

Influence of bioproducts and mycorrhizal fungi on the growth and yielding of sweet cherry trees

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Citation: Gluszek S., Derkowska E., Sas-Paszt L., Sitarek M., Sumorok B. (2020): Influence of bioproducts and mycorrhizal fungi on the growth and yielding of sweet cherry trees. Hort. Sci. (Prague), 47: 122–129.

Abstract: The experiment assessed the influence of various biofertilizers and biostimulants on the growth characteristics of the root system, its colonization by arbuscular mycorrhizal fungi and the yielding of sweet cherry trees in field conditions. The experiment, conducted in Pomological Orchard of Research Institute of Horticulture located in Skierniewice during 2011–2014, involved the use of a mycorrhizal substrate, organic fertilizers and biostimulant in randomized block design. The control combination consisted of plants fertilized with mineral fertilizers (NPK). The use of the organic fertilizer BF Ekomix in dose 100 g per tree each year in the spring significantly increased the number of root tips in comparison with the control trees. There was also a tendency for the roots to lengthen and increase their surface area under the influence of this biofertilizer. In addition, the inoculation of roots with the mycorrhizal substrate in dose 200 g per tree per year stimulated the colonization of the roots of sweet cherry trees by arbuscular mycorrhizal fungi, which in turn led to improved root growth parameters.

Keywords: organic fertilizer; mycorrhizal inoculum; biopreparation; sweet cherry; root growth; mycorrhiza

As a cultivated species, the sweet cherry is not as important in Poland as, for example, sour cherry. The production efficiency increased year by year from 40.1 thousand tonnes at 2010 to 48.1 thousand tonnes in 2015 (GUS 2017). Such a tendency may be evidence of the development of cultivation technology of species. Nutritional requirements of sweet cherry during fruiting are similar to sour cherry (50 kg N, 5 kg P, 60 kg K, 70 kg Ca and 10 kg Mg/kg). There are a number of risks associated with the cultivation of sweet cherry. The trees can be damaged by frosts during the winter, and spring frosts often reduce potential yields by destroying flowers and fruit buds. Another, weather associated problem is fruit cracking after rainfalls. Other

important problems associated with the cultivation of sweet cherry are diseases, especially bacterial canker (*Pseudomonas syringae* pv. *syringae* van Hall and *P.s.* pv. *morsprunorum* Wormald) (Sobiczewski, Schollenberger 2002), as well as numerous pests, such as the cherry fruit fly (*Rhagoletis cerasi* L.) (Rozpara et al. 2010) or the spotted-wing drosophila (*Drosophila suzuki* Matsumura) (Łabanowska, Piotrowski 2015), whose larvae feed on the flesh of the fruit. Another problem in sweet cherry production are birds, which can destroy yield by eating and damaging of fruits. 'Vanda' is a cultivar derived in the Czech Republic and quite commonly grown in Poland. Trees of this variety grow moderately vigorously and are very produc-

Supported by the Research Institute of Horticulture, Poland, Statutory Found, Project No. 3.3.5.

<https://doi.org/10.17221/102/2018-HORTSCI>

tive. The fruits reach a mass of 8 g, are dark red, with a tasty intensely red, juicy flesh and are not susceptible to cracking during rainfall (Grzyb et al. 2008; Stachowiak et al. 2014).

'GiSelA 5' is a dwarfing rootstock of German selection, whose importance in the cultivation of sweet cherry in Europe is growing steadily. As demonstrated by the research of many authors (De Salvador et al. 2005; Franken-Bembenek 2005; Sitarek, Bartosiewicz 2012), this rootstock is effective in reducing the growth vigour of trees and increases their productivity, without lowering the quality of the fruit. The restriction of the growth of the aboveground part by the 'GiSelA 5' rootstock results from the differences in the structure of the root system in comparison with a vigorously growing rootstock, and may also be due to the small active surface area of the root system compared with its overall size (Ljubojević et al. 2014; Sitarek, Sas-Paszt 2014). The growth and yielding of sweet cherry trees, their health and also the diversity of associated rhizosphere microorganisms are significantly influenced by the method of plant fertilization. The high level of mineral fertilizers used in intensive conventional agriculture, has a negative impact on the development of most beneficial soil microorganisms, including the growth and colonization of roots by mycorrhizal fungi (Smith, Read 2008). Preparations of organic origin, compared with artificial fertilizers, are characterized by a much lower rate at which nitrogen ions and minerals are released to the soil, especially at low temperatures (Kelderer et al. 2008). Therefore, biofertilizers, apart from traditional organic fertilizers such as manure or compost, are one of the solutions to the problem of environment eutrophication as an alternative to chemical fertilizers in fruit tree cultivation (Maksoud et al. 2009; Sas Paszt et al. 2015). Biofertilizers are preparations containing, among others, humic compounds, hydrolysates, plant extracts and beneficial microorganisms (including mycorrhizal fungi), which have the ability to colonize the rhizosphere of plants, increase the supply and availability of organic matter and nutrients in the soil and reduce the negative impact of soil pathogens, thus stimulating the growth and development of plants (Bhattacharjee, Dey 2014).

Suitably developed bioproducts with microbiological ingredients like beneficial bacteria or mycorrhizal fungi increase the effectiveness of plant fertilization with both synthetic mineral fertilizers and organic

fertilizers and reduce the susceptibility of cultivated plants to various environmental stresses, both biotic and abiotic (Mosa et al. 2016; French 2017; Turan et al. 2017). Colonization of the root system by arbuscular mycorrhizal fungi (AMF) can change its morphological structure, e.g. the size of the root system, its topographic distribution as well as the surface area and volume of roots, and, when used in crops, they increase the yield and improve its quality (Kapoor et al. 2008; Baum et al. 2015).

Both mycorrhizal fungi and beneficial rhizosphere bacteria (PGPR) increase the effectiveness of plant feeding based on the use of organic fertilizers. Their widespread use in agriculture can lead to a reduction in the use of chemical means of plant production and to improving the efficiency of utilization of mineral compounds contained in the soil, and in the long term, to a permanent improvement in the quality of soils (Lingua et al. 2013; Singh et al. 2015). The combined use of organic fertilizers and beneficial microorganisms allows the obtaining of good quality yields even under unfavourable growing conditions (Bharti et al. 2016). It is therefore advisable to use mycorrhizal and bacterial consortia in which the microorganisms mutually increase their beneficial effects on the growth and yielding of plants, and on the quality of the obtained crops (Bona et al. 2015, 2017).

The aim of the research was to determine the influence of a few biofertilizers and biostimulants on the extent of root colonization by arbuscular mycorrhizal fungi and the growth characteristics of the sweet cherry root system.

MATERIALS AND METHODS

One-year-old maiden sweet cherry trees of the cultivar 'Vanda' grafted onto the 'GiSelA 5' rootstock were planted in the spring of 2007 in the Experimental Orchard of the Research Institute of Horticulture in Skierniewice Central Poland (latitude 51.9625N, longitude 20.1624E, 128 meters a.s.l.). Amount of rain is showed on Figure 1. The experiment was established in three replications with 3 trees per plot. The trees grew at a spacing of 4.0 m × 2.0 m on graded Class IV soil (medium quality pseudo-podsolic soil), with pH 6.0, 1.3% of organic matter and macroelements content (% DW): 2.37% N, 0.15% P, 1.35% K, 0.22% Mg, 1.64 Ca%. Microelements content in soil was (mg/kg): 3.2 mg/kg of B, 13.5 mg/kg of Cu, 1 084 mg/kg of Fe,

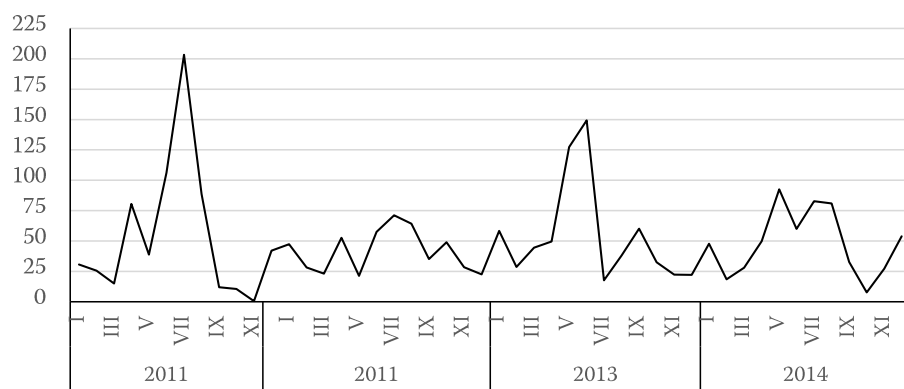
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Figure 1. Amount of rain in Skierniewice (IO Pomological Orchard, 2011–2014)

96.6 mg/kg of Mn and 11.8 mg/kg of Zn. Trees were irrigated via a computer-controlled irrigation system. Plant protection treatments were carried out in accordance with the recommendations for commercial sweet cherry orchards. After second spray with insecticide against cherry fruit fly (*Rhagoletis cerasi* L.) trees were covered with green, plastic net against birds. In the first years after planting, weeds were removed manually and later with the use of herbicides. The experiment included the following combinations:

Control – trees fertilized with NPK in doses: ammonium nitrate (35.1 g/tree), granular triple superphosphate (57.3 g/tree), and potassium sulphate (7.7 g/tree) applied manually in the spring at the beginning of April.

Mycorrhizal substrate – product of Mykoflor, Końskowola – 1 g containing 10^6 propagules of arbuscular mycorrhizal fungi: *Rhizophagus intraradices*, *Funneliformis mosseae*, *Claroideoglossum etunicatum*, *Rhizophagus clarus* on an organic carrier (pure high peat). The substrate was applied manually to the root system zone at the time of establishing the experiment at 200 g per tree, and afterwards to the soil each year in the spring.

Organic fertilizer Biollsa – commercial product of ILSA Group Arzignano, Vicenza, Italy, of animal origin (12 : 0:2), used in the form of granules applied manually to the soil at a dose of 2.4 g per tree each year in the spring at the beginning of April.

Organic fertilizer BioFeed Ekomix – commercial plant origin product of AgroBio Products, Wageningen, NL, (7.5 : 2 : 4), used in the form of granules applied manually to the soil at a dose of 100 g per tree each year in the spring at the beginning of April.

Organic biostimulant Ausma – original name of the preparation by Biolat (Latvia), containing an extract from steam distilled conifer needles with

0.29 N; 0.12 P and 0.95% K, applied to the leaves in the form of a water solution: 3 foliar applications at a concentration of 0.1% – 9, 6, and 3 weeks before the estimated fruit harvest date.

The following measurements and observations were made in the experiment:

Determination of root growth characteristics.

The roots together with the rhizosphere soil were sampled at September of every year, at a distance of a few centimeters away from the sour cherry tree trunk with the use of a soil auger with a capacity of approx. 0.5 l. The portion of the root system from three replicates per treatment, obtained in that way was placed on a sieve, cleaned of soil, rinsed, dried and scanned using an Epson Expression 10000 XL root scanner. Root growth characteristics were determined by means of WinRhizo software (Regent Instruments, Quebec, Canada) (Arsenault et al. 1995), followed by the calculations of root length, surface area, diameter, volume and number of root tips. Fresh and dry root weight was determined by the weight method.

Assessment of the extent of root colonization by arbuscular mycorrhizal fungi.

The sample fragments of the roots of sweet cherry trees (10 g from each replicate) were stained according to the method developed by Derkowska et al. (2015). The 30 approx. 1-cm-long stained roots fragments were placed parallel to one another on a glass slide containing glycerin and crushed with a cover glass. The prepared histological preparations were examined using a Nikon 50i optical microscope (with 20×, 40×, 60×, 100× objectives) and the observed mycorrhizal structures were photographed with Nikon DS-Fi1 digital camera. The assessment of the extent of colonization of the roots of sweet cherry trees by arbuscular mycorrhizal fungi was made by the Trouvelot method (Trouvelot et al. 1986). Mycor-

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Table 1. Influence of bioproducts on root growth characteristics of ‘Vanda’ sweet cherry trees (IO Pomological Orchard, 2011–2014)

Treatment	Root length (cm/plant)	Root surface area (cm ² /plant)	Root diameter (mm/plant)	Root volume (cm ³ /plant)	Number of root tips (per plant)
Control	347 ± 88 ^{ab}	90 ± 15 ^a	0.88 ± 0.12 ^{ab}	2.02 ± 0.36 ^a	742 ± 114 ^a
Mycorrhizal substrate	290 ± 96 ^a	90 ± 28 ^a	1.09 ± 0.20 ^c	2.42 ± 0.98 ^b	651 ± 164 ^a
Biollsa	305 ± 20 ^a	89 ± 15 ^a	0.96 ± 0.14 ^{bc}	2.14 ± 0.63 ^{ab}	706 ± 72 ^a
BF Ekomix	540 ± 113 ^b	118 ± 22 ^b	0.77 ± 0.12 ^a	2.17 ± 0.58 ^{ab}	1 249 ± 359 ^b
Ausma	386 ± 87 ^{ab}	93 ± 15 ^a	0.79 ± 0.15 ^a	1.87 ± 0.47 ^a	981 ± 176 ^{ab}

Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey’s multiple test

rhizal frequency ($F\%$) was calculated with Mycocalc software (Table 1).

Statistical analysis. The results were statistically analyzed by one-way analysis of variance in a random block design. Multiple comparisons of means for the combinations were performed with Tukey’s test at a significance level of $\alpha = 0.05$ using Statistica v.10 software (StatSoft, Inc. 2011).

RESULTS AND DISCUSSION

Assessment of the extent of root colonization by arbuscular mycorrhizal fungi. Applications of the bioproducts had a positive influence on the degree of mycorrhizal association in the roots of sweet cherry trees. The largest increase in root colonization by arbuscular mycorrhizal fungi occurred under the influence of the mycorrhizal substrate. In comparison with the control combination, the other bioproducts increased the occurrence of mycorrhizal fungi in the roots of sweet cherry trees, but these differences were not statistically proven (Table 2 and Figures 2–7).

The use of arbuscular mycorrhizal fungi significantly affects the development of the root system of inoculated trees, but this influence depends on the species of the mycorrhizal fungus used. Aka-

Kacar et al. (2010) studied the influence of various AMF species on the uptake of minerals and the development of the ‘Tabel Edabriz’ and ‘GiSelA 5’ rootstocks grown in containers. They observed that the ‘GiSelA 5’ rootstock was characterized by the highest dry weight of roots after inoculation with the *Funneliformis mosseae* (*Glomus mosseae*) fungus, while the roots of plants inoculated with *Funneliformis caledonium* (*Glomus caledonium*) had the lowest dry weight of roots. These authors also found that the ‘GiSelA 5’ rootstock, regardless of the inoculating species of AMF, grew best in the compost-containing substrate, while the mycorrhizal frequency depended on the fungal species and the growth substrate used. Most of the tested species of mycorrhizal fungi colonized to the greatest extent the roots of the ‘GiSelA 5’ rootstock grown in a tuff : peat (1 : 1) mixture. In addition, the inoculation of sweet cherry rootstocks with AMF increased the uptake of phosphorus and zinc. Compared with plants fertilized with the recommended doses of mineral fertilizers, application of reduced doses of mineral fertilization accompanied by inoculation with AMF strains increases the growth of sweet cherry trees. The effectiveness of mycorrhizal fungi is also increased by the PGPR bacteria used in conjunction with them (Sharma 2016).

Table 2. Influence of bioproducts on the fresh and dry weight of roots and mycorrhizal frequency of ‘Vanda’ sweet cherry trees (IO Pomological Orchard, 2011–2014)

Treatment	Root fresh weight (g/plant)	Root dry weight (g/plant)	Mycorrhizal frequency ($F, \%$)
Control	5.08 ± 1.07 ^a	2.57 ± 0.42 ^a	8.34 ± 2.72 ^a
Mycorrhizal substrate	6.44 ± 2.73 ^b	3.16 ± 1.38 ^b	29.45 ± 2.98 ^b
Biollsa	4.72 ± 1.68 ^a	2.40 ± 0.91 ^a	17.78 ± 3.51 ^{ab}
BF Ekomix	5.90 ± 1.24 ^{ab}	2.70 ± 0.9 ^{ab}	17.78 ± 1.92 ^{ab}
Ausma	4.67 ± 1.68 ^a	2.48 ± 0.59 ^a	20.0 ± 3.07 ^{ab}

Means in columns marked with the same letter do not differ significantly at $P = 0.05$ according to Tukey’s multiple test

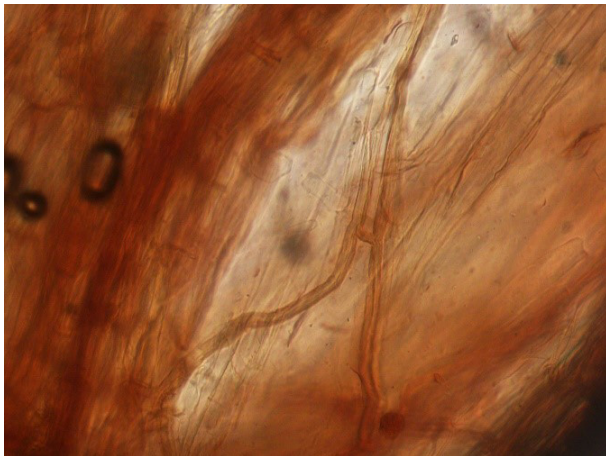


Figure 2. Mycorrhizal mycelium in the; roots of 'Vanda' sweet cherry trees (Pomological Orchard 2014): (A) control, (B) treated with the mycorrhizal substrate



Figure 3. A vesicle in the roots of 'Vanda' sweet cherry trees (Pomological Orchard 2014): (A) control, (B) treated with the mycorrhizal substrate

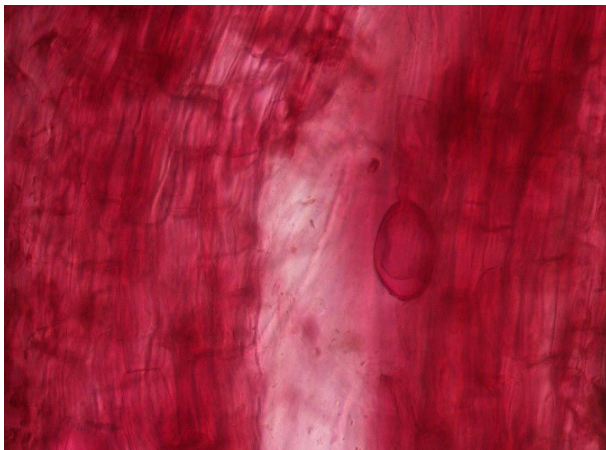


Figure 4. Vesicles in the roots of 'Vanda' sweet cherry trees treated with the mycorrhizal substrate (Pomological Orchard 2014)

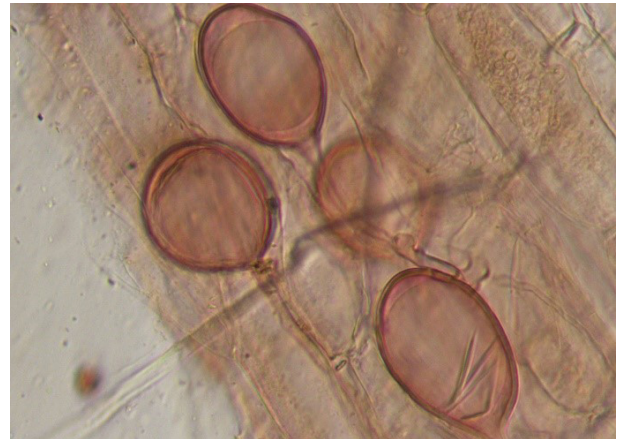
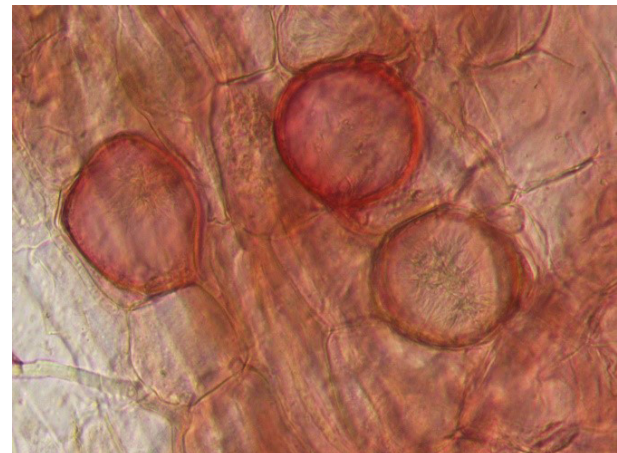
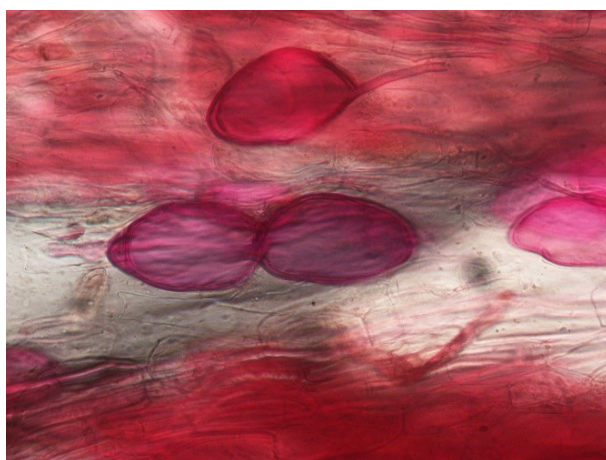


Figure 5. Vesicles in the roots of 'Vanda' sweet cherry trees treated with the mycorrhizal substrate (Pomological Orchard 2014)



Figures 6 and 7. Vesicles in the roots of 'Vanda' sweet cherry trees treated with the mycorrhizal substrate (Pomological Orchard 2014)

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A better supply of plants with minerals resulting from the application of mycorrhizal fungi as well as inoculation with PGPR bacteria has also been observed in other fruit tree species and in wild plants. This effect is particularly pronounced when growing plants in unfavourable conditions. Xiao et al. (2014) found that inoculation with AMF of the roots of 'Newhall' citrus (*Citrus sinensis* Osbeck cv. Newhall) and 'Ponkan' tangerine (*Citrus reticulata* Blanco cv. Ponkan) grafted onto the trifoliolate orange (*Poncirus trifoliata* L. Raf.) rootstock had a positive effect on the growth of plants, and increased the magnesium content in the tissues of plants grown in a substrate with a low Mg content. Mycorrhizal fungi also increase the efficiency of the photosynthesis process, but this effect depends on the species being cultivated. Similar results were obtained in the case of a deficit of other mineral components in the growth substrate (Chen et al. 2014; Mohamed et al. 2016). The mycorrhizal fungi *Funneliformis mosseae* (*Glomus mosseae*) and *Claroideoglomus etunicatum* (*Glomus etunicatum*) improved the uptake of minerals from the soil and the growth of Beach Plum (*Prunus maritima*) plants under conditions of salinity stress. After inoculation with AMF, the plants had greater biomass and total leaf surface area compared with the control plants (Zai et al. 2014). The cited authors found that application of mycorrhizal fungi was an alternative method of alleviating the stress associated with soil salinity in plum (*P. maritima*) trees.

Boyer et al. (2015) observed that strawberry plants showed stronger growth and produced fruit more abundantly after applications of mycorrhizal fungi under the conditions of both optimal moisture levels and drought stress. Application of a consortium containing 5 species of mycorrhizal fungi and an isolate of a bacterium of the genus *Pseudomonas* increased the number of flowers and fruits, the weight and size of strawberries and their dry matter content compared with non-inoculated plants (Bona et al. 2015). Among other organic products the strongest impact on mycorrhizal frequency had Ausma (20.00%), the next ones were BioIlsa and BioFeed Ekomix (both 17.78%). Ausma used in long time experiment on grapevines cv. 'Solaris' and 'Regent' grafted on SO4 rootstock, gave the highest average values of mycorrhizal frequency on roots of 'Solaris' cultivar (28.15%). In the same experiment, preparations BioFeed Ekomix and BioIlsa gave similar values of mycorrhizal frequency (respectively 25.73 and 25.56) for 'Solaris' vines. In case of 'Regent' biggest values

of mycorrhizal frequency were obtained in treatment with BioFeed Ekomix (30.00%), whereas for Ausma it was 27.96% and for BioIlsa 26.48% (Sas Paszt et al. 2019). Beneficial effects of different organic preparations on mycorrhizal association in plant roots was observed in other works. Increased mycorrhiza formation in plant roots is associated with organic compounds produced by plants or microorganisms used as ingredient of biopreparations (Kuwada et al. 2005; Horii et al. 2009).

Root growth characteristics. The bioproducts had also beneficial influence on the growth of sweet cherry roots (Table 2). In particular, applications of the mycorrhizal substrate to the root zone of plants significantly increased the fresh and dry weight of roots (PGPR bacteria 1), as well as their diameter and volume (Table 1). In turn, the use of the organic fertilizer BF Ekomix caused an increase in the number of root tips compared with the control trees. There was also an observable tendency to increase the length of roots and their surface area under the influence of the BF Ekomix bio-fertilizer; however, these differences were not statistically significant (Table 1).

Flores et al. (2015), in an experiment on 'Lapins' sweet cherry trees after application of a bio-inoculant and organic matter/organic preparations, observed a 15% increase in the dry weight of roots. Organic fertilizers and preparations are known for modification of root growth parameters also in other pomological crops, but these effects are associated with used product. Increase of fresh root weight in comparison to mineral fertilization was observed in apple cv 'Topaz' and 'Ariva' after application of manure or Humus UP product whereas other products reduced fresh mass of roots. Similar effects were observed in root dry weight (Derkowska et al. 2017).

CONCLUSION

The use of organic fertilization and mycorrhization has a positive influence on yield performance of 'Vanda' sweet cherry trees grafted onto the 'GiSelA 5' rootstock.

The highest effectiveness in the growth and development of the root system of sweet cherry trees on the 'GiSelA 5' rootstock is shown by the biofertilizer BF Ekomix and inoculation with the mycorrhizal substrate.

Mycorrhization has a beneficial influence on the formation of structures of arbuscular mycorrhizal

<https://doi.org/10.17221/102/2018-HORTSCI>

fungi in the roots of sweet cherry trees growing on the 'GiSela 5' rootstock.

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Received: July 17, 2018
Accepted: January 24, 2020