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Colonisation of apple and blackcurrant roots by arbuscular mycorrhizal fungi following mycorrhisation and the use of organic mulches

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ABSTRACT

The aim of the study was to determine the effect of mycorrhisation and mulching on the colonisation of the roots of 'Gold Milennium' apple trees and 'Ojebyn' and 'Tiben' blackcurrant bushes by arbuscular mycorrhizal fungi. In order to assess mycorrhizal frequency, samples of roots were collected in the 2012 season from all of the experimental combinations (control, peat substrate, bark, sawdust, manure, compost, mycorrhizal substrate and straw). The results of the analyses showed that all of the different mulches had a positive effect on increasing the degree of mycorrhizal association. In apple, the highest mycorrhizal frequency (F% – mycorrhizal frequency for the entire sample) and mycorrhizal intensity (M% – relative mycorrhizal frequency for the entire sample) were observed in the roots of trees inoculated with the mycorrhizal substrate (F = 24.40%, M = 0.24%) and those mulched with compost (F = 16.67%, M = 0.17%). In blackcurrant, the highest values of mycorrhizal frequency and mycorrhizal intensity were recorded in the roots of 'Tiben' bushes inoculated with the mycorrhizal substrate (F = 37.78%, M = 0.38%) and those mulched with sawdust (F = 21.11%, M = 0.21%).

Key words: AMF, mycorrhiza, mycorrhizal frequency, rhizosphere, staining method

INTRODUCTION

Poland contains a large percentage of not very fertile soils (89% of Polish soils are of poor quality). They are characterised by low organic matter content (1.25%, on average), which reduces their fertility and microbial biodiversity indicators (Jones et al. 2005). In this context, it is very important to conduct research on optimising and increasing the levels of organic matter and humus in agricultural soils by means of organic mulches. An increase in the humus content primarily increases the biological activity of soils, their water capacity and sorption capacity, reduces soil density and improves the gaseous exchange between the atmosphere and the soil.

The proper development of the root system and the activity of the processes taking place in the rhizosphere, including the activity of symbiotic mycorrhizal fungi and rhizosphere bacteria, are of great importance for the proper development of plants in all natural communities as well as in orchards and berry fruit plantations (Sas Paszt and Mercik 2004, Sas Paszt and Żurawicz 2004, 2005). Symbiotic microbial activity in the rhizosphere is a factor conditioning the growth of plants and their resistance to pathogens (Azcón-Aguilar and Barea

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1992, Smith and Read 1997, Linderman 2000). Mycorrhizal fungi are an important component of the rhizosphere; because of the presence of mycelium the absorptive surface of roots increases and so does the availability of phosphorus for plants (Cox and Tinker 1976). Rhizosphere bacteria support the development of mycorrhizal fungi and plants (Vessey 2003).

In order to obtain high yields in intensive horticultural and agricultural production, the common practice is to use mineral fertilisers at high application rates, combined with the application of plant protection chemicals. An alternative to this production method is the use of manure and the application of straw and natural bioproducts to the soil, i.e. biofertilisers, biostimulators, and microbiologically enriched composts. Research on the development of organic methods of plant cultivation and fertilisation is aimed at the production of high quality crops and the protection of the natural environment and human health. In the era of new, eco-friendly cultivation technologies in a sustainable environment, it is necessary to increase the efficiency of the uptake and assimilation of nutrients from organic fertilisers (natural mulches) and organo-mineral ones, with limited use of mineral fertilisers and plant protection chemicals. This is necessitated by the need to maintain agrobiocenoses in the natural ecological equilibrium.

Data from international literature indicate the beneficial effects of organic compounds in natural mulches on the development of symbiotic soil microorganisms, including mycorrhizal fungi, and vascular plants, which include crop plants. Natural mulches are primarily a source of minerals (C, N and P), which, after the mineralisation of organic matter, are available to plants. Macro- and microorganisms in the soil derive the energy and minerals necessary for them to live from organic matter, which increases the biological activity of soils (Esperschütz et al. 2007). The humus compounds in mulches also have a significant impact on the physiological processes of crop plants, such as water balance, respiration and photosynthesis (Ngosong et al. 2010).

The aim of the presented study was to determine the effect of mycorrhisation and mulching on the colonisation of the roots of 'Gold Millennium' apple trees and 'Ojebyn' and 'Tiben' blackcurrant bushes by arbuscular mycorrhizal fungi.

MATERIAL AND METHODS

One-year-old apple maidens of the cultivar 'Gold Milennium' were planted in August 2003 in the Pomological Orchard of the Institute of Horticulture (IO) in Skierniewice. The experiment was conducted in four replications (three trees per replication). The spacing between the trees was 4.0 m × 1.5 m, with a one-metre-wide isolation strip between the plots. The experiment included control trees (not mulched, fertilised with NPK), trees inoculated with a mycorrhizal substrate (applied in the spring of each year, 1 million propagules per 100 g of substrate, with inoculum applied at 10 ml per tree, 1 g substrate per tree), and mulching (applied in the spring of each year in the amount of 25 L of organic mulches per plot of three apple trees). All of the experimental combinations were fertilised in the spring of each year with the standard dose of NPK (80 kg N ha^{-1}, 80 kg K ha^{-1}, 60 kg P ha^{-1}).

Blackcurrant bushes of the cultivars 'Ojebyn' and 'Tiben' were planted in the Pomological Orchard in the spring of 2003 in a randomised block design in three replications. A replication consisted of three blackcurrant bushes growing at a spacing of 3.25 m × 0.50 m, with a one-metre-wide isolation strip between the plots. Organic mulches were applied at 25 L per plot, and the mycorrhizal inoculum (applied in the spring of each year, 1 million propagules per 100 g of substrate, 1 g substrate per tree) at 10 ml under every bush. All of the experimental combinations were fertilised with standard NPK doses (60 kg N ha^{-1}, 80 kg K ha^{-1}, 60 kg P ha^{-1}).

The apple trees and blackcurrant bushes were irrigated by a computer-controlled irrigation system. Plant protection treatments were carried out according to the recommendations for commercial orchards/plantations; weeds were removed by hand or by using herbicides.

In both experiments, the following treatments, arranged in a random block design, were used:

1. control (no mulching, no mycorrhisation),
2. peat substrate (available from garden centres),
3. tree bark (shredded pine bark),
4. sawdust (coniferous),
5. manure (bovine),
6. compost (decomposed plant debris),
7. mycorrhizal substrate (a commercial product by Mykoflor, Końskowola, containing strains of mycorrhizal fungi: *Glomus intraradices, G. mosseae, G. etunicatum*),
8. straw (cereals, mostly rye).

In order to determine the extent of colonisation of the roots of apple trees and blackcurrant bushes by mycorrhizal fungi, samples of the roots were collected in the 2012 season from the rhizosphere of blackcurrant bushes (in July) and apple trees (in August). In order to perform a laboratory assessment of the degree of mycorrhizal association in the roots of apple trees and blackcurrant bushes, the root samples were stained and used to prepare microscopic specimens, which were then analysed by microscopy.

Roots were stained according to a new procedure developed at the Rhizosphere Laboratory of the Institute of Horticulture, based on the cold staining method by Phillips and Hayman (1970) (per Turnau et al. 2001). The newly developed method significantly reduced the time needed to prepare root samples for further analyses compared with the method used previously (Phillips and Hayman 1970), and made it easier to determine the extent of colonisation of roots by mycorrhizal fungi at the stage of examining the microscopic specimens. Modification in Phillips and Hayman's (1970) procedure for staining roots were as follow: treatment with 10% NaOH for 30 min. at 65°C, washing out NaOH for about 5 min., acidification with 10% lactic acid for 10 min., staining with carbolic fuchsine for 5-10 min., rinsing out the dye for about 10 min., preservation and storage of roots in glycerol.

After staining the root samples, 30 one-centimetre-long segments of the roots were selected from each replication and laid parallel to one another on a microscope slide, and then carefully crushed with a cover glass. The prepared specimens were examined using a Nikon Eclipse 50i microscope (lenses: 20×, 40×, 60×, 100×), and the mycorrhizal structures observed in the roots were photographed using a Nikon DS - Fi1 microscope camera.

The degree of mycorrhizal association was assessed using the method developed by Trouvelot et al. (1986), by assigning a degree of mycorrhizal colonisation to the one-cm-long segments of roots. Based on the microscopic observations, mycorrhizal frequency (F% – total number of root segments in which mycelium had formed) and mycorrhizal intensity (M% – total number of root segments in which the degree of colonisation by mycorrhizal structures was 5-1) were determined using the program MYCOCALC (http://www2.dijon.inra.fr/mychintec/Protocole/Workshop_Procedures.html#1.5).

Data were analysed using ANOVA. The Duncan multiple range test at p = 0.05 was used for specific comparisons of the means. All calculations were done by means of the STATISTICA v.10 package (StatSoft, Inc. 2011).

RESULTS

The results of this study showed that all of the mulches had a positive effect on increasing the degree of mycorrhizal association in comparison to the control with chemical fertilisation. Inoculation of apple trees with arbuscular mycorrhizal fungi (AMF) increased the degree of mycorrhizal association in the roots more than five-fold compared with the roots of the control trees. As a result of the laboratory analyses, we observed that mycorrhizal fungi most often and most intensely colonised the roots of the trees inoculated with the mycorrhizal substrate (F = 36.66%, M = 0.36%) and those mulched with compost (F = 25.00%, M = 0.26%). The lowest mycorrhizal frequency and intensity were observed in the roots of the control trees (F = 6.67%, M = 0.06%) and those mulched with sawdust (F = 8.34%, M = 0.09%) (Tab. 1).

The roots of the 'Tiben' and 'Ojebyn' blackcurrant cultivars also showed a positive effect of mulching on mycorrhizal frequency and the intensity of colonisation by AMF. In 'Tiben', a more than eight-fold increase was observed in the colonisation of roots after the application of the mycorrhizal inoculum compared with the roots of the control trees, while in the roots of 'Ojebyn' there was about a six-fold increase. The highest mycorrhizal frequency and intensity occurred in the roots of 'Tiben' bushes inoculated with the mycorrhizal substrate (F = 56.67%, M = 0.57%) and in those mulched with sawdust (F = 31.67%, M = 0.32%). The lowest level of mycorrhizal association was

Table 1. Effect of mulching and application of AMF inoculum on mycorrhizal frequency [F%] and relative mycorrhizal intensity [M%] in the roots of 'Gold Milennium' apple trees following mycorrhisation and the use of organic mulches

Treatment	F%	M%
Control	6.67 a*	0.06 a
Peat	15.00 bc	0.15 bc
Bark	16.67 c	0.17 c
Sawdust	8.34 ab	0.09 ab
Manure	15.00 bc	0.15 bc
Compost	25.00 d	0.26 d
Mycorrhizal substrate	36.66 e	0.36 e
Straw	13.35 abc	0.14 bc

*Values marked with the same letter do not differ significantly at p = 0.05

Table 2. Effect of mulching and application of AMF inoculum on mycorrhizal frequency [F%] and relative mycorrhizal intensity [M%] in the roots of 'Ojebyn' and 'Tiben' blackcurrant bushes following mycorrhisation and the use of organic mulches

Treatment	Tiben		Ojebyn	
	F%	M%	F%	M%
Control	6.66 a*	0.06 a	6.68 a	0.08 a
Peat	16.67 bc	0.17 b	16.67 bc	0.17 b
Bark	23.34 de	0.24 bc	15.00 bc	0.15 ab
Sawdust	31.67 f	0.32 c	20.00 cd	0.20 b
Manure	28.34 ef	0.29 c	23.34 de	0.24 bc
Compost	23.34 de	0.24 bc	16.67 bc	0.17 b
Mycorrhizal substrate	56.67 h	0.57 e	40.00 g	0.41 d
Straw	11.67 ab	0.15 ab	20.00 cd	0.20 b

*Explanations: see Table 1

shown by the roots of the control bushes of 'Tiben' (F = 6.66%, M = 0.06%) and 'Ojebyn' (F = 6.68%, M = 0.08%), and of 'Tiben' mulched with straw (F = 11.67%, M = 0.15%) (Tab. 2).

Comparing the results of mulching and inoculation of the roots of 'Gold Millennium' apple trees with the average values for 'Tiben' and 'Ojebyn' blackcurrant bushes, we found that the roots of the blackcurrant bushes were characterised by a higher degree of mycorrhizal association than the roots of the apple trees (except when compost was used). As a consequence of using the mycorrhizal inoculum, the mycorrhizal frequency recorded in the roots of the blackcurrant bushes (average for the two cultivars) was about 1.5 times higher than in the roots of the apple trees.

DISCUSSION

Mycorrhisation and the use of organic mulches positively affected the colonisation of the roots of 'Gold Milennium' apple trees and 'Ojebyn' and 'Tiben' blackcurrant bushes by arbuscular mycorrhizal fungi (apple – 36.66%; blackcurrant: 'Tiben' – 56.67%, 'Ojebyn' – 40.00%). Mulching apple trees with compost and blackcurrant bushes with manure and sawdust increased the degree of mycorrhizal association in the roots of these two fruit plant species (apple – 25.00%; blackcurrant: 'Tiben' – 31.67%, 'Ojebyn' – 23.34 %).

Sumorok et al. (2011) obtained similar results in a study in which the use of mulches and a mycorrhizal inoculum increased the degree of mycorrhizal association in the roots of apple trees (44.44%) and blackcurrant bushes ('Tiben' – 12.22%). Meikle and Amaranthus (2008) had mycorrhised plants of golden currant (*Ribes aureum* Pursh) in an experiment with low levels of phosphates in the soil. They observed that standard fertilisation reduced mycorrhizal frequency (0-20%), whereas following the use of organic fertilisation mycorrhizal frequency increased significantly (92%). Purin et al. (2006) investigated the activity and diversity of mycorrhizal fungi in apple orchards cultivated by conventional methods and in organic orchards. They found that mycorrhizal frequency in the roots of apple trees in organic orchards was higher (48.8%), and lower in the roots of trees growing in orchards managed by conventional methods (43.7%). Matsubara et al. (1996) inoculated *Malus pumila* Mill. var. *domestica* apple seedlings of the cultivars 'Macintosh', 'American Summer Pearmain', 'Jonathan', 'Golden Delicious', 'Fugji', 'Mutsu', 'Red Gold', and *Malus sieboldii* seedlings with two species of mycorrhizal fungi: *Gigaspora margarita* and *Glomus etunicatum*. In all of the tested cultivars, they noted the presence of mycorrhizal fungi. The highest degree of mycorrhizal association was observed in the following cultivars: 'Golden Delicious' – 31.7%, and 'Jonathan' – 50.5%. In 2001, Forge et al. studied the effect of mycorrhisation of the roots of domestic apple *Malus domestica* Borkh. with six species of AMF (*Glomus aggregatum, G. clarum, G. etunicatum, G. intraradices, G. mosseae* and *G. versiforme*) and the effect of mycorrhizal fungi on the growth of nematode populations. They found that the colonisation of the roots of apple trees was 6% for the control plants fertilised with NPK, and 63-86% in the roots of apple trees inoculated with the six fungal species.

Organic fertilisation increases the number and diversity of soil microorganisms (Plencette et al. 2005, Toyota and Kuninaga 2006, Esperschütz et al. 2007), including mycorrhizal fungi (Ryan et al. 1994, Miller and Jackson 1998) to a greater extent than conventional mineral fertilisation. Arbuscular mycorrhizal fungi play an important role in the growth and development of various plant species, including many species of crop plants (Ngosong et al. 2010). Research in this area has been carried out in different systems of crop cultivation and fertilisation, and has also included observations of the growth of plant roots and their colonisation by mycorrhizal fungi (Sas Paszt et al. 2011, Sumorok et al. 2011).

CONCLUSIONS

1. All of the organic mulches used in the experiments increased the degree of mycorrhizal association of the roots of the apple and blackcurrant plants studied.
2. The roots of blackcurrant bushes were more frequently colonised by mycorrhizal fungi than the roots of apple trees.
3. Inoculation with a mycorrhizal substrate and mulching with compost, sawdust or manure significantly increased mycorrhizal frequency in the roots of the two cultivars of blackcurrant bushes and the roots of apple trees.

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ZASIEDLANIE PRZEZ ARBUSKULARNE GRZYBY MIKORYZOWE KORZENI JABŁONI I PORZECZKI CZARNEJ PO ZASTOSOWANIU MIKORYZACJI I ŚCIÓŁEK ORGANICZNYCH

Streszczenie: Celem doświadczenia było określenie wpływu mikoryzacji i ściółkowania na zasiedlanie korzeni drzew jabłoni odmiany Gold Milenium oraz porzeczki czarnej odmian: Ojebyn i Tiben przez arbuskularne grzyby mikoryzowe. W celu oceny stopnia frekwencji mikoryzowej, w sezonie 2012 pobrano próby korzeni ze wszystkich kombinacji doświadczalnych (kontrola, substrat torfowy, kora, trociny, obornik, kompost, substrat mikoryzowy, słoma). Wyniki przeprowadzonych badań wykazały, iż wszystkie ściółki miały pozytywny wpływ na zwiększenie stopnia asocjacji mikoryzowej. Najwyższy stopień frekwencji mikoryzowej (F% – stopień frekwencji mikoryzowej w całej próbce) i intensywności mikoryzowej (M% – intensywność mikoryzowa w całej próbce) odnotowano w korzeniach jabłoni inokulowanych substratem mikoryzowym (F = 24,40%, M = 0,24%) i ściółkowanych kompostem (F = 16,67%, M = 0,17%). W korzeniach porzeczki czarnej odmiany Tiben inokulowanych substratem mikoryzowym (F = 37,78%, M = 0,38%) oraz ściółkowanych trocinami (F = 21,11%, M = 0,21%) odnotowano najwyższe wartości frekwencji mikoryzowej i intensywności mikoryzowej.

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