The soil core samples, app. 500 cm^3, were taken from a depth of 0-15 cm with the help of metal tubes, in six replications for each combination.

Setting up trap cultures

The soil samples, collected together with cut-up fragments of the roots, were mixed with autoclaved sand in the proportion of 1:1 (v/v), then placed in 500 cm^3 (0.5 l) pots and seeded with narrowleaf plantain (*Plantago lanceolata* L.) at a rate of 30 seeds per pot. To avoid mixing up spores from different traps, the pots were placed inside semi-permeable plastic SUNBAG bags (Sigma B7026). The trap cultures were established in three replications (3 pots) for each combination (Błaszkowski, 2003). The plants grew in a greenhouse (RIH) at a temp. of 20 °C, under 8/16 hr irradiance (70-100 $\mu M\ m^{-2}\ s^{-1}$) with a sodium light from a SON-T AGRO 400W lamp. The plants were watered with distilled water once every two weeks. After 6 months, the propagated spores were isolated for examination.

Isolation of spores in sucrose solution

A 100 g of the substrate, taken from 3 places in the pot was put in a 1 l plastic bottle and mixed vigorously for about 5 minutes with 800 ml of deionised water. The suspension was placed in a refrigerator at -4 °C for 24 h. The spores were isolated by filtering the suspension (mesh size 50 μm) and centrifuging the filtrates in a 20% sucrose solution followed by centrifugation in a 60% sucrose (Brundrett et al., 1996).

Preparation of microscopic specimens and analysis of spores

The spores from the pellets were picked with a preparation needle or an automatic pipette and placed on a Petri dish, where they were divided into morphotypes (taking into consideration: size, shape, colour, number of walls). Next, the samples were suspended in a drop of Polyvinyl-Lacto-Glycerol (PVLG) and then mixed with a drop of a mixture of PVLG and Meltzer's reagent dyes (4:1, v/v) on a microscope slide (Omar et al., 1979). The specimen were analysed/identified according to Błaszkowski (2003, 2008).

Determination of mycorrhizal frequency

In order to calculate mycorrhizal frequency, root pieces were cold-stained using the Phillips and Hayman method (1970) and modified by Turnau et al. (2001). In particular, bleaching with 10% KOH and acidification with 5% lactic acid were carried out for 24 hours each. Staining was performed with 0.01% aniline blue.

The microscopic analyses of the roots were carried out according to Trouvelot's method (1986). Thirty 1-cm-long root segments were selected randomly from each of the stained samples. The segments were examined under a Nikon Eclipse E200 microscope. Mycorrhizal frequency (F%), absolute mycorrhizal intensity (m%) and the abundance of arbuscules present in the root fragments (a%) were assessed in each root segment. The mycorrhizal parameters were calculated using the Mycocalc software (http://www2.dijon.inra.fr.mychintec/Mycocalc-pgr/download.html). For each experimental treatment three replicates were analyzed, constituting in total 90 root segments (Derkowska et al., 2008).

Determination of soil chemical parameters

The amounts of available P and K were determined by Egner-Rhiem method, whereas available Mg was detected according to Schachtschabel method ICP spectrometry method (Mercik, 2002). Soil pH was determined in KCl.

The results were statistically evaluated with an analysis of variance. Mean values were assessed at $p \leq 0.05$ with the Duncan's test.

RESULTS

From the trap cultures set up with the rhizosphere soil of apple trees cv. 'Gold Milenium', 6 species of fungi of the phylum *Glomeromycota* were identified in all the experimental combinations. In the trap cultures from the rhizosphere soil mulched with manure and the mycorrhized soil 5 AMF species were identified; in the soil mulched with compost 4 species; 3 species of AMF were found in the combination treated with peat and in the control (Tab. 1).

Glomus aggregatum, G. claroideum, G. macrocarpum were observed in the trap cultures from all the tested combinations, *G. constrictum* in the combination mulched with compost, in the inoculated combination and in the control. *G. mosseae* was identified only in the inoculated combination. *Gigaspora margarita* was observed in the combination mulched with manure. *G. claroideum* was also found in the rhizosphere soil taken from the inter-rows in the Pomological Orchard.

In the trap culture with the rhizosphere soil taken together with the roots from under blackcurrant bushes cv. 'Tiben' there were found 6 species of AMF. Five AMF species were found in the mycorrhized soil. In the combination with straw there were present 4 species. In the soil mulched with compost, peat and in the control 3 species were identified (Tab. 2).

Gigaspora margarita, *Glomus caledonium* and *G. macrocarpum* were identified in all the trap cultures established with soil samples from blackcurrant bushes, whereas *G.intraradices* and *G. mosseae* were found in the inoculated soil only. The species *G. claroideum* was found only in the soil mulched with straw.

In the rhizosphere of apple, the largest number of species was found in the combinations with manure, the mycorrhizal inoculum (5 species), and compost (4 species). In the case of blackcurrant, the use of the mycorrhizal substrate and straw resulted in the greatest number of AMF species (5 and 4 species, respectively).

Table 1. Species of arbuscular mycorrhizal fungi (AMF) identified with the use of trap cultures in the soil samples collected from the root zone of apple trees 'Gold Milenium'

Mulching combinations	Species of AMF
Control	*Glomus aggregatum* Schenck & Smith *G. claroideum* Schenck & Smith *G. macrocarpum* Tulasne & Tulasne
Manure	*Glomus aggregatum* Schenck & Smith *G. claroideum* Schenck & Smith *G. constrictum* Trappe *G. macrocarpum* Tulasne & Tulasne *Gigaspora margarita* Becker & Hall
Peat	*Glomus aggregatum* Schenck & Smith *G. claroideum* Schenck & Smith *G. macrocarpum* Tulasne & Tulasne
Mycorrhizal inoculum	*Glomus aggregatum* Schenck & Smith *G. claroideum* Schenck & Smith *G. constrictum* Trappe *G. macrocarpum* Tulasne & Tulasne *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe
Compost	*Glomus aggregatum* Schenck & Smith *G. claroideum* Schenck & Smith. *G. constrictum* Trappe *G. macrocarpum* Tulasne & Tulasne

Table 2. Species of arbuscular mycorrhizal fungi (AMF) identified with the use of trap cultures in the soil samples collected from the root zone of blackcurrant bushes 'Tiben'

Mulching combinations	Species of AMF
Control	*Glomus caledonium* (Nicol. & Gerd.) Gerdemann & Trappe *G. macrocarpum* Tulasne & Tulasne *Gigaspora margarita* Becker & Hall
Straw	*Glomus caledonium* (Nicol. & Gerd.) Gerdemann & Trappe *G. claroideum* Schenck & Smith *G. macrocarpum* Tulasne & Tulasne *Gigaspora margarita* Becker & Hall
Peat	*Glomus caledonium* (Nicol. & Gerd.) Gerdemann & Trappe *G. macrocarpum* Tulasne & Tulasne *Gigaspora margarita* Becker & Hall
Mycorrhizal inoculum	*Glomus caledonium* (Nicol. & Gerd.) Gerdemann & Trappe *G. intraradices* N.C. Schenck & G.S. Sm *G. macrocarpum* Tulasne & Tulasne *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe *Gigaspora margarita* Becker & Hall
Compost	*Glomus caledonium* (Nicol. & Gerd.) Gerdemann & Trappe *G. macrocarpum* Tulasne & Tulasne *Gigaspora margarita* Becker & Hall

In the apple orchard there were species of fungi different from those on the blackcurrant plantation. In the apple orchard, in the combination mulched with compost there was *G. constrictum*, whereas on the blackcurrant plantation in the combination with compost this species was not found. On the other hand, in the combination fertilized with manure there were the same fungi as those in the inoculated soil, except for *G. mosseae*, which had been introduced with the mycorrhizal inoculum. In the inoculated combination there was *G. constrictum*, which was not present in the control combination, nor in the MYKOFLOR inoculum. Two species of AMF, *G. intraradices* and *G. etunicatum*, which were present in the inoculum, were not found in the inoculated soil.

In the 2006 growing season, the highest mycorrhizal frequency (F%), intensity of colonization (m%) and arbuscule abundance (a%) in the roots of apple cv. 'Gold Milenium' were recorded for the combinations mulched with the peat substrate and bark. In these combinations, an abundance of vesicles and mycorrhizal mycelia was observed, while in the roots from the other combinations tested very few arbuscules were found. The lowest mycorrhizal frequency was recorded for manure- and sawdust-treated plans (Tab. 3). In the 2008 season, the highest mycorrhizal frequencies were recorded for the inoculated soil, and the lowest for the control fertilized with NPK only (Tab. 3). In that season, no arbuscules were observed, while vesicles and mycelia occurred at low abundance.

In the 2006 growing season, the highest mycorrhizal frequency (F%) and intensity of colonization (m%) in the roots of blackcurrant cv. 'Tiben' were recorded in the combination mulched with the peat substrate and in the inoculated soil. The highest value of arbuscule abundance was recorded in the combination mulched with bark. In the remaining combinations, vesicles, mycelia and arbuscules were present at low abundance. The lowest values of mycorrhizal frequency were recorded for sawdust, manure, compost and the control (Tab. 4). In the 2008 season, the highest values of mycorrhizal frequency were recorded for the mycorrhizal inoculum, sawdust and compost, and the lowest for the NPK-fertilized control (Tab. 4). In that season, no arbuscules were found, while vesicles (and mycelia) occurred at low abundance.

Mycorrhizal frequency in the roots of mulched and inoculated apple trees and blackcurrant bushes reached higher values for both plant species in the 2008 season than in 2006. In both seasons, however, the mycorrhizal frequency in the roots of apple cv. 'Gold Milenium' was higher than in the roots of blackcurrant cv. 'Tiben'.

The mulches and mycorrhizal inoculum applied to apple trees cv. 'Gold Milenium' contributed to lowering soil pH in comparison with the control. Mulching apple trees with bark and the application of manure contributed to an increase in the

Table 3. Mycorrhizal colonization parameters determined in the roots of apple ‘Gold Milenium’. The parameters are: F% – mycorrhizal colonization frequency, m% – intensity of colonization within individual mycorrhizal roots, a% – arbuscule abundance in root fragments where arbuscules were present (different letters in columns referring to the same parameter indicate statistically significant differences at $p < 0.05$)

Mulching combination	2006			2008		
	F%	M%	a%	F%	m%	a%
Control	2.5 a	1.75 ab	1.29 a	5.56 ab	1.0 a	0.0 a
Peat substrate	25.83 c	2.87 b	24.40 a	11.11 ab	1.0 a	0.0 a
Bark	23.33 bc	1.40 ab	10.59 a	14.44 abc	1.33 a	0.0 a
Sawdust	3.33 a	0.25 a	1.25 a	16.67 bc	1.0 a	0.0 a
Manure	5.0 ab	0.5 a	3.75 a	15.55 bc	1.0 a	0.0 a
Compost	10.83 abc	2.0 ab	2.44 a	18.89 bc	1.0 a	0.0 a
Mycorrhizal inoculum	9.17 abc	1.0 ab	6.25 a	44.44 c	1.0 a	0.0 a
Straw	15.0 abc	1.0 ab	2.13 a	13.33 abc	1.0 a	0.0 a

Table 4. Mycorrhizal colonization parameters determined in the roots of blackcurrant bushes ‘Tiben’. The parameters are: F% – mycorrhizal colonization frequency, m% – intensity of colonization within individual mycorrhizal roots, a% – arbuscule abundance in root fragments where arbuscules were present (different letters in columns referring to the same parameter indicate statistically significant differences at $p < 0.05$)

Mulching combination	2006			2008		
	F%	M%	a%	F%	m%	a%
Control	3.21 a	1.0 a	2.22 a	1.67 a	1.47 a	0.0 a
Peat substrate	11.11 abc	1.0 a	6.95 ab	5.55 ab	2.78 a	0.0 a
Bark	4.44 a	1.0 a	10.0 abc	9.17 ab	5.06 ab	0.0 a
Sawdust	3.33 a	1.0 a	0.0 a	10.0 abc	1.67 a	0.0 a
Manure	3.33 a	0.67 a	6.67 ab	4.44 a	0.33 a	0.0 a
Compost	3.33 a	0.67 a	5.0 ab	10.0 abc	4.56 a	0.0 a
Mycorrhizal inoculum	5.56 ab	1.0 a	8.33 ab	12.22 abc	5.37 ab	0.0 a
Straw	0.0 a	0.0 a	0.0 a	2.22 a	1.0 a	0.0 a

phosphorus content of the soil. Mulching with manure contributed also to a higher level of potassium in the soil (Tab. 5). Compared with the control, mulching and mycorrhization of the trees had a considerable effect on lowering magnesium and potassium content in the soil (Tab. 5).

Mulching blackcurrant bushes with compost and manure contributed to an increase in the rhizosphere soil pH. The application of manure and compost significantly increased the phosphorus content of the soil in comparison with the control and the other mulches (Tab. 6). The highest potassium content, in relation to the control, was recorded after the use of manure, compost and straw, and the lowest after the use of the peat

T a b l e 5. pH level and phosphorus, potassium, magnesium content in the rhizosphere soil of apple trees 'Gold Milenium' after treatment with different organic fertilizers (n = 3; different letters referring to the same nutrient or pH level indicate statistically significant differences at $p < 0.05$)

Mulching combination	pH in KCl	P [mg·100 g^{-1}]	K [mg·100 g^{-1}]	Mg [mg·100 g^{-1}]
Control	6.83 g	5.32 b	35.10 g	12.01 h
Peat substrate	5.58 e	7.83 e	23.40 e	6.47 f
Bark	4.82 a	9.89 h	25.90 f	4.54 a
Sawdust	4.89 b	7.55 d	23.10 d	5.24 d
Manure	4.93 c	9.50 g	42.50 h	7.32 g
Compost	4.82 a	6.26 c	19.10 b	4.93 c
Mycorrhizal inoculum	5.47 d	5.00 a	15.90 a	4.87 b
Straw	5.71 f	8.15 f	21.80 c	6.40 e

T a b l e 6. pH level and phosphorus, potassium, magnesium content in the rhizosphere soil of blackcurrant bushes 'Tiben' after treatment with different organic fertilizers (n = 3; different letters referring to the same nutrient or pH level indicate statistically significant differences at $p < 0.05$)

Mulching combination	pH in KCl	P [mg·100 g^{-1}]	K [mg·100 g^{-1}]	Mg [mg·100 g^{-1}]
Control	4.96 g	6.20 a	15.27 d	4.20 h
Peat substrate	4.82 d	6.33 ab	12.17 b	2.81 a
Bark	4.79 bc	6.52 abc	13.22 c	2.88 ab
Sawdust	4.89 e	6.84 bcd	15.38 d	3.34 e
Manure	5.01 h	12.14 g	38.38 h	6.60 l
Compost	5.25 j	8.55 e	19.91 f	4.90 i
Mycorrhizal inoculum	4.82 d	6.85 bcd	15.49 d	3.20 d
Straw	4.83 d	7.00 cd	17.26 e	2.96 bc

substrate and bark. The use of manure, and compost to a lesser extent, contributed to a significant increase in the magnesium content of the soil in comparison with the control and the other mulches (Tab. 6).

DISCUSSION

Organic mulches and mycorrhizal inocula are frequently used in order to develop innovative eco-friendly methods of fruit growing (Sas Paszt and Żurawicz, 2004, 2005; Sas Paszt and Głuszek, 2007). In the work presented, a total of eight species of AMF: *Glomus aggregatum*, *G. caledonium*, *G. claroideum* *G. constrictum*, *G. intraradices*, *G. macrocarpum*, *G. mosseae* and *Gigaspora margarita*, were identified in the soil samples taken from the root zone of apple trees cv. 'Gold Milenium' and blackcurrant bushes cv. 'Tiben'. In the trap cultures established with the rhizosphere soil of apple, the greatest number of species was found in the combinations with manure and the mycorrhizal inoculum (5 species), and compost (4 species). In the case of blackcurrant, the use of the mycorrhizal substrate and straw resulted in the greatest number of the species of arbuscular mycorrhizal fungi (5 and 4 species, respectively).

In the experiments done in southern Brazil, a greater species diversity of AMF was found in organic orchards – 30 species, than in conventional orchards – 20 species. However, sporulation of those fungi was greater in the orchards fertilized in a conventional way (Purin et al., 2006). In natural ecosystems, in marine sand dunes, the number of the recorded species can be as high as 30 (Błaszkowski and Czerniawska, 2011), but it can also be lower, e.g. about 17 species in the soil of native grassland (Purin et al., 2006).

The species: *Glomus mosseae*, *G. intraradices* and *G. etunicatum* applied in our experiment to inoculate the apple trees and blackcurrant bushes are often used in the mycorrhization of crop plants. *G. mosseae* was detected in both apple and blackcurrant. Other authors described this AMF species in apple (Pinochet et al., 1993) and other plants (Vijayalakshmi and Rao, 1988; Şesan et al., 2010). This species is often used to inoculate apple trees in pot and field experiments all over the world (e.g. Covey et al., 1981; Fortuna et al., 1996; Schubert and Lubraco, 2000; Błaszkowski, 2003; Ridgway et al., 2008). *G. intraradices* was found in blackcurrant soil only. However, *G. etunicatum*, used in the mycorrhizal inoculum in our experiment, was not found in the rhizosphere soil of apple or blackcurrant. The species: *Glomus aggregatum*, *G. caledonium* *G. claroideum* *G. constrictum*, *G. macrocarpum* and *Gigaspora margarita*, identified in our experiment, are the species commonly found in various regions of Poland and in other countries worldwide (Vijayalakshmi and Rao, 1988; Matsubara et al., 1996; Błaszkowski, 2003; Lee and Eom, 2009; Şesan et al., 2010).

The mulches used varied in their influence on AMF root colonization. The highest mycorrhizal frequency in apple was obtained in the mycorrhized roots in the 2008 season (44.4%), lower in the combinations with compost, sawdust, manure, bark, straw, peat, and the lowest in the roots of the control plants (5.56%). Matsubara et al. (1996) carried out inoculation of seedlings of apple *Malus pumila* Mill. var. *domestica* (cvs. 'Macintosh', 'American Summer Pearmain', 'Jonathan', 'Golden Delicious', 'Fuji', 'Mutsu' and 'Red Gold') and *Malus sieboldii* Rehd. using *Gigaspora margarita* and *Glomus etunicatum*. The presence of AMF was noted in all the experimental combinations. Mycorrhizal frequency, as a result of inoculation with *G. etunicatum*, was 31.7% in 'Golden Delicious', and 50.5% in 'Jonathan'. In the apple varieties inoculated with *Gigaspora margarita*, the mycorrhizal frequency was 24.0% in *Malus sieboldii* Rehd. and 50.7% in 'Starking Delicious'.

In this paper, the highest mycorrhizal frequency in the roots of the blackcurrant bushes cv. 'Tiben' was recorded following the application of the mycorrhizal inoculum in the 2008 season (12.22%), lower in the combinations with sawdust, compost and straw, peat, manure and bark, and the lowest in the control (1.67%). Meikle and Amaranthus (2008) inoculated the roots of golden currant (*Ribes aureum* Pursh.) grown in a container experiment, in a soil with a reduced phosphorus content. The degree of mycorrhizal frequency with traditional fertilization was from 0 to 20%, whereas with organic fertilization from 16 to 92%.

The results obtained in the Pomological Orchard (RIH) in 2006-2008 are an indication of a favourable effect of mycorrhization and mulching on AMF species number, mycorrhizal frequency (colonization) and nutrient uptake and acquisition.

The study will be continued to enable characterization and identification of the species of AMF by means of molecular techniques. The most valuable species of arbuscular mycorrhizal fungi that colonize the roots of apple and blackcurrant in nature will be used in fruit-growing practice, in particular those species and strains that improve plant nutrition and growth, fruit yield and fruit quality.

CONCLUSIONS

1. In total, eight species of AMF were identified in the samples of rhizosphere soil of apple trees 'Gold Milenium' and blackcurrant bushes 'Tiben': *Glomus aggregatum*, *G. caledonium*, *G. claroideum* *G. constrictum*, *G. intraradices*, *G. macrocarpum*, *G. mosseae* and *Gigaspora margarita*.
2. In the trap cultures set up with the rhizosphere soil of apple, the greatest number of AMF species was found in the combinations with manure and the mycorrhizal inoculum (5 species), and compost (4 species). In the case of blackcurrant, the use of the mycorrhizal substrate and straw resulted in the greatest number of the species of arbuscular mycorrhizal fungi (5 and 4 species, respectively).
3. The highest mycorrhizal frequency in apple was obtained in the mycorrhized roots in the 2008 season (44.4%), lower in the combinations with compost, sawdust, manure, bark, straw, peat, and the lowest

in the roots of NPK control plants (5.56%).

4. In 2008, the highest mycorrhizal frequency in the roots of the blackcurrant bushes 'Tiben' was recorded following the application of the mycorrhizal inoculum (12.22%), lower in the combinations with sawdust, compost, straw, peat, manure and bark, and the lowest in the NPK control (1.67%).

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WPŁYW MIKORYZACJI I ŚCIÓŁKOWANIA DRZEW JABŁONI 'GOLD MILENIUM' I KRZEWÓW PORZECZKI CZARNEJ 'TIBEN' NA WYSTĘPOWANIE ARBUSKULARNYCH GRZYBÓW MIKORYZOWYCH

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S T R E S Z C Z E N I E

Badania prowadzono w latach 2006-2008 w Sadzie Pomologicznym i kompleksie szklarniowym Instytutu Ogrodnictwa w Skierniewicach (IO). Ich celem była identyfikacja arbuskularnych grzybów mikoryzowych (AMF) występujących w glebie rizosferowej oraz określenie stopnia frekwencji mikoryzowej AMF w korzeniach jabłoni odmiany 'Gold Milenium' i porzeczki czarnej odmiany 'Tiben'. Drzewa jabłoni i krzewy porzeczek czarnych były ściółkowane (substratem torfowym, korą, trocinami, obornikiem, kompostem, słomą) i mikoryzowane (inokulum firmy MYKOFLOR).

Próbki gleby i korzeni były pobierane spod drzew jabłoni i krzewów porzeczek czarnych z wybranych kombinacji doświadczalnych i kontroli. W celu identyfikacji spor arbuskularnych grzybów mikoryzowych założono kultury pułapkowe (z gleby rizosferowej i piasku) z użyciem babki lancetowatej (*Plantago lanceolata* L.). Z zarodników wyizolowanych z kultur pułapkowych przygotowano preparaty mikroskopowe do identyfikacji gatunków AMF i oznaczono je na podstawie cech morfologicznych. Frekwencję mikoryzową określono w preparatach z korzeni jabłoni i porzeczek czarnych barwionych błękitem aniliny.

Osiem gatunków AMF zidentyfikowano w próbkach gleby z kultur pułapkowych zawierających glebę rizosferową drzew jabłoni 'Gold Milenium' i krzewów porzeczki czarnej 'Tiben': *Glomus aggregatum, G. caledonium, G. claroideum*, *G. constrictum*, *G. intraradices*, *G. macrocarpum*, *G. mosseae* i *Gigaspora margarita*. Pięć gatunków AMF odnotowano w kombinacji z obornikiem i inokulm mikoryzowym, a 4 w kombinacji z kompostem. W przypadku porzeczki czarnej w kombinacji z inokulum mikoryzowym znaleziono 5 gatunków, a w kombinacji ze słomą 4 gatunki AMF.

W sezonie 2008 najwyższą wartość frekwencji mikoryzowej u jabłoni 'Gold Milenium' stwierdzono w korzeniach mikoryzowanych (44,4%), niższe w kombinacji z kompostem, trocinami, obornikiem, korą, słomą, substratem torfowym, a najniższą w korzeniach roślin kontrolnych nawożonych NPK (5,56%). Najwyższą wartość frekwencji mikoryzowej stwierdzono w mikoryzowanych korzeniach porzeczki czarnej 'Tiben' (12,22%), niższe wartości w kombinacjach z trocinami, kompostem, słomą, substratem torfowym, obornikiem i korą, a najniższe w kontroli NPK (1,67%).

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Słowa kluczowe: arbuskularne grzyby mikoryzowe (AMF), ściółkowanie, kultury pułapkowe, gatunki AMF, frekwencja mikoryzowa w korzeniach