https://doi.org/10.17221/97/2021-HORTSCI

Effect of microbiologically enriched fertilizers on soil microorganisms in the rhizosphere of apple trees

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Citation: Sas-Paszt L., Smolińska U., Trzciński P., Mika A., Głuszek S., Derkowska E., Lisek A., Górnik K., Sumorok B., Szczech M., Kowalska B., Treder W. (2023): Effect of microbiologically enriched fertilizers on soil microorganisms in the rhizosphere of apple trees. Hort. Sci. (Prague), 50: 189–198.

Abstract: In long-term cultivation of apple trees, replantation disease may occur, caused by a set of biotic and abiotic factors, occurring in the soil, e.g. the accumulation of pathogenic and harmful microorganisms. Beneficial microorganisms can be of great importance in limiting orchard replant disease. In our study, the Urea fertilizer was enriched with *Aspergillus niger* and *Purpureocillium lilacinum* fungi, while the Polifoska 6 and Super Fos Dar 40 fertilizers with strains of the bacteria *Bacillus* spp., *Bacillus amyloliquefaciens*, and *Paenibacillus polymyxa*. The aim of the three-year study was to determine what effects the application of mineral fertilizers enriched with beneficial microorganisms, applied in 100% doses and in doses lower by 40% had on the microorganisms in the rhizosphere of apple trees. The number of bacteria of the genus *Pseudomonas* in the rhizosphere of apple trees was the highest in combination with Urea 60% + fungi and Polifoska 60% + bacteria. These values were 2–3 times higher compared with the control. In combination with Polifoska 60% + bacteria, the number of fluorescent *Pseudomonas* bacteria was five times higher compared to the control. The highest number of actinomycetes was observed in the third year of the study, in combination with Polifoska 60% + bacteria. The use of this fertilizer increased the number of these bacteria more than five-fold compared with the control. The beneficial effect of Polifoska 60% + bacteria, Super Fos Dar 100% + bacteria, and Urea 60% + fungi on phosphate-solubilizing bacteria was observed in the third year of cultivation. The additional application of filamentous fungi together with Urea did not have a significant effect on this group of microorganisms. The obtained results show that in many cases the application of the selected fertilizers positively influenced the microorganisms inhabiting the apple-tree rhizosphere. Particularly noteworthy is Polifoska enriched with the selected bacteria, the use of which significantly increased the number of beneficial bacteria of the genus *Pseudomonas*.

Keywords: beneficial microorganisms; biofertilizers; *Malus domestica* Borkh.; soil biodiversity

The apple tree (*Malus domestica*) is the most cultivated fruit tree in the world. In Poland, apple orchards occupy 175 431 hectares, constituting over 70% of the horticultural acreage (GUS 2020). The apple tree is a species well adapted to the climatic conditions of Poland. Nowadays, very intensive orchards are cultivated, where apple trees are grafted on dwarf rootstocks and require proper training and fertilization, which makes it possible to obtain a sufficiently high crop of good quality fruit.

Apple trees grow best in soils with a pH of approx. 6.4. The nutritional requirements of apple

Supported by the National Centre for Research and Development under the BIOSTRATEG program, contract number BIOSTRATEG3/347464/5/NCBR/2017.

https://doi.org/10.17221/97/2021-HORTSCI

trees for nitrogen are moderate. The efficient use of this element by plants depends on the form of nitrogen present in the fertilizer, the date and method of application, soil pH and moisture content, among other things. Apple trees take up nitrogen mostly in the form of a nitrate ion. Most of the fertilizers used in the cultivation of apple trees cause a significant decrease in soil pH, which in turn promotes the accumulation of Mn and Al ions and increases their toxic effect both on plant roots and soil microorganisms (Rabikowska, Wilk 1991).

Fruit trees, including apple trees, grow and bear fruit best in positions not previously used for orchards. Fruit growers thus face problems with the phenomenon known as replant disease. It is related to the deterioration of plant growth and yielding caused by a disturbance in the functioning of the soil biocenosis (Čatská et al. 1982). Previous studies have shown that replant disease is caused by a set of biotic and abiotic factors, unfavourable for plant growth, occurring in the soil, e.g. the accumulation of pathogenic and harmful microorganisms, phytotoxic compounds, inadequate soil pH and soil structure (Hoestra 1988; Blok, Bollen 1993). In particular, changes in microbial populations, especially of fungi, and the production of toxic compounds by them, are cited as the cause of replant disease (Nicola et al. 2018; Tilston et al. 2018). Recent studies indicate that, in addition to soil microorganisms, soil nematodes are also involved in the occurrence of replant disease because they can act as vectors for pathogenic microorganisms in the soil (Kanfra et al. 2018). Various techniques were first used to combat the symptoms of replant disease, including soil fumigation and mulching with various materials (Jensen, Buszard 1988). It turned out, however, that microorganisms can be of great importance in limiting orchard replant disease. Previous studies have shown that increasing the number of certain groups of microorganisms in the soil, e.g. from the genera *Pseudomonas* or *Bacillus*, can contribute to reducing the effects of this disease (Utkhede, Smith 1992; Biró et al. 1998; Utkhede, Smith 2000; van Schoor et al. 2009; Mehta et al. 2010). The impetus for the development of microbiological technologies for limiting replant disease came from the 'discovery' of soils known as 'replant disease suppressive soils', in which, despite cultivating them in monoculture, soil fatigue does not occur (Mazzola 2002; Weller 2006). Proper fertilization is important for plant growth, development, and yielding (Treder 2003). It affects the extent of flowering and the size, colour, and flavour of the fruit. In addition to nitrogen, phosphorus is also a very important element, which affects the growth of roots and the firmness and storability of fruit. Phosphorus has difficulty in penetrating deep into the soil, therefore it is not easy to increase its amount in a growing orchard by surface-applied fertilizers. Phosphorus, and also potassium, fertilizers cannot be effectively used at low soil pH. Sharma et al. (2017) studied the bacteria of the genus *Pseudomonas* inhabiting the roots of apple trees in terms of their ability to dissolve insoluble phosphorus compounds. They believe that organic compounds secreted by apple roots can stimulate the multiplication of phosphorolytic (fluorescent) *Pseudomonas* bacteria and *Bacillus* bacteria.

As mentioned earlier, the presence of beneficial microorganisms plays an essential role in apple cultivation. They can, for example, compete with pathogenic and/or unfavourable fungi or bacteria for nutrients and prevent them from multiplying (Gupta et al. 2015). Other mechanisms of action of beneficial microorganisms include the induction of defence mechanisms against pathogens inside plants, the formation of a biofilm on the roots and aboveground parts of plants, the production of phytohormones, the production of antibiotics and other biocides, and hyperparasitism (Olanrewaju et al. 2017).

In recent years, research has been intensified to limit the use of mineral fertilizers (due to environmental costs) and to improve the fertility of arable soils through the use of various types of biofertilizers (Mosa et al. 2016; Pešaković et al. 2017; Zhu et al. 2020). Derkowska et al. (2014; 2017) observed that the use of Micosat F, a preparation containing microorganisms, had a positive effect on the root system of apple trees. Kuzin et al. (2020), in the cultivation of apple trees of the cultivar 'Berkutovskoye', using half the dose of NPK fertilizers ($N_{45}P_{15}K_{60}$) together with a consortium of microorganisms (*Azotobacter chroococcum, Bacillus subtilis, B. megaterium, Trichoderma harzianum*), obtained an apple yield of the same amount as that produced with a full NPK ($N_{90}P_{30}K_{120}$) dose. The important role of bacteria from the genera *Pseudomonas* and *Bacillus* in limiting soil pathogens of apple trees and reducing the severity of replant disease was demonstrated in the study by Jiang et al. (2017). The positive effect of microorganisms

https://doi.org/10.17221/97/2021-HORTSCI

applied together with mineral fertilizers in full and reduced doses has also been noted in other cultivated plant species (Zafar-ul-Hye et al. 2015; Ahmad et al. 2017; Karpenko et al. 2020).

In the study describe here, the Urea fertilizer was enriched with *Aspergillus niger* and *Purpureocillium lilacinum* fungi, while the Polifoska 6 and Super Fos Dar 40 fertilizers with strains of the bacteria *Bacillus* spp., *Bacillus amyloliquefaciens*, and *Paenibacillus polymyxa*. It was found that isolates belonging to these species can colonize roots, increase plant yields and reduce harmful pathogen presence through production of toxic compounds (Ishaq 2017; Yadav et al. 2011; Yin et al. 2015). So far, the impact of strains of these species of beneficial microorganisms, selected from Polish soils and applied together with phosphate fertilizers or urea, on rhizosphere microorganisms in apple cultivation has not been assessed yet.

For this reason, the aim of the study was to determine what effects the application of mineral fertilizers enriched with beneficial microorganisms had on the microorganisms in the rhizosphere of apple trees.

MATERIAL AND METHODS

The experiment was carried out in 2018–2020 in the open Experimental Field of the Warsaw University of Life Sciences in Skierniewice (Central Poland, latitude 51.9625'N, longitude 20.1624'E, 128 metres a.s.l.).

Apple trees cv. 'Sampion' grafted on M9 rootstocks, about 130 cm tall, were planted at the beginning of April 2018, individually in ceramic stoneware pots, 0.40 m in diameter and 1.20 m tall, sunk into the ground. The stoneware pots were filled with about 120 l of podzolic soil. Before starting the experiment, the concentrations of minerals, organic matter content, and soil pH were determined. The concentrations of macronutrients were as follows: P – 7.5, K – 12.4, Mg – 5.8 mg/100 g, and of microelements: B – 2.4, Cu – 4.8, Fe – 862, Mn – 5.5, Na – 4.35, Zn – 3.7 mg/1 000 g. The soil pH was 6.2, and the organic matter content was approx. 1.2%. Before the trees were planted, an additional 30 g of calcium oxide and 6 g of magnesium sulphate per tree were applied to the soil.

Throughout all the years of the experiment, soil moisture in the stoneware pots was monitored with 5TE capacitive probes (Decagon, USA). From 2020, the trees were irrigated automatically using the Agreus system (Inventia, Poland).

Three commercial fertilizers were used in the experiment: 1. Polifoska 6 (Grupa Azoty S.A., Poland) – 6% nitrogen in the form of NH_4, 20% phosphorus (P_2O_5), 30% potassium (K_2O), 7% sulphur trioxide (SO_3); 2. Super Fos Dar 40 (Grupa Azoty S.A., Poland) – 40% phosphorus pentoxide (P_2O_5) soluble in mineral acids and 25% P_2O_5 soluble in a neutral citrate solution, 10% calcium oxide (CaO), microelements (Cu, Ca, Fe, Mn, Zn); 3. Urea (Grupa Azoty S.A., Poland) – 46% nitrogen in the amide form.

The experiment was carried out in 13 fertilization combinations, with 3 repetitions (3 stoneware pots per combination).

Fertilization combinations (treatments) used in the experiment with apple trees:

1. Control – no fertilization (K-0)

2. Standard NPK – soil fertilization before planting in dose 20 g of granulated fertilizer Super Fos Dar, 160 g of potassium salt per stoneware container (12 m^2) and 55 g of urea were applied under an individual tree (6 m^2). (K-NPK)

3. Control + beneficial fungi – beneficial soil fungi on their own in the amount of 5.25 g per per stoneware container were applied immediately after planting the plants, thoroughly mixing them with the soil. The mixture of beneficial soil fungi contained two species: *Aspergillus niger* and *Purpureocillium lilacinum*. (K-0 + F)

4. Control + beneficial bacteria – beneficial soil bacteria on their own in the amount of 3.83 g per stoneware container was applied immediately after planting the trees, thoroughly mixing them with the soil. The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* sp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*). (K–0 + B)

5. Standard NPK + beneficial fungi – soil fertilization as in point 2 with the beneficial soil fungi listed in point 3. (K-NPK + F)

6. Standard NPK + beneficial bacteria – soil fertilization as in point 2 and the beneficial bacteria applied to the soil as in point 4. (K-NPK + B)

7. Standard NPK – soil fertilization before planting in dose 40 g of Polifoska 6. Urea, in the amount of 48 g was applied once before planting into stoneware container, potassium salt was used in the amount of 100 g. (P100%)

8. NPK with fungi enriched Urea ($NPK_{f.e.\ Urea}$) – Urea enriched with strains of filamentous fungi

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of the species and quantitative composition as in point 3. For each stoneware container, before planting, 160 g of potassium salt, 55 g of urea, and 20 g of Super Fos Dar 40 fertilizer were used. (U100% + F)

9. NPK with bacteria enriched Polifoska 6 ($NPK_{b.e.Polifoska\ 6}$) – Polifoska 6 enriched with three strains of *Bacillus* bacteria in the amount and species composition as in point 4. Fertilizers were applied before planting in a dose 40 g of Polifoska 6, 100 g of potassium salt, and 48 g of urea were used per one stoneware container. (P100% + B)

10. NPK with bacteria enriched Super Fos Dar 40 ($NPK_{b.e.Super\ Fos\ Dar\ 40}$) – Super Fos Dar 40 (dose (20 g) enriched with three strains of *Bacillus* bacteria was applied in the amount of 3.83 g per stoneware container. In addition, before planting the trees, the soil was fertilized with 100 g of potassium salt and 55g of Urea (SFD100%+B)

11. 60% – NPK with fungi enriched Urea ($NPK_{f.e.\ Urea\ (0.6)}$) – 60% of the variant dose number 8. Urea enriched with strains of filamentous fungi of the species and quantitative composition as in point 3. Applied to each stoneware container before planting the plants, 96 g of potassium salt, 35 g of urea, and 12 g of Super Fos Dar 40 were used. (U60% + F)

12. 60% – NPK with bacteria enriched Polifoska 6 ($NPK_{b.e.Polifoska\ 6\ (0.6)}$) – 60% of the variant dose number 9. Polifoska enriched with three strains of bacteria of the genus *Bacillus* was used in the same way as in point 9. Before planting the plants, 14 g of Polifoska 6, 60 g of potassium salt and 30 g of urea were used for each stoneware container (P60% + B)

13. 60% – NPK with bacteria enriched Super Fos Dar 40 ($NPK_{b.e.Super\ Fos\ Dar\ 40\ (0.6)}$) 60% of the variant dose number 10. Super Fos Dar enriched with three *Bacillus* bacterial strains was used in the same way as in point 12. Before planting: 12 g of Super Fos Dar 40, 60 g of potassium salt and 33 g of urea were applied per plot. (SFD60% + B)

The Urea mineral fertilizer was enriched with selected strains of the fungi *Aspergillus niger* and *Purpureocillium lilacinum*. Polifoska 6 and Super Fos Dar 40 were enriched with strains of the bacteria *Bacillus* spp., *Bacillus amyloliquefaciens*, and *Paenibacillus polymyxa*. The selected isolates of microorganisms came from the collection of the Department of Microbiology and Rhizosphere, Institute of Horticulture – National Research Institute in Skierniewice. Dry formulations of the bacteria, in which maltodextrin was the carrier, were prepared by the production company Skotan S.A., Poland. The product containing conidial spores of *A. niger* and *P. lilacinum* fungi was prepared in the Department of Microbiology and Rhizosphere of the Institute of Horticulture. The fungi were multiplied on corn and rice flour mixed at 1 : 8 in 2018 and at 1 : 10 in 2019. In the combinations in which the selected isolates were applied, the following doses of fungal inoculum per one stoneware pot were used: 1.3 g of substrate containing approx. 2×10^8 cfu/g (in 2018) and 1×10^7 cfu/g (in 2019). In the case of bacterial strains, 3 g of the dry formulation was used for each stoneware pot, with a bacterial population density of approx. $1–2 \times 10^8$ cfu/g.

The study of microorganisms in the rhizosphere of apple trees was conducted in 2018–2020. The stoneware soil was collected in October with a sampling stick 1.5 cm in diameter. The soil samples were stored in a cold store at 5 °C for about 24 hours. Before microbiological analysis, the soil was thoroughly mixed and ground in a mortar. Three 10-gram portions were poured into flasks containing 100 ml of sodium chloride physiological solution (0.85% NaCl) with glass balls each, the fourth 10-gram portion was placed in a thermostat and dried for 24 hours at 104 °C. The soil in the flasks was shaken on a shaker for 20 minutes, then appropriately diluted suspensions were plated on selective media. The total bacterial population was assessed on soy agar (TSA 10%, Merck). The number of bacteria of the genus *Pseudomonas* was determined on Gould's medium, while *Pseudomonas* secreting fluorescent dyes on the same medium under UV light (Gould et al. 1985). Fungal colonies were analyzed on Rose Bengal Chloramfenicol Agar (BTL Sp. z o.o.) commercial medium. Picovska's medium was used to determine the number of P-solubilizing spore bacteria (Picovska 1948). Those counted were bacterial colonies forming transparent 'halo' zones, proving their ability to dissolve calcium phosphate. Actinomycetes were analyzed on colloidal chitin agar (Hsu, Lockwood 1975). The colonies counted were those of bacteria decomposing chitin (creating a transparent 'halo' around them) and showing morphological features of actinomycetes.

The obtained results were statistically processed using the analysis of variance for univariate experiments. The Newman-Keuls test with the Statistica 13.1 software was used to evaluate the differences between the means.

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RESULTS AND DISCUSSION

In 2019, in the combinations with Polifoska 100% + bacteria, there was a much greater total number of bacteria (41.6 × 10^6 cfu/g soil DW) compared with the control combinations K-0 (20.9 × 10^6 cfu/g soil DW) and K-NPK (11.4 × 10^6 cfu/g soil DW) (Table 1). A slightly smaller number was also observed between these combinations and the soil fertilized with Polifoska alone, not enriched microbiologically. A similar number of bacteria was found in the samples fertilized with Super Fos Dar 100% + bacteria (41.5 × 10^6 cfu/g soil DW). It was significantly greater than, for example, in the soil with the addition of Urea 60% + fungi.

When analyzing the population of *Pseudomonas* bacteria in 2019, it can be noticed that the trends observed a year earlier became more evident (Table 1). In the soil with the addition of Polifoska 60% + bacteria, the number of bacteria of this genus was 35.6 × 10^3 cfu/g soil DW, significantly more than in K-0 and K-NPK.

In 2020, the number of bacteria of the genus *Pseudomonas* was the highest in the combination with Urea 60% + fungi and Polifoska 60% + bacteria, and amounted to, respectively, 30.0 × 10^3 cfu/g soil DW and 25.5 x 10^3 cfu/g soil DW (Table 2). These values were 2–3 times higher compared with the control K-0 (8.6 × 10^3 cfu/g soil DW) and K-NPK (7.3 × 10^3 cfu/g soil DW). Even greater, almost five times larger, was the difference in the case of fluorescent *Pseudomonas* bacteria. In the combination with Polifoska 60% + B, the number of these bacteria was 48.0 × 10^3 cfu/g soil DW, while in K-0 – 10.0 × 10^3 cfu/g soil DW, and in K-NPK – 7.3 × 10^3 cfu/g soil DW (Table 2). The ability to maintain a higher level of *Pseudomonas* in the fertilization with Polifoska + bacteria than in the other combinations is essential because there was evidence of a decline in the number of bacteria of this genus with the ageing of the apple trees.

The yielding and health of apple trees is influenced by a number of biotic and abiotic factors; however, the important role of rhizosphere microorganisms is indisputable (dos Passos et al. 2014; Singh et al. 2019). Evidence of the importance of microorganisms for the growth of apple trees is provided by the fact that 94% of bacterial isolates from the rhizosphere of this species are capable to produce phytohormones that stimulate root growth (Karakurt, Aslantas 2010; dos Passos et al. 2014). In studies on the evaluation of the effectiveness of biological preparations, it is very important to know their impact not only on the cultivated plants, but also on native microorganisms living in the soil. This is not easy in the case of the rhizosphere of trees, because the make-up of microorganisms, depending on the type of soil, climatic conditions, and plant development phase, also differs depending on the 'zone along the root', which is mainly related to the differ-

Table 1. Selected groups of microorganisms in the rhizosphere zone of apple trees – 2019

Fertilization	Bacteria total count	*Pseudomonas* total count	Fluorescent *Pseudomonas*	Actinomycetes	Filamentous fungi	Phosphate-solubilizing bacteria
	(cfu × 10^5/g soil DW)					
K-0	209^{ab}	0.48^{ab}	0.17^{ab}	26.9^{a}	1.09^{ab}	9.2^{ab}
K-0 + F	277^{ab}	0.43^{ab}	0.13^{ab}	32.3^{a}	1.69^{bc}	10.4^{ab}
K-0 + B	343^{ab}	0.26^{ab}	0.06^{a}	31.0^{a}	2.37^{bc}	8.4^{ab}
K-NPK	114^{ab}	0.35^{ab}	0.14^{a}b	42.6^{a}	1.30^{ab}	9.4^{ab}
K-NPK + F	305^{ab}	0.55^{b}	0.15^{ab}	44.7^{a}	2.30^{bc}	14.8^{ab}
K-NPK + B	252^{ab}	0.38^{ab}	0.21^{abc}	45.5^{a}	1.15^{ab}	10.8^{ab}
P100%	220^{ab}	0.39^{ab}	0.13^{ab}	42.5^{a}	2.35^{bc}	12.2^{ab}
P100% + B	416^{b}	0.42^{ab}	0.21^{abc}	37.0^{a}	2.72^{c}	28.5^{c}
P60% + B	395^{ab}	0.33^{ab}	0.36^{c}	46.1^{a}	1.85^{bc}	12.7^{ab}
SFD100% + B	415^{b}	0.47^{ab}	0.30^{bc}	29.0^{a}	2.04^{bc}	20.1^{b}
SFD60% + B	273^{ab}	7.9^{a}	0.24^{abc}	42.6^{a}	1.50^{bc}	13.0^{ab}
U100% + F	218^{ab}	40.8^{ab}	0.17^{ab}	33.0^{a}	1.49^{abc}	8.5^{ab}
U60% + F	51^{a}	32.3^{ab}	0.2^{abc}	25.0^{a}	0.41^{a}	6.0^{a}

$^{a–c}$Marked with the same letter in the columns do not differ significantly according to the Newman-Keuls test (P = 0.05)

https://doi.org/10.17221/97/2021-HORTSCI

Table 2. Selected groups of microorganisms in the rhizosphere zone of apple trees – 2020

Fertilization	Bacteria total count	*Pseudomonas* total count	Fluorescent *Pseudomonas*	Actinomycetes	Filamentous fungi	Phosphate-solubilizing bacteria
	(cfu × 10^5/g soil DW)					
K-0	110^{ab}	0.09^{ab}	0.1^{a}	23.0^{a}	3.71^{a}	9.3^{a}
K-0	131^{ab}	0.12^{b}	0.14^{a}	16.2^{a}	3.01^{a}	14.2^{ab}
K-0 + F	127^{abc}	0.14^{b}	0.06^{a}	21.1^{a}	3.33^{a}	10.8^{ab}
K-0 + B	171^{bcd}	0.09^{ab}	0.07^{a}	22.1^{a}	3.84^{a}	11.2^{ab}
K-NPK	130^{ab}	0.10^{ab}	0.04^{a}	34.5^{a}	3.99^{a}	20.2^{ab}
K-NPK + F	78^{a}	0.12^{b}	0.09^{a}	27.6^{a}	4.35^{a}	14.5^{ab}
K-NPK + B	230^{d}	0.03^{a}	0.08^{a}	25.0^{a}	4.34^{a}	17.9^{ab}
P100%	317^{e}	0.08^{ab}	0.06^{a}	24.1^{a}	3.44^{a}	14.5^{ab}
P100% + B	285^{e}	0.26^{c}	0.48^{b}	63.1^{b}	5.38^{a}	24.8^{b}
P60% + B	120^{ab}	0.11^{ab}	0.05^{a}	25.5^{a}	4.63^{a}	25.0^{b}
SFD100% + B	220^{d}	0.14^{b}	0.08^{a}	20.0^{a}	3.52^{a}	13.9^{ab}
SFD60% + B	118^{ab}	0.09^{ab}	0.03^{a}	17.5^{a}	3.53^{a}	15.4^{ab}
U100% + F	194^{cd}	0.3^{c}	0.05^{a}	32.3^{a}	4.89^{a}	24.4^{b}

$^{a–c}$Marked with the same letter in the columns do not differ significantly according to the Newman-Keuls test ($P = 0.05$)

ent intensity of nutrient secretion along this organ (Lagos et al. 2015).

Soil microorganisms are good indicators of soil fertility because changes in their number and activity are considered an early signal of a decrease or improvement in soil quality (Brzezińska 2009). There is a close relationship between the condition of the plant, the size of the root system, and the intensity of the impact on soil microorganisms (Jankowska, Swędrzyńska 2016). In this study, the numbers of selected microbial groups were determined by the methods of plating on selective media. Although these methods allow the isolation of only a small amount (the so-called 'cultivated' part) of soil microorganisms, these microorganisms, due to their rapid growth and large size, account for 80–90% of the bacterial biomass in the soil (Olsen, Bakken 1987).

In this study, conducted over three consecutive years, microbiological analyses were performed at the same time of the growing season (October), because a close relationship has been observed between the date of sampling and the number of microorganisms in the samples (Rumberger et al. 2007). The use of mineral fertilizers enriched with microorganisms had, in many cases, a beneficial effect on soil microorganisms in the rhizosphere of apple trees. It should be emphasized that this effect varied depending on the year of cultivation. In the first year of cultivation, apple trees were transplanted into stoneware pots filled with 'fresh', microbiologically unstabilized, soil. For this reason, in 2018, due to the large dispersion in the numbers of microorganisms between individual replications in combinations, no statistical differences were observed (Table 3). However, some trends could be seen, such as a marked increase in the total number of bacteria in the combination with Polifoska 60% + bacteria, and of bacteria of the genus *Pseudomonas* in the combinations with this fertilizer.

The observed positive effect of the use of some of the biofertilizers on increasing the population of bacteria of the genus *Pseudomonas* is very important because, from the point of view of broadly understood soil fertility, their presence is considered extremely beneficial (Garbeva et al. 2004). The research conducted by Dos Passos et al. (2014) showed that *Pseudomonas* was one of the most numerous groups of microorganisms isolated from the rhizosphere of apple trees and accounted for 18.7% of isolated species. These are bacteria that, having the ability to produce hormones (e.g. auxins, gibberellins, cytokinins), can stimulate plant growth through, *inter alia*, root development and by increasing the efficiency of water and nutrient uptake. Biologically active compounds produced by these bacteria, e.g. antibiotics, lytic enzymes, siderophores, etc., can inhibit the development of pathogens and induce systemic resistance in plants (Singh 2018). *Pseudomonas* bacteria have also been observed to increase soil suppressiveness in relation to apple root disease caused by *Rhizocto-*

https://doi.org/10.17221/97/2021-HORTSCI

Table 3. Selected groups of microorganisms in the rhizosphere zone of apple trees – 2018*

Fertilization	Bacteria total count	*Pseudomonas* total count	Fluorescent *Pseudomonas*	Actinomycetes	Filamentous fungi	Phosphate-solubilizing bacteria
	(cfu × 10^5/g soil DW)					
K-0	453	4.0	0.7	97	0.72	5.0
K-0 + F	402	3.5	4.4	45	0.94	4.9
K-0 + B	732	5.7	3.0	161	1.47	19.4
K-NPK	507	8.4	3.4	89	0.95	10.2
K-NPK + F	274	2.1	0.7	19	0.61	2.9
K-NPK + B	821	16.8	4.6	87	1.69	11.8
P100%	696	14.6	3.2	88	1.09	11.3
P100% + B	464	16.1	13.7	54	1.10	5.8
P60% + B	1 610	29.4	10.4	65	1.28	7.9
SFD100% + B	448	14.2	5.1	37	1.13	7.4
SFD60% + B	712	8.9	2.1	46	0.9	9.0
U100% + F	870	8.5	6.1	76	1.38	8.1
U60% + F	444	9.4	4.0	70	1.18	11.1

*In the 2018 no statistical differences between combinations were observed

nia solani (Mazzola, Gu 2002). Koczorowski (2019) showed the beneficial effect of cultivating apple trees cv. 'Sampion' fertilized with biofertilizers containing *Pseudomonas fluorescens* on fruit yield and quality. The role of antagonistic *Pseudomonas* in the prevention of apple replant disease is not always clear-cut, since Rumberger et al. (2007) did not observe any relationship between its intensity and bacteria of the genus *Pseudomonas* to produce biologically active compounds, especially antibiotics or cyanogenic compounds. Stimulation of apple-tree root growth under the influence of the addition of Plant Growth Promoting Rhizobacteria (PGPR) bacterial strains was also observed by Singh (2018).

The results obtained from the research presented here show a relatively small effect of the applied fertilization on the number of actinomycetes. This group of bacteria plays an important role in nature due to their ability to break down various chemical compounds and participation in the mineralization of organic substances. They produce a huge number of antibacterial and antifungal compounds (Lenart-Boroń, Banach 2014). The highest number of actinomycetes in the apple-tree rhizosphere was observed for the combination with Polifoska 60% + bacteria in the third year. The fertilizer increased the number of the bacteria more than five-fold, if compared with K-0 and K-NPK (Table 2). When analyzing the population of filamentous fungi, their numbers were increased only in the combination with Polifoska 100% + bacteria in 2019. The additional application of filamentous fungi together with Urea did not have a significant effect on this group of microorganisms.

The study also analyzed the populations of bacteria capable of dissolving insoluble phosphorus compounds and converting them into forms available to plants. It was found that the use of Polifoska 100% + bacteria almost tripled the number of this group of bacteria in the second year of apple-tree cultivation. The analyses performed in the third year of cultivation showed the beneficial effect of Polifoska 60% + bacteria, Super Fos Dar 100% + bacteria, and Urea 60% + fungi on phosphate-solubilizing bacteria. It is known that the ability of bacteria to dissolve phosphorus compounds varies and depends on, among others, the amount and type of the released organic acids, which may affect this process with varying intensity (Kurek, Ozimek 2008; Ciopińska, Bezak-Mazur 2018). Increased numbers of phosphate-solubilizing bacteria are beneficial because they can contribute to increasing the pool of 'mobile' phosphorus available to plants.

CONCLUSION

The obtained results show that in many cases the application of the selected biofertilizers positively influenced the microorganisms inhabiting

https://doi.org/10.17221/97/2021-HORTSCI

the apple-tree rhizosphere. Particularly noteworthy is Polifoska enriched with the selected bacteria, the use of which significantly increased the number of beneficial bacteria of the genus *Pseudomonas*. However, on the basis of the obtained results, it is not possible to state whether it is a direct or indirect effect of the applied biofertilizers on the plant, and requires further research. It is necessary to verify the variety dependent response on the applied biofertilizers. This is important because the use of appropriate biofertilizers would make it possible to alter the microbiome of the apple-tree rhizosphere in a way that would reduce or prevent the replant disease.

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Received: October 19, 2022
Accepted: January 31, 2023