

The influence of bioproducts on root growth and mycorrhizal occurrence in the rhizosphere of strawberry plants cv. 'Elsanta' under controlled conditions

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Abstract

An experiment was carried out in a greenhouse of the Research Institute of Horticulture (RIH) to evaluate the effect of new organic fertilizers and amendments on root growth and mycorrhizal abundance and biodiversity in the rhizosphere of strawberry plants cv. 'Elsanta'. The plants were grown in rhizoboxes (sized 37 cm x 1.8 cm x 20 cm), filled with 1.85 kg of a podsollic soil collected from an uncultivated field of an experimental organic orchard of the RIH. The soil characteristics were: pH 5.5, organic matter content 1.5%, P content 51 mg P · kg⁻¹, K content 158 mg K · kg⁻¹. The plants were treated with different organic fertilizers and amendments: dry granulated bovine manure (Doctor O'grodnik), extract of vermicompost (Humus UP), extract of humates (Humus Active + Aktywit PM), plant extract (BioFeed Amin), extract from several seaweed species reinforced with humic and fulvic acids (BioFeed Quality), a consortium of beneficial soil organisms (Micosat), a stillage from yeast production (Vinassa) and a solution of titanium (Tytanit). Plants treated with BioFeed Amin, BioFeed Quality, Micosat, Vinassa and Tytanit received also half dose of dry manure. A standard NPK fertilization (NPK control) and a not fertilized control were also included. The following parameters were measured: root growth and morphological parameters, number of Arbuscular Mycorrhizal Fungi (AMF) spores, mycorrhizal frequency of AMF in the roots. The chemical composition of the applied products and of soil were also determined. The treatment inducing the highest development of mycorrhizas in the roots of strawberry plants cv. 'Elsanta' were Micosat and BF Amin. The treatments BF Quality and BF Amin had the most beneficial effect on the formation of AMF spores in the rhizosphere. Application of the bioproducts had a positive effect on root growth parameters in comparison with the plants fertilized with NPK. The use of BF Quality and Humus UP induced a reduction of the amount of mineral nutrients in the soil.

Keywords: bioproducts, mycorrhizal frequency, AMF, root morphology, strawberry

Introduction

Organic farming is considered an important element of the Polish and EU's strategy for the development of the agricultural sector. The production of organic fruits has been increasing in recent years. However, the limited availability of traditional organic fertilizers (i.e. manure), and scarce information about the effects of new kinds of organic fertilizers, such as plant extracts (Sas Paszt *et al.*, 2009) or microbial inocula (Malusà *et al.*, 2007), are serious obstacles threatening the future development of the sector. Application of mycorrhizal inocula can increase species diversity of these fungi in the rhizosphere and consequently improve the growth, yielding and yield quality of cultivated fruit crops (Sas Paszt & Żurawicz, 2005; Sas Paszt & Głuszek, 2007). Establishing the best conditions for plant-fungus symbiosis is the key factor to obtaining efficient mycorrhization (Estaún *et al.*, 1994).

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The continuous cultivation of one crop reduces the diversity and richness of AMF species in the soil (An *et al.*, 1993). Studies of indigenous mycorrhizal fungi colonizing the roots of strawberry plants have also been conducted (Nemec, 1974; Didier *et al.*, 2003; Derkowska *et al.*, 2008; Botham *et al.*, 2009). Much of the research work has concentrated on the effects of mycorrhizal fungi on the growth and yield characteristics of strawberry plants (Niemi & Vestberg, 1992). Plants of the cultivar 'Elsanta' have not yet been the subject of many studies on their symbiosis with AM fungi (Varma & Schüepp, 1994). Roots of the strawberry cultivar 'Elsanta' readily form symbiotic associations with arbuscular fungi (Varma & Schüepp, 1994). Maintaining species diversity of these fungi in the rhizosphere environment of an organic orchard may have an effect on the growth and health status of the plants and the quality of the fruit they produce. New innovative products and technologies for organic fruit production are under development, including the use of beneficial soil microorganisms and fertilizers or amendments of organic origin (Sas Paszt *et al.*, 2008; Malusà & Sas Paszt, 2009). The influence of organic amendments on the development of mycorrhizal fungi has been reported by several authors. The technologies rely on the use of these bioproducts as they are or in a form enriched with beneficial soil microorganisms (Malusà *et al.*, 2007). Several organic fertilizers and amendments have been recently tested and introduced, which are also acting as natural stimulators of plant growth and development (Chelariu & Ionel, 2005; Gousterova *et al.*, 2008; Khan *et al.*, 2009; Chelariu *et al.*, 2009; Meszka & Bielenin, 2009). These are preparations of natural (plant or animal) origin, harmless to humans and the environment, which contain nutrient elements and biologically active substances (i.e. plant hormone-like substances, enzymes) as well as other compounds that stimulate plant growth, yield, quality or tolerance to abiotic stresses. Enrichment of organic bioproducts with beneficial soil bacteria and mycorrhizal fungi could enhance the effectiveness of the organic products in plant growth stimulation and fruit production.

The study, carried out within the framework of a project aimed at developing new products and technologies for organic fruit production in Poland, evaluated the effect of new organic fertilizers and amendments on root growth and mycorrhizal abundance and biodiversity in the rhizosphere of strawberry plants 'Elsanta'.

Material and Methods

The experiment was carried out in a greenhouse over a 5-month period with the use of frigo-plants of the strawberry cultivar 'Elsanta'. The plants were planted in rhizoboxes (37 x 1.8 x 20 cm), filled with 1.85 kg of a podsolic soil collected from an uncultivated field of an experimental organic orchard of the RIH. The plants were subjected to the following growing conditions: photoperiod 16/8 h (day/night), light intensity 70 $\mu\text{M m}^{-2} \text{s}^{-1}$, temperature 25/20 C and air humidity approx. 50% (Dinkelaker *et al.*, 1993; Sas *et al.*, 2003; Sas Paszt and Żurawicz, 2005). The levels of nutrient elements in the soil were: organic matter 1.5%, P 51 mg P kg⁻¹, K 158 mg K kg⁻¹, pH 5.5.

The following experimental treatments were applied:

- 1) Control (no-treatment) (organic matter 1.5%, P 51 mg P kg⁻¹, K 158 mg K kg⁻¹, pH 5.5).
- 2) Standard NPK soil fertilization: 4 g NH₄NO₃ plant⁻¹, 3 g triple superphosphate and 6 g K₂SO₄ per rhizobox.
- 3) Dry granulated bovine manure for organic farming (Doktor O'grodnik) – 1 g per rhizobox.
- 4) Micosat (CCS Aosta s.r.l.) – a mixture of AM fungi: *Glomus* species, *Trichoderma viride* and rhizosphere bacteria species (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces* spp.) – 10 g per rhizobox.

5) Humus UP (Ekodarpol) – an extract from a vermicompost – 2% solution to the soil per rhizobox.

6) Humus Active + Aktywit PM (Ekodarpol) – Humus Active is a soil improver with active humus and a population of beneficial microorganisms – 2% solution of Humus Active and 1% solution of Aktywit PM to the soil per rhizobox.

7) BioFeed Quality (Agrobio Products B.V.) – an extract from several seaweed species reinforced with humic and fulvic acids – 0.5% solution + 0.5 g manure to the soil per rhizobox.

8) BioFeed Amin (Agrobio Products B.V.) – an extract of 100% vegetal amino-acids – 0.5% solution + 0.5 g manure to the soil per rhizobox.

9) Tytanit (Intermag) – titanium (Ti) 0.8% (5 g Ti in 1 l of working solution), pH 3.4, containing 3163 mg kg⁻¹ Ti – 0.05% solution + 1 g manure to the soil per rhizobox.

10) Vinassa – molasses residue from yeast production – 0.5% solution + 0.5 g manure to the soil per rhizobox.

Determination of root growth and morphological parameters:

- Dry weight (Ostrowska *et al.* 1991).
- Root morphological parameters (total root length, root diameter, root surface area, root volume and total number of root tips) - image analysis system with an Epson scanner, controlled by WinRhizo software (Regent Instruments Inc.).

Determination of soil chemical parameters:

- The amount of N and C in the soil - Dumas method using a TruSpec CNS analyzer. Available P and K - the Egner-Rhiem method of emission spectrometry (Cygański 1997). The electrochemical method in KCl was used to measure soil pH.

Determination of mycorrhizal frequency:

- Roots were cold-stained using the Phillips & Hayman method (1970).
- The microscopic analysis of the roots - Trouvelot's method (1986).
- The mycorrhizal frequency (F%) and mycorrhizal intensity (both relative – M%, and absolute – m%) 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>

Identification of spores:

- Trap cultures were set up with narrowleaf plantain in 0.5 L pots filled with a mixture of rhizosphere soil and autoclaved sand, at a ratio of 1:1 v/v (Błaszowski 2003). Pots were placed in SunBags (Sigma).
- After six months, 200g samples of the pot substrate were taken from the trap culture combinations and spores were isolated by wet sieving and centrifuging in a sucrose gradient (Brundrett *et al.* 1996).
- The isolated spores were divided into morphotypes according to size, shape, and colour of spores.
- The layer thickness of spore walls and germination walls was measured in freshly isolated spores which were crushed in PVLG or PVLG+Melzer's reagent (1:1, v/v) and observed under a light microscope equipped with a micrometer eyepiece (Błaszowski 2003, 2008).
- The observed AMF species were named according to Schüßler *et al.* (2001) and Błaszowski (2003).

All the results were statistically evaluated with analysis of variance. Comparisons of means were at $p \leq 0.05$ with the Duncan test.

Results

The highest values of mycorrhizal frequency and relative intensity were recorded in the roots of the plants inoculated with the microbial consortium (Micosat), followed by the plants from the treatments with: BF Amin + manure and Humus UP (Tab. 1). The lowest value of mycorrhizal frequency was determined for the NPK standard fertilization. Treatment of strawberry plants with bioproducts increased formation of mycorrhizal structures in the roots.

Table 1: Mycorrhizal colonization parameters determined in the roots of strawberry plants 'Elsanta' grown in rhizoboxes. (Means of 90 replicates; different letters referring to the same parameter indicate statistically significant difference $p \leq 0.05$).

Experimental treatments	F [%]	M [%]	m [%]
Control	11.11 ab	0.11 a	1.0 ab
NPK	2.22 a	0.02 a	0.67 a
Manure	3.33 a	0.03 a	1.00 ab
Micosat	45.55 d	1.63 b	3.33 bc
Humus UP	21.11 bc	0.48 a	2.37 b
Humus Active + Aktywit PM	15.56 b	0.70 a	4.00c
BF Quality + ½ manure	3.33 a	0.03 a	1.00 a
BF Amin + ½ manure	28.89 c	1.67 b	6.74 d
Tytanit + manure	3.33 a	0.08 a	0.78 a
Vinassa + ½ manure	16.67 b	0.26 a	1.74 ab

Table 2: Root growth and morphological parameters of strawberry plants cv. 'Elsanta' treated with different bioproducts (means of 3 replicates; different letters referring to the same parameters indicate statistically significant differences $p \leq 0.05$).

Treatment	Root dry weight [g]	Root surface area [cm ²]	Root diameter [mm]	Root volume [cm ³]	Root length [cm]	Number of root tips
Control	2.2 bc	726 ef	0.64 b	11.93 d	3582.49 bc	6723.5 d
NPK	0.93 a	574 d	0.66 c	4.54 a	1348.82 a	2787.7 a
Manure	2.7 d	277 a	0.64 b	9.85 c	3007.95 b	5609.8 bc
Micosat	2.7 d	608 cd	0.61 a	10.19 c	3323.66 b	6236.2 c
Humus UP	2.5 cd	648 e	0.62 ab	9.17 c	2945.50 b	6202.7 c
Humus Active + Aktywit PM	1.6 ab	578 d	0.66 c	8.00 b	2297.18 ab	4388.3 b
BF Quality + ½ manure	1.7 b	479 bc	0.60 a	9.28 c	3198.24 b	6978.0 d
BF Amin + ½ manure	1.9 b	609 cd	0.61 a	10.14 c	3323.34 b	6708.4 d
Tytanit + manure	3.2 e	649 e	0.62 ab	12.16 d	4087.93 c	9028.2 e
Vinassa + ½ manure	2.4 c	802 f	0.67 c	9.56 c	3582.49 bc	5298.8 bc

The highest dry weight of roots was recorded in the treatment with Tytanit + manure, followed by Manure and Micosat (Tab. 2). The plants fertilized with NPK had the lowest dry root weight. The largest surface area was found in the roots of the plants treated with Vinassa + manure, followed by the Control, Tytanit + manure, and Humus Active with Aktywit PM, but the smallest surface area of roots - after the application of Manure (Tab.2). Root diameter ranged from 0.60 to 0.66mm with the largest value found in the plants treated with NPK, Humus Active + Aktywit PM and Vinassa (Tab. 2). The plants not fertilized and those treated with Tytanit + manure showed the largest volume of roots, while those treated with NPK had the lowest (Tab. 2). The highest induction of elongation of roots was produced by Tytanit + manure; all other treatments except the NPK, also favoured to some extent elongation of roots (Tab.2). Consequently, Tytanit showed the highest numbers of root tips, also in this case, all organic products increased the branching of roots, while this parameter was not influenced by NPK treatment (Tab. 2).

In comparison to the control, BF Quality + manure, BF Amin + manure, Micosat, Humus Active + Aktywit PM, and manure induced the formation of the highest number of AMF spores in the trap cultures containing the rhizosphere soil of strawberry plants 'Elsanta' grown in rhizoboxes (respectively: 199.32; 173.53; 160.04; 155.47 and 150.66). The lowest average and total numbers of spores were recorded for the treatments Vinassa + manure, NPK, and the no-treatment control (respectively: 89.68; 117.42 and 101.97). Several AM fungi species, ranging from 3 to 6, were found in the trap cultures containing rhizospheric soil from the diverse treatments. The most common species of AM fungi found in the experimental combinations were: *Glomus claroideum* (found in all 10 treatments), *Scuteliospora dipurpurescens* (found in 9 treatments), *G. mosseae* (identified in 7 treatments), *G. fasciculatum* (present in 6 treatments). *Glomus caledonium* was found in three treatments and *G. macrocarpum* in two. On the other hand, four AMF species were found in only one treatment: *Glomus microaggregatum* and *G. constrictum* were present only with Manure, *G. pallidum* with Vinassa, and *G. drummondii* in the NPK control.

Discussion

The highest mycorrhizal frequency (45.55) was achieved for the application of the microbial consortium Micosat. Such result supports findings showing the efficacy of inoculation of plants with AMF (Jeffries *et al.*, 2003; Malusá *et al.*, 2007). The inoculation of strawberry plants with Micosat also resulted in an increased number of spores produced by the fungi. However, only one species *Glomus mosseae* of the four present in the inoculum was found as a spore. This species was also among the indigenous fungi present in the podsollic soil, used for all of the experimental treatments. This fact could be due to the differences in either sporulation or various ability of the fungal species to inoculate the plant roots. However, the plants treated with BF Quality (a seaweed extract) had one of the lowest mycorrhizal frequency rate (3.33%), whereas the plants treated with BF Amin (a plant extract enriched with humic acids) had a higher mycorrhizal frequency rate (28.89%).

The largest number of spores in the trap cultures was obtained after the combined applications of manure and plant-derived preparations: BF Quality or BF Amin (obtained from processed seaweed or herbaceous plants). The results show a favourable impact of the organic products on the sporulation of AM fungi. Results of other studies confirm the positive effect of seaweed-derived substances (Kuwada *et al.*, 2005; Kuwada *et al.*, 2006a,b) and other compounds of plant origin (Poulin *et al.*, 1993; Ishii *et al.*, 1997; Gryndler & Hřelová, 1998; Gryndler *et al.*, 2005; Horii *et al.*, 2009) on the development of AMF.

All bioproducts had a positive effect on the growth parameters of the roots, particularly in comparison to NPK. Interestingly, these effects were found also from the treatments with bioproducts applied to the leaves (Vinassa and Tytanit). Effect of foliar fertilization on root growth and morphology was already noted (Malusá *et al.*, 2007). Interestingly, Tytanit, which is supposed to enhance the photosynthetic metabolism, induced the highest root elongation and also number of root tips. Previous experiments with preparations containing titanium concerned primarily the impact of this element on the size and quality of the yield produced by various fruit plants (Serrano *et al.*, 2004), including strawberry (Laszlov-szky-Zmarlicka, 2006; Skupień & Oszmiański, 2007ab; Michalski, 2008), and also on the chemical composition of plants (Borkowski *et al.*, 2007; Wallace *et al.*, 1977). The positive effect of the biofertilizer Vinassa, which is the stillage resulting from the processing of sugar beet molasses in the production of yeast, on the development of the root system of strawberry plants confirms the data from the other studies (Chelariu & Ionel, 2005; Chelariu *et al.*, 2009). Vestberg (1992ab) evaluated suitability of *Glomus mosseae* and also *G. intraradices* for inoculation of strawberry and reported that the latter was found to be the most efficient fungus species as it increased shoot growth several-fold.

Generally, the organic fertilizers induced a considerable branching of the root system, as derived from the high total root length and number of tips. On the contrary, the NPK treatment was always causing the smallest root system, which is consistent with the observations of the other authors (Haynes & Goh, 1987; Sas *et al.*, 2003; Glinicki, verbal communication, 2011). This feature is common in chemically fertilized plants and it is considered also a possible factor affecting the plant ability to tolerate environmental stresses (e.g. water deficiency).

We found that in the rhizosphere of strawberry plants 'Elsanta' fertilized with various bioproducts there were spores of arbuscular mycorrhizal fungi of several species of the genus *Glomus* and also of the fungus *Scutellospora dipurpureascens*. However, their occurrence was not uniform. For example, *Glomus claroideum*, a species frequently found in Polish sandy soils (Błaszowski, 2003), was present in all the experimental treatments. The *Glomus mosseae*, a very common species found also in the rhizosphere of strawberry plants (Didier *et al.*, 2003), was present in soil from several treatments. This species is also used as inoculum to increase the resistance of strawberry plants to stress caused by excess phosphorus (Stewart *et al.*, 2005) and drought (Yin *et al.*, 2010). Four other species, namely *Glomus microaggregatum*, *G. constrictum*, *G. pallidum* and *G. drummondii*, were found in the soil treated with only one bioproduct. This difference in the presence of AMF species in rhizospheric soil could be related to the effect of the treatment. Indeed, the plant is actively interacting with the soil to promote the establishment of mycorrhizal symbiosis for nutritional purposes (Hartmann *et al.*, 2009). Changes in its physiology caused by differences in availability of nutrients could thus modify the chemical communication between the plant and the different AMF species, leading to a selective establishment of the symbiosis (Allen *et al.*, 2003). Such hypothesis could be supported by findings from several authors. *Glomus macrocarpum* had also been recorded by Didier *et al.* (2003) in trap cultures of the rhizosphere soil of strawberry plants, but they found no spores of this species in soil samples from strawberry field crops. *Glomus caledonium*, identified in the rhizosphere of the strawberry plants fertilized with Tytanit, was found to occur in intensively and semi-intensively cultivated agricultural soils (Oehl *et al.*, 2003).

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