

# THE INFLUENCE OF MYCORRHIZATION AND ORGANIC MULCHES ON MYCORRHIZAL FREQUENCY IN APPLE AND STRAWBERRY ROOTS

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## A B S T R A C T

The aim of the experiments was to study the natural colonization of the roots of apple trees cv. 'Gold Milenium' and the roots of strawberry plants cv. 'Kent' by arbuscular mycorrhizal (AM) fungi. One-year-old apple maidens cv. 'Gold Milenium' were planted in 4 replicates, each consisting of 3 apple trees planted 4 m x 1.2 m apart, with a 1-metre-wide isolation strip between the plots. Strawberry plants cv. 'Kent' were planted in 3 replicates, each consisting of 20 plants planted at 1.0 m x 0.25 m spacing, with 0.5-metre-wide breaks between the plots.

For each of the experimental combinations: control, peat mulch, bark, sawdust, manure, compost, straw and mycorrhizal inoculum, root samples were collected with a cork borer for microscopic analyses. The roots were cold-stained using a method involving treatment with 10% KOH, acidification with 5% lactic acid, staining with 0.01 aniline blue and treatment with 80% lactic, and observed under microscope. The extent of micorrhization was assessed according to Trouvelot by determining the following parameters: **F %** – mycorrhizal frequency, **M %** – relative mycorrhizal intensity (for the whole sample), **m %** – absolute mycorrhizal intensity (for the segments in which there was some evidence of colonization by mycorrhizal fungi) **a %** – absolute abundance of arbuscules (for the segments in which arbuscules were found), **A %** – relative abundance of arbuscules (for the whole sample). The results obtained were analyzed using MycoCalc computer program.

On the basis of the results obtained it was concluded that mycorrhizal fungi colonized the roots of strawberry cv. 'Kent' more often than the roots of apple cv. 'Gold Milenium', and that the mycorrhizal frequency in strawberry roots, in individual combinations, was significantly higher than in apple roots (32-87% in strawberry, and 3-25% in apple). Other parameters, i.e. the abundance of arbuscules and mycorrhizal intensity, also had higher values for strawberry roots than for apple roots (0.31-91% and 0-24% for arbuscule abundance, respectively, and 0.9-13% and 0.25-4.5% for mycorrhizal intensity, respectively). The differences resulted from the different morphological characteristics of the roots in apple and strawberry, and from the significant difference in the size of the respective root systems.

**Key words:** mycorrhiza, rhizosphere, mycorrhizal frequency, arbuscular fungi, mulches

## INTRODUCTION

Mycorrhizal fungi are a very important component within the rich biodiversity of microorganisms occurring in the rhizosphere (Turnau et al., 2002). Xavier and Boyetchko (2002) have found that mycorrhizal, in particular endomycorrhizal fungi have a beneficial effect on plant growth and development, and that that effect can be likened to the effects of biostimulators and biofertilizers on plants. Al-Karaki (2004) showed that mycorrhizal fungi colonized more readily the roots of plants growing in an area with high water deficiency, and that the use of mycorrhizal inocula in dry areas had a favourable effect on the size and quality of the crop. Likewise, Kaldorf and Ludwig-Müller (2000) observed that mycorrhiza-covered roots were better developed; especially the number of lateral and fine roots was significantly greater. The presence of mycorrhiza in the roots intensifies uptake of water and minerals from the soil by the root system.

There is little data and ongoing research on the rhizosphere of fruit-

bearing plants despite the fact that this field of science has in recent years been developing quite intensively in case of forest and agricultural species. The studies of mycorrhizas of fruit plants concern mainly plant species of the tropical, subtropical and Mediterranean climates, and to a lesser degree those of the temperate climate (Sas Paszt and Głuszek, 2007a). Therefore, there is an urgent need to develop this field of research for the fruit plants grown in the Polish climate.

The roots of fruit trees and bushes can live and function for many months, and even years (especially the skeleton and lateral roots). The apple roots, in particular fine roots and root hairs, interact actively with the rhizosphere by secreting various organic compounds. These compounds affect, directly or indirectly, the level and the course of the bio-physico-chemical processes taking place in the rhizosphere (Sas Paszt and Głuszek, 2007a). In phosphorus-deficient soils, the mycorrhiza that occurs in apple roots increases the efficiency of phosphorus uptake, whereas in soils richer in phosphorus

the mycorrhiza facilitates the uptake of zinc and copper ions (Gnekov and Marschner, 1989). It should also be mentioned that in the roots of apple trees the most commonly occurring mycorrhizas are arbuscular (AM), which in the conditions of severe environmental stresses protect the trees from stresses (e.g. draught, salinity, acidification). Thanks to the beneficial effects associated with symbiotic soil microorganisms, such as mycorrhizal fungi and rhizosphere bacteria, it is possible to develop ecological methods of plant fertilization and implement them in fruit production. This direction of research complies with the thematic priorities of the European Union and is of a great importance in the production of high quality fruit (Sas Paszt, Głuszek 2007b).

The roots of strawberry plants, like those of apple trees, are most often colonized by arbuscular mycorrhizal fungi. The association is called endomycorrhiza, and consists in colonizing the roots of fruit plants by fungi of the genus *Glomus*. Inoculation of micropropagated strawberry plants with mycorrhizal fungi and rhizosphere bacteria increases plant growth and the enzymatic activity of the hyphae that penetrate the soil (Gryndler et al., 2002). Mycorrhizal fungi protect the root system of strawberry plants against infection by soil pathogens. The mycelium grows into the root cells forming arbuscular structures that facilitate uptake and assimilation of water and minerals by the plants, and make it difficult for pathogenic mycelia to develop.

Mycorrhization of plants improves their resistance to some diseases, e.g. verticillium wilt (Borkowska and Sowik, 2005). Inoculation of parent plants of some strawberry cultivars with mycorrhizal fungi has a positive effect on the formation of runner plants (Steward et al., 2005). The benefits arising from the high activity of mycorrhizal fungi and their participation in plant nutrition are the reason why research work on mycorrhizas and the rhizosphere of fruit plants is of major importance in the development of ecological methods of cultivation and fertilization of strawberry plants.

## MATERIAL AND METHODS

The experiments were carried out on two species of fruit-bearing plants, i.e. apple (arborescent/woody plant) and strawberry (perennial/herbaceous plant), in order to determine variations in the occurrence of mycorrhizal fungi that naturally colonize plant roots.

### Experiment 1

The aim of the experiment was to study the natural colonization by AM fungi of the roots of apple trees 'Gold Milenium' of control plants (non-inoculated) and those that had been mycorrhized or mulched. After cultivating the soil (according to the recommendations for commercial orchards), one-year-old apple maidens cv. 'Gold Milenium' were planted in August 2003 in the Experimental Field of the Pomological Orchard in Skiernewice. The experiment was set up

in 4 replicates, each consisting of 3 apple trees planted 4 m x 1.2 m apart, with a 1-metre-wide isolation strip between the plots. Mycorrhization of the plants was carried out by sprinkling the mycorrhizal substrate (200 g/plant) within the reach of the roots and covering it with a layer of soil. The mulches, 25 litres per a plot, were spread around 3 trees, and then lightly mixed with the soil. The trees are watered by a computer-operated irrigation system. This is an ongoing experiment that will be continued until the year 2010. Plant protection is carried out in accordance with the recommendations for commercial orchards. Weeds are removed by hand or by means of soil-applied herbicides.

The following treatments were applied to the trees:

1. Control (not mulched nor inoculated).
2. Deacidified peat (pH 6.5).
3. Hard wood bark mulch.
4. Sawdust mulch.
5. Horse manure mulch.
6. Plant kompost mulch.
7. Mycorrhizal substrate containing five *Glomus* species, produced by Mykoflor, Poland.
8. Straw mulch.

In order to determine the degree of colonization of the roots of apple trees 'Gold Milenium' by mycorrhizal fungi, samples of the roots were taken in August 2006 and subjected to a laboratory assessment.

## Experiment 2

The aim of the second experiment was to study the natural coloni-

zation by AM fungi of the roots of strawberry plants cv. 'Kent', of control plants (non-inoculated) and those that had been mycorrhized or mulched. Strawberry plants cv. 'Kent' were planted in April 2003 in the Experimental Field of the Pomological Orchard in Skierniewice. The experiment was set up in 3 replicates, each consisting of 20 plants planted at 1.0 m x 0.25 m spacing, with 0.5-metre-wide breaks between the plots. Inoculation with the mycorrhizal substrate was carried out in the same way as for apple trees, i.e. by scattering the substrate within the reach of the roots at 100 g per plant. Mulching was carried out by spreading and lightly mixing with the soil 25 litres of organic mulches per plot, each with 20 strawberry plants. A computer-controlled irrigation system was used to water the plants. Plant protection was carried out in accordance with the recommendations for commercial strawberry plantations. Weeds were removed by hand or by means of soil-applied herbicides.

The following combinations were used in the experiment:

0. Control (non-inoculated).
1. Deacidified peat (pH 6.5).
2. Hard wood bark mulch.
3. Sawdust mulch.
4. Plant compost.
5. Mycorrhizal substrate containing five *Glomus* species, prepared by Mykoflor, Poland.
6. Straw mulch.

In order to determine the degree of colonization of the roots of strawberry plants by mycorrhizal fungi, samples of the roots were

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### Layout of Experiment 1:

Combinations	Comb. /Repeat	Combinations	Comb. /Repeat
Control	1/3	Straw	8/3
Compost	6/4	Peat	2/3
Manure	5/4	Mycorrhizal substrate	7/3
Mycorrhizal substrate	7/4	Sawdust	4/3
Bark	3/4	Manure	5/3
Sawdust	4/4	Bark	3/3
Control	1/4	Compost	6/3
Peat	2/4	Manure	5/2
Straw	8/4	Mycorrhizal substrate	7/2
		Straw	8/2
		Compost	6/2
		Bark	3/2
		Control	1/2
		Sawdust	4/2
		Peat	2/2
		Compost	6/1
		Bark	3/1
		Manure	5/1
		Sawdust	4/1
		Mycorrhizal substrate	7/1
		Peat	2/1
		Straw	8/1
		Control	1/1

### Layout of Experiment 2:

Combinations	Comb /Repeat	Combinations	Comb /Repeat	Combinations	Comb /Repeat	Combinations	Comb /Repeat
Compost	5/1	Mycorrhizal substrate	6/1	Sawdust	4/3	Peat substrate	2/3
Sawdust	4/1	Straw	7/1	Control	1/3	Bark	3/2
Bark	3/1	Peat substrate	2/2	Compost	5/2	Mycorrhizal substrate	6/3
Peat substrate	2/1	Control	1/2	Straw	7/2	Compost	5/3
Control	1/1	Sawdust	4/2	Mycorrhizal substrate	6/2	Straw	7/3

taken in August 2006 and subjected to a laboratory assessment.

### Laboratory assessment

The laboratory assessment of the root samples consisted in staining the roots, making microscopic prepara-

tions, and examining them under the microscope.

For the two experiments described above a cold-staining method was used. The method was that developed by Philips and Hayman (1970) and modified by Turnau et al. (2001).

Staining was carried out in several stages:

1. Treatment with 10% potassium hydroxide (KOH) for 24 hrs.
2. Removing KOH by rinsing for approx. 15 min.
3. Acidification with 5% lactic acid for 24 hrs.
4. Staining in 0.01% aniline blue for 24 hrs.
5. Rinsing the dye out for approx. 20 min.
6. Preservation and storage of the roots in 80% lactic acid.

Although it is a long process, the possibility of damaging root structures is minimal in comparison with other methods (hot staining). Cells in the roots of herbaceous and perennial plants may become damaged during hot staining (effect of high temperature), which consequently makes it impossible to analyze mycorrhizal structures.

The microscopic analysis of the mycorrhizal preparations of the roots taken from the experiments was carried out in accordance with Trouvelot's method (1986). Thirty 1-cm-long root segments were selected randomly from each of the stained samples. The segments were placed parallel to one another on a microscopic slide, in 2 rows with 15 pieces each, and then carefully crushed with the cover glass. The obtained preparations were examined under Nikon Eclipse E200 microscope (using 100x, 400x and 1000x magnifications); the observed mycorrhizal formations: fungal hyphen, arbuscules and vesicles, were photographed.

Under assessment was the degree to which the roots were colonized, that is, mycorrhizal frequency, as well as mycorrhizal intensity, and the abundance of arbuscule formations in 1-cm-long segments of the roots. For that purpose, the preparations were examined under the microscope, and the results of the assessment were tabulated.

Degrees of colonization:

0. No structures of arbuscular fungi have formed within the root segment.
1. Structures of arbuscular fungi have formed, which occupy less than 1% of the root segment.
2. Structures of arbuscular fungi occupy less than 10% of the root segment.
3. Structures of arbuscular fungi occupy less than 50% of the root segment.
4. Structures of arbuscular fungi occupy more than 50% of the root segment.
5. structures of arbuscular fungi occupy more than 90% of the root segment.

Abundance of arbuscules:

- A0** – no arbuscular structures,
- A1** – single arbuscular structures observed,
- A2** – small groups of arbuscular structures observed,
- A3** – large groups of arbuscular structures observed.

The results relating to the abundance of arbuscules and mycorrhizal colonization were used to calculate the following parameters:

**F** – mycorrhizal frequency,  
**M** – relative mycorrhizal intensity  
 (for the whole sample),  
**m** – absolute mycorrhizal intensity  
 (for the segments in which there  
 was evidence of colonization by  
 mycorrhizal fungi),  
**a** – absolute abundance of arbuscules  
 (for the segments in which  
 arbuscules were found),  
**A** – relative abundance of arbuscules  
 (for the whole sample).

Using the following formulae:

$$F\% = I_m \times I_t^{-1} \times 100$$

where:

**F** – mycorrhizal frequency,  
**I<sub>m</sub>** – total number of root segments in  
 which mycelium had formed,  
**I<sub>t</sub>** – total number of the segments  
 examined.

$$M\% = (95 \times n_5 + 70 \times n_4 + 30 \times n_3 + 5 \times n_2 + n_1) \times I_t^{-1}$$

where:

**M** – relative mycorrhizal frequency,  
**n<sub>5</sub>– n<sub>1</sub>** – total number of root segments  
 in which the degree of colonization  
 by mycorrhizal structures was 5-1,  
**I<sub>t</sub>** – total number of the segments  
 tested.

$$m\% = M\% \times I_t \times I_m^{-1} = M\% \times 100 \times F^{-1}$$

where:

**m** – absolute mycorrhizal intensity  
**F** – mycorrhizal frequency,  
**M** – relative mycorrhizal frequency,  
**I<sub>m</sub>** – total number of root segments in  
 which mycelium had formed,  
**I<sub>t</sub>** – total number of the segments  
 examined.

$$a\% = (100 \times m\%A3 + 50 \times m\%A2 + 10 \times m\%A1) / 100$$

where:

$$m\%A3 = (95 \times n_5A3 + 70 \times n_4A3 + 30 \times n_3A3 + 5 \times n_2A3 + n_1A3) \times I_m^{-1} \times 100 \times m\%^{-1}$$

where:

**a** – absolute abundance of arbuscules,  
**m** – absolute mycorrhizal intensity,  
**I<sub>m</sub>** – total number of root segments in  
 which mycelium had formed,  
**n<sub>5</sub>– n<sub>1</sub>** – total number of root segments in  
 which the degree of colonization by  
 mycorrhizal structures was 5-1.

$$A\% = a\% \times 0.01 M\%$$

where:

**A** – relative abundance of arbuscules,  
**M** – relative mycorrhizal intensity,  
**a** – absolute abundance of arbuscules.

Calculations were performed with the help of MycoCalc computer program provided on the Internet at <http://www2.dijon.inra.fr/mychintec/MycoCalc-pgr/download.html>. The results were evaluated statistically by means of the STAT program used to perform analysis of variance. The differences between mean values were assessed with Duncan's t test at  $p = 0.05$ .

## RESULTS

The differences among mycorrhizas that can be seen while comparing the results presented on Tables 4 and 5 are a consequence of species-related differences between apple and strawberry. The two species belong to

two different groups of plants. Apple represents arborescent/woody plants, whereas strawberry is a perennial/herbaceous plant. The differences in the anatomy and morphology and in the mineral's content of the root system of the plants studied are very large, which significantly affects the results of the experiments. A larger number of fine and hair roots, and a larger surface area of the roots in strawberry facilitated easy penetration by mycorrhizal fungi and a higher mycorrhizal frequency in the roots of strawberry plants (Tab. 1).

The size of the root system also has a significant effect on mycorrhizal intensity. The number of root tips in strawberry can be a dozen or so times higher than in apple (studies conducted in the Rhizosphere Laboratory of ISK). Mycorrhizal intensity of the examined samples of the roots of strawberry plants cv. 'Kent' was also higher than in apple trees 'Gold Milenium' (Tab. 2).

The abundance of arbuscules in the examined root samples was also much greater in strawberry 'Kent' than in apple 'Gold Milenium' (Tab 3).

An analysis of the obtained results indicates that large differences can be found in the extent of colonization of the roots of strawberry plants cv. 'Kent' by mycorrhizal fungi (Tab. 4).

The highest mycorrhizal frequency was obtained in the roots of the plants treated with the mycorrhizal substrate and in the roots of the control plants, whereas the lowest frequency was recorded for the roots of the plants mulched with sawdust and peat. Inoculation also had a beneficial effect

on the adaptation of the plants to the changing environmental conditions. The plants mulched with peat and sawdust showed only a small amount of mycorrhizal structures in their roots. The roots of the plants mulched with bark, compost and straw were colonized by mycorrhizal fungi to an intermediate extent. Like in strawberry, the differences in the colonization by mycorrhizal fungi of the roots of apple trees cv. 'Gold Milenium' are obvious and varied in the individual experimental combinations (Tab. 5).

The highest mycorrhizal frequency was recorded for the roots of the plants mulched with peat and bark, and the lowest for those mulched with manure and sawdust. The other combinations (including the mycorrhizal substrate) were marked by an intermediate level of mycorrhizal frequency in the roots.

The results related to the colonization of plant roots by mycorrhizal fungi may change from one year to the next because of the age of the roots and the influence of various environmental factors on the plants and mycorrhizal frequency.

Depending on the environmental conditions, the expected results of mycorrhization may be delayed and will not become apparent for a few years (Borkowska, 2007).

## DISCUSSION

More and more research centres in Poland and the world are working on mycorrhization of various plant species. It thus appears that this research topic is extremely important



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Table 1. Comparison of mycorrhizal frequencies in the roots of apple 'Gold Milenium' and strawberry 'Kent'

Combination	Mycorrhizal frequency [%]	
	Apple	Strawberry
Control	12.5 abc	67.58 c
Peat	25.83 c	35.55 a
Bark	23.33 bc	47.78 ab
Sawdust	3.33 a	32.22 a
Manure	5 ab	-
Compost	10.83 abc	48.89 ab
Mycorrhizal substrate	9.17 abc	87.78 bc
Straw	15 abc	45.55 ab

Table 2. Comparison of relative mycorrhizal intensities in the roots of apple 'Gold Milenium' and strawberry 'Kent'

Combination	Mycorrhizal intensity [%]	
	Apple	Strawberry
Control	0.19 a	4.52 a
Peat	0.80 b	3 a
Bark	0.37 a	4.12 a
Sawdust	0.03 a	4.35 a
Manure	0.05 a	-
Compost	0.14 a	2.85 a
Mycorrhizal substrate	0.09 a	3 a
Straw	0.15 a	0.9 a

Table 3. Comparison of the relative abundance of arbuscules in the roots of apple 'Gold Milenium' and strawberry 'Kent'

Combination	Abundance of arbuscules [%]	
	Apple	Strawberry
Control	0.03 a	3.3 a
Peat	0.32 b	0.63 a
Bark	0.06 a	3.34 a
Sawdust	0.0025 a	3.98 a
Manure	0.0025 a	-
Compost	0 a	2.16 a
Mycorrhizal substrate	0.0025 a	1.17 a
Straw	0.0025 a	0.31 a

Table 4. Parameters of mycorrhizal colonization and the abundance of arbuscules in the roots of strawberry 'Kent'

Combination	F [%]	M [%]	m [%]	a [%]	A [%]	Remarks
Control	67.58 c	4.52 a	6.13 ab	55.86 abc	3.3 a	None
Peat	35.55 a	3 a	3.21 ab	50.18 ab	0.63 a	None
Bark	47.78 ab	4.12 a	8.95 bc	81.76 bc	3.34 a	None
Sawdust	32.22 a	4.35 a	13.74 c	91.22 c	3.98 a	None
Compost	48.89 ab	2.85 a	6.5 ab	59.52 abc	2.16 a	None
Mycorrhizal substrate	87.78 bc	3 a	3.35 ab	51.29 ab	1.17 a	None
Straw	45.55 ab	0.9 a	1.99 a	34.56 a	0.31 a	None

where:

- F – mycorrhizal frequency,
- M – relative mycorrhizal intensity
- m – absolute mycorrhizal intensity
- a – absolute abundance of arbuscules
- A – relative abundance of arbuscules

Table 5. Parameters of mycorrhizal colonization and the abundance of arbuscules in the roots of apple trees 'Gold Milenium' (field experiment, ISK Pomological Orchard, 2006)

Combination	F [%]	M [%]	m [%]	a [%]	A [%]	Remarks
Control	12.5 abc	0.19 a	1.75 ab	13.29 a	0.03 a	very large groups of vesicles
Peat	25.83 c	0.80 b	2.87 b	24.40 a	0.32 b	single vesicles
Bark	23.33 bc	0.37 a	1.40 ab	10.59 a	0.06 a	very large groups of vesicles
Sawdust	3.33 a	0.03 a	0.25 a	1.25 a	0.0025 a	single vesicles
Manure	5 ab	0.05 a	0.5 a	3.75 a	0.0025 a	large groups of vesicles
Compost	10.83 abc	0.14 a	2 ab	2.44 a	0 a	very large groups of vesicles
Mycorrhizal substrate	9.17 abc	0.09 a	1 ab	6.25 a	0.0025 a	large groups of vesicles
Straw	15 abc	0.15 a	1 ab	2.13 a	0.0025 a	large groups of vesicles

where :

- F – mycorrhizal frequency,
- M – relative mycorrhizal intensity
- m – absolute mycorrhizal intensity
- a – absolute abundance of arbuscules
- A – relative abundance of arbuscules

and interesting, giving great opportunities to learn more about the effects of mycorrhization on the plant growth and yielding. Identification of the strains of mycorrhizal fungi that colonize the roots of apple trees and strawberry plants in natural conditions and their subsequent isolation will contribute to the development of research on their beneficial effects on the health and yield size and quality of these types of plants.

The results of experiments carried out in the Rhizosphere Laboratory in 2002-2006 indicate that, in terms of morphological features, the root system of strawberry plants differs significantly from that of apple trees. When expressed per 1 g of both fresh and dry root weight, the root system of a strawberry plant is 14 times bigger than the root system of an apple-tree. The differences are also in the number of root tips, which are 14 times more numerous in strawberry than in apple. Equally, strawberry roots can be as much as 8 times longer than those of an apple tree. Root surface area in strawberry is 6.5 times larger than that in apple, which has a significant effect on water balance and plant nutrition status. The results obtained at ISK also show beneficial effects of mycorrhiza and organic mulches on the growth and yielding of strawberry plants and apple trees, as well as on improving their mineral nutrition status.

Fidelibus et al. (2000) revealed varied effects of various strains of fungi of the genus *Glomus* on mycorrhizal frequency in plant roots and the stimulation of the longitudinal

growth in roots in comparison with control plants. The results obtained in the study presented in this paper confirm these relationships. Mycorrhizal frequency was from 1.5 to 10 times higher in strawberry roots than in apple roots. Mycorrhizal intensity in the roots of strawberry plants increased from 3 to 145 times in comparison with apple trees. And the abundance of arbuscules in strawberry roots was also greater, in some cases even as much as 1000 times, compared with apple roots. These results revealed large differences in mycorrhizal frequency, the size of the root system and the number of root tips of apple and strawberry plants. Kaldorf and Ludwig-Müller (2000) had proved in their studies that AM fungi modify the growth, morphology and the number of roots in maize, making the root system more efficient in the uptake of water and mineral compounds from the soil. In many cases, in their experiment, the roots colonized by mycorrhizal fungi were better formed and had more lateral roots. These results indicate that mycorrhization of plants has a significantly beneficial effect on the growth and development of the root system, plant mineral nutrition status, and plant growth and yielding. Al-Karaki (2004) found that the roots of plants growing in dry habitats are colonized by AM fungi more often, and that mycorrhization of crop plants grown in such areas can increase the size and quality of the crop. Xavier and Boyetchko (2002) noted that the action of mycorrhizal fungi had a

beneficial effect on the growth and development of crop plants similar to the effects produced by biostimulators.

The studies conducted in the Rhizosphere Laboratory in the years 2002-2006 on strawberry plants treated with organic mulches revealed that mycorrhization stimulated the vegetative growth of strawberry plants, while the organic mulches had a favourable effect on the mineral and organic matter content in the soil. The studies also showed that peat and mycorrhizal inoculum were the best in improving root growth parameters: root length, the number of root tips, root diameter, surface area and volume. In the study presented, mycorrhizal frequency in the roots of strawberry plants following the application of mycorrhizal inoculum had the highest value, which was of benefit for plant growth. The results presented here, i.e. those pertaining to mycorrhizal frequency affected by inoculation, confirm its beneficial effect on the growth and yielding of strawberry plants (Rhizosphere Laboratory, ISK).

At the same time, during the period 2002-2006, experiments were carried out in the Rhizosphere Laboratory of ISK on apple trees with the use of mycorrhization and organic mulches. The experiments revealed that mycorrhization and mulching of apple trees had a greater effect on improving tree growth and mineral nutrition status of apple trees than NPK mineral fertilization. The use of compost and peat had a positive effect on improving root growth

parameters: the number of root tips and root length, and, to a lesser degree, root volume and surface area. The results of the experiments presented demonstrate that mycorrhizal frequency in the roots of the plants mulched with compost was lower than in those mulched with peat, and that peat was found to affect mycorrhizal frequency to a larger extent. These results agree with the data collected in the Rhizosphere Laboratory, especially with respect to the effects of mycorrhization and mulching on plant growth and mycorrhizal frequency in the plant roots.

Studies of the colonization of plant roots by arbuscular mycorrhizal fungi have also been carried out at the Institute of Botany, Czech Academy of Sciences, under the direction of Püschel et al. (2007). The studies have concerned the natural occurrence of AM fungi colonizing plants of the *Chenopodiaceae*, *Asteraceae* and *Poaceae* families. Plant species such as: wood small-reed (*Calamagrostis epigejos*), glossy-leaved orache (*Atriplex sagittata*), and scentless mayweed (*Tripleurospermum inodorum*), which are dissimilar in the mycotrophic sense, were planted on a mine waste-heap (an area of land destroyed by mining operations) and had their roots examined for successive stages of colonization by three species of mycorrhizal fungi: *Glomus mosseae* BEG95, *Glomus claroideum* BEG96, *Glomus intr-aradices* BEG140, and a mycorrhizal inoculum mixture of the three species. In control plants (non-inoculated), arbuscular mycorrhizal fungi were not present. The lowest

mycorrhizal frequency, from 0.2% to 16%, was found in the roots of glossy-leaved orache. Mycorrhizal intensity in the roots of this species was also at the lowest level – 0.1% to 5.1%, and there was no evidence of the presence of arbuscules. In wood small-reed, mycorrhizal frequency was considerably higher, from 91.1% to 97.3% (both for the individual species of *Glomus* and the inoculum mixture). Mycorrhizal intensity in the roots of glossy-leaved orache was from 38.2% to 63%. By comparison, the presence of arbuscules in the roots of wood small-reed was at a level of 16.2% to 36.4%. The highest mycorrhizal frequency was found in the plants of the species scentless mayweed, from 97.8% to 99.8%, both when the *Glomus* species were used individually and as an inoculum mixture. Mycorrhizal intensity in the roots of scentless mayweed was also the highest, at a level of 65.9% – 83.6%, in comparison with the other two plant species. The abundance of arbuscules ranged from 39.5% to 56.4%. The reported values indicate the least mycotrophic character of the species glossy-leaved orache (*A. sagittata*), which had the lowest values of the parameters describing the colonization of plant roots by AM fungi. It was noted that the inoculation with a mixture of mycorrhizal fungi resulted in the highest levels of colonization by AM fungi of the roots of all three plant species (*A. sagittata*, *C. epigejos* and *T. inodorum*).

The results of the studies conducted at the Institute of Botany of

the Czech Academy of Sciences are in agreement with the results of the experiments presented. In the case of strawberry plants 'Kent', the mycorrhizal substrate was found to affect the value of mycorrhizal frequency to 87.78%. By contrast, mycorrhizal frequency in the roots of apple trees 'Gold Milenium' was, in comparison with the other combinations, at an intermediate level of 9.17%. The remaining two parameters, i.e. mycorrhizal intensity and the abundance of arbuscules, also varied. Their values were at an intermediate level as follows: for strawberry 'Kent', mycorrhizal intensity – 3%, the abundance of arbuscules – 1.17%; for apple 'Gold Milenium', mycorrhizal intensity – 0.09%, the abundance of arbuscules – 0.0025%. Significant differences were also noted in the extent of colonization by AM fungi of the roots of strawberry plants cv. 'Kent' and apple trees 'Gold Milenium'. Mycorrhizal frequency in the roots of the studied cultivars was 10 times higher in strawberry than in apple. Mycorrhizal intensity in turn was 145 times higher in strawberry than in apple, while the abundance of arbuscules was as much as 1000 times greater in the roots of strawberry plants than in those of apple trees.

## CONCLUSIONS

The results of the experiments presented here allow one to conclude that the roots of apple trees 'Gold Milenium' and those of strawberry plants cv. 'Kent' exhibit large differences in the extent of colonization by

arbuscular mycorrhizal fungi, with the differences in the number of mycorrhizal fungi that naturally colonize the roots of those apple and strawberry cultivars resulting from species-related differences because of the dissimilar root morphologies and significant differences in the size of the roots of those plants. The roots of strawberry plants cv. 'Kent' are characterized by a greater abundance of arbuscules and a higher mycorrhizal frequency than the roots of apple trees cv. 'Gold Milenium'.

The extent of root colonization by mycorrhizal fungi and mycorrhizal intensity in apple 'Gold Milenium' and strawberry 'Kent' is closely connected with the size of the root system of the two plant species.

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## WPLYW MIKORYZACJI I ŚCIOŁEK ORGANICZNYCH NA FREKWENCJĘ MIKORYZOWĄ W KORZENIACH JABŁONI I TRUSKAWKI

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

Przeprowadzone badania miały na celu określenie naturalnego zasiedlenia korzeni jabłoni 'Gold Milenium' i truskawki 'Kent' przez arbuskularne grzyby mikoryzowe (AMF) oraz określenia stopnia frekwencji mikoryzowej. Z kombinacji doświadczalnych: kontrolnej i ściółkowanych torfem, korą, trocinami, obornikiem, kompostem, słomą oraz inokulowanej substratem mikoryzowym, pobrano korkoborem próby korzeni do analiz mikroskopowych. Korzenie wybarwiono metodą na zimno, w kolejnych etapach odbarwiano 10% KOH, zakwaszono 5% kwasem mlekowym, barwiono 0,01% błękitem aniliny oraz traktowano po barwieniu 80% kwasem mlekowym i obserwowano pod mikroskopem oraz wykonano preparaty mikroskopowe do dalszych analiz. Kolejnym

etapem była mikroskopowa ocena preparatów, którą wykonano metodą Trouvelot, określając takie parametry, jak: **F %** – frekwencję mikoryzową, **M %** – względną intensywność mikoryzową, **m %** – bezwzględną intensywność mikoryzową, **a %** – bezwzględną obfitość arbuskul, **A %** – względną obfitość arbuskul. Obliczenia wykonano za pomocą programu komputerowego Myccalc,

Na podstawie uzyskanych wyników stwierdzono, iż grzyby mikoryzowe częściej zasiedlały korzenie truskawki 'Kent' niż korzenie jabłoni 'Gold Milenium', a frekwencja mikoryzowa w korzeniach truskawki, w poszczególnych kombinacjach, była istotnie wyższa niż w korzeniach jabłoni (korzenie truskawki – 32-87%, a korzenie jabłoni – 3-25%). Również pozostałe parametry, to jest obfitość arbuskul oraz intensywność mikoryzowa wykazywały wyższe wartości w korzeniach truskawki niż w korzeniach jabłoni (obfitość arbuskul w korzeniach truskawki wynosiła 31-91%, a w korzeniach jabłoni – 0-24%), (intensywność mikoryzowa w korzeniach truskawki wynosiła 9-13%, a w korzeniach jabłoni – 0.25-4.5%). Różnice te wynikały z odmiennych cech morfologicznych korzeni oraz istotnych różnic w wielkości systemu korzeniowego truskawki i jabłoni.

**Słowa kluczowe:** mikoryza, rizosfera, frekwencja mikoryzowa, grzyby arbuskularne, ściółki