



# Mycorrhizal frequency, physiological parameters, and yield of strawberry plants inoculated with endomycorrhizal fungi and rhizosphere bacteria

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## Abstract

Due to the impoverishment of agricultural and horticultural soils and replant diseases, there is a need to use bioproducts and beneficial microorganisms in order to improve the quality of soils and growth substrates. For this reason, research was undertaken to assess the impact of arbuscular mycorrhizal fungi and rhizosphere bacteria on changes in soil microbiology, the degree of colonization of plant roots by mycorrhizal fungi, selected physiological parameters, and fruit quality and yield of the strawberry cultivar “Rumba.” The plants were inoculated with the mycorrhizal preparation Mykoflor (*Rhizophagus irregularis*, *Funneliformis mosseae*, *Claroideoglossum etunicatum*), MYC 800 (*Rhizophagus intraradices*), and the bacterial preparation Rhizocell C (*Bacillus amyloliquefaciens* IT45). The applied preparations increased the total number of bacteria and fungi in the soil and mycorrhizal frequency in the roots of the strawberry plants. They increased the chlorophyll “a” and total chlorophyll concentrations in the leaves as well as the rate of transpiration and CO<sub>2</sub> concentration in the intercellular spaces in the leaves. The plants treated with Rhizocell C and MYC 800 exhibited a higher CO<sub>2</sub> assimilation rate than control plants. The biopreparations increased chlorophyll fluorescence parameters such as maximum fluorescence ( $F_M$ ) and the maximum potential photochemical reaction efficiency in PS II ( $F_v/F_M$ ). The influence of the species of rhizosphere bacteria and mycorrhizal fungi used in the experiment on the physiological traits of strawberry plants contributed, especially in the second year of the study, to increase the yield and mean weight of strawberry fruit.

**Keywords** Arbuscular mycorrhizal fungi · Plant growth promoting rhizobacteria · Chlorophyll · CO<sub>2</sub> assimilation · Fluorescence parameters · Total soluble solids

## Introduction

The strawberry fruit (*Fragaria × ananassa* Duch) is one of the most popular berry fruits due to its taste quality and nutritional

values (Khan et al. 2010). From an economic point of view, strawberries are the most important soft fruits cultivated in the world, mainly in the northern hemisphere, in the temperate climate zone (Hummer and Hancock 2009). At present, special attention is paid to sustainable and environmentally friendly production. The technologies related to sustainable and ecological production methods ensure high fruit yields of proper quality in terms of taste and nutritional value, while limiting the use of mineral fertilizers and synthetic plant protection agents (Bona et al. 2015; Kurokura et al. 2017). Owing to the impoverishment of mineral nutrient availability or an increase of soil organic matter of agricultural and horticultural soils as well as replant diseases, there is a need to use bioproducts and beneficial microorganisms (bacteria, mycorrhizal fungi, and filamentous fungi). These might increase soil biodiversity and stimulate growth and yield in addition to having antagonistic effects on microorganisms that are

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harmful to plants. According to many authors (Pešaković et al. 2013; Sinclair et al. 2014; Bona et al. 2015; Derkowska et al. 2015b; Cecatto et al. 2016; Sowik et al. 2016), the activity of microorganisms in the soil is a factor largely determining the growth and productivity of plants and their resistance to pathogens and abiotic stress. Beneficial microorganisms colonizing roots improve the health of plants in different ways: some produce toxic metabolites while others compete for space or nutrients (Khan and Lee 2013). Arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizosphere bacteria (PGPR) favorably affect, among other attributes of plants, the absorptive surface of roots, the availability of nutrients to plants (Sas-Paszt et al. 2011; Cavagnaro et al. 2015; Leifheit et al. 2015), and also the biosynthesis of phytohormones and plant metabolism (Torelli et al. 2000; Bona et al. 2010, 2011; Ciatelli et al. 2012; Baslam et al. 2013). They can also indirectly affect the rate of photosynthesis by increasing stomatal conductance to CO<sub>2</sub> and can improve the efficiency of photochemical processes. They increase yield and crop quality, especially under stress conditions (Borkowska 2002; Khade and Rodrigues 2009; Karlidag et al. 2013).

Microbiologically enriched bioproducts are increasingly used in the cultivation of strawberries. Meanwhile, little is known about the impact of beneficial microorganisms on the soil microbiota and the physiological state of strawberry plants and consequently on their yield.

The aim of this study was to assess the impact of arbuscular mycorrhizal fungi and rhizosphere bacteria on changes in soil microbiology, degree of colonization of roots by mycorrhizal fungi, selected physiological parameters, fruit quality, and yield of strawberry plants.

## Materials and methods

The 2-year study (14 May 2013–30 September 2014) was conducted in the West Pomeranian University of Technology in Szczecin (53°26′51″ N, 14°31′50″ E) located in northwestern Poland. A 1-factor pot experiment (10-dm<sup>3</sup> Kick's pot with 8 dm<sup>3</sup> of substrate) designed for complete randomization was set up with five replications of each treatment. One replication consisted of 3 plants per pot. The research was conducted on the strawberry cultivar “Rumba.” Frigo plants were planted on May 14, 2013, in a clayey-sandy soil with a pH 5.8 (in KCl). Soil nutrient levels (mg 100 g<sup>-1</sup>), determined in the soil, were P—2.21, K—14.7, Ca—4.06, and Mg—0.41. The concentrations of phosphorus and potassium in the soil were determined by the method of Egner-Riehm (Egner et al. 1960), calcium by the method of Scheibler (ISO 10693 1995), and magnesium by the method of Schachtschabel (Schachtschabel 1954). Water potential in the soil was maintained at a level -10 kPa. The experimental factor was the

inoculation of plant roots with mycorrhizal fungi and rhizosphere bacteria. The plants were inoculated with mycorrhizal preparations Mykoflor (Mykoflor, Końskowola, Poland) or MYC 800 (Lallemand Plant Care S.A.S.) and bacterial preparation Rhizocell C (Lallemand Plant Care S.A.S.). Preparation Mykoflor contained 1000 propagules per 1 g of arbuscular mycorrhizal fungi: *Rhizophagus irregularis*, *Funneliformis mosseae*, and *Claroideoglossum etunicatum*. Preparation MYC 800 contained 800 propagules per 1 g of arbuscular mycorrhizal fungus *Rhizophagus intraradices*. Bacterial preparation Rhizocell C contained 10<sup>9</sup> cfu of *Bacillus amyloliquefaciens* IT45 per 1 g. The mycorrhizal preparation Mykoflor was applied once during planting, by introducing 1 g of the preparation per plant, in the vicinity of the strawberry root system (Mykoflor treatment). Inoculation with the mycorrhizal preparation MYC 800 was performed once, 6 days after planting, by applying 0.5 g of the preparation per one plant (MYC 800 treatment). Inoculation was performed by watering plants with water (100 cm<sup>3</sup> of distilled water intended for watering one plant contained 0.5 g of MYC 800). The preparation Rhizocell C, in the first year of the study, was applied 6 days after planting, and in the second year, a week after the vegetative growth of strawberry plants had started (Rhizocell C treatment). Before the inoculation with Rhizocell C, the product was mixed with a small amount of water at room temperature and left for an hour, after which time water was added until the desired volume was reached. To water one plant, 100 cm<sup>3</sup> of the suspension was used, which corresponds to a dose of 1 g of the preparation per plant. The controls consisted of plants growing in the soil without the addition of any biopreparations (control treatment).

## Microbiological analysis of soil

Rhizosphere soil samples were collected at the beginning of September of each year (2013–2014) for both years of the study. After separating the plants from the soil, their root system was gently shaken to remove the soil particles still adhering to it. Five plants were taken from each experiment treatment (one plant from each pot—replication). The soil was shaken onto filter paper. The weight of the soil sample taken from a replication was 100 g. The samples were mixed thoroughly, and 5 g of each sample was transferred to 100-ml Erlenmeyer flasks containing 45 g of sterile distilled water. The suspended samples were homogenized at 190 rpm for 45 min. Serial tenfold dilutions (10<sup>-2</sup>, 10<sup>-3</sup> ... 10<sup>-5</sup>) were prepared from each suspension. Soil dry weight was determined by oven-drying the soil samples, at 90 °C for 4 days.

Sterile Petri dishes were inoculated with 100-μl aliquots of each dilution prepared from the soil suspensions. The inoculated dishes were flooded with a liquid agar medium at a

temperature of approx 50 °C. All analyses were made in triplicate. To estimate the number of microorganisms, the following microbial media were used: for estimation of the total number of culturable bacteria—10% tryptic soy agar (TSA) (Biocorp, cat. number: PS22); for estimation of the total number of spore-forming bacteria (to obtain the bacterial spores, the soil samples were incubated at 80 °C for 30 min before being transferred into Petri dishes)—10% TSA; and for estimation of the number of filamentous fungi—rose bengal chloramphenicol agar (Biocorp, cat. number: PS66).

Incubation of inoculated Petri plates was performed at 26 °C for 5 to 7 days to estimate the number of spore-forming bacteria and filamentous fungi and for 10 to 14 days for estimation of an overall population of bacteria.

To estimate the number of bacterial or fungal colonies, Petri dishes containing between 30 and 300 colonies were selected. The results were expressed as colony-forming units per 1 g of dry weight of soil (Schinnerk et al. 1995).

### Assessment of root colonization by arbuscular mycorrhizal fungi

The roots of strawberries (10 g from each replication), collected in September, were stained according to the method described by Derkowska et al. (2015b). Microscopic specimens were prepared and examined with a Nikon 50i microscope (objectives with magnifications of  $\times 20$ ,  $\times 40$ ,  $\times 60$ ,  $\times 100$ ). The assessment of the degree of colonization of the roots by arbuscular mycorrhizal fungi was performed with the Trouvelot method (Trouvelot et al. 1986). Based on the results, mycorrhizal frequency ( $F\%$ ) and relative mycorrhizal intensity ( $M\%$ ) were calculated using the computer program MYCOCALC, available from the website: <http://www2.dijon.inra.fr/mychintec/Mycocalcprg/MYCOCALC.EXE>.

### Concentrations of chlorophyll and carotenoids in leaves

The concentrations of chlorophyll a, b, and total chlorophyll in leaves were determined by the method of Arnon et al. (1956) modified by Lichtenthaler and Wellburn (1983) and the concentrations of carotenoids in leaves by the method of Hager and Mayer-Berthenrath (1966). The concentration of chlorophyll and carotenoids was determined for plants in each pot on the same test dates and for same leaves on which gas exchange measurements were made. The absorbance of extracts was determined using a Marcel Mini spectrophotometer at wavelengths of  $\lambda = 440$  nm (carotenoids), 645 nm (chlorophyll), and 663 nm (carotenoids and chlorophyll).

### Gas exchange parameters of plants

The parameters of gas exchange of plants ( $\text{CO}_2$  assimilation intensity— $A$ , transpiration— $E$ , stomatal conductance for water— $g_s$ , and  $\text{CO}_2$  concentration in chlorenchyma intercellular spaces— $c_i$ ) were measured twice in each year of the experiment (at the full flowering stage and during fruit ripening), with a TPS-2 (PP Systems) portable gas analyzer (with standard settings) equipped with a PLC4 measuring chamber operating as an open system. The measurements were performed on healthy, fully grown leaves of each plant. On the basis of the results of  $\text{CO}_2$  assimilation intensity and transpiration, the photosynthetic water use efficiency ( $\omega_w$ ) was calculated by the ratio of assimilation intensity to transpiration (Candolfi-Vasconcelos and Koblet 1991).

### Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were recorded in the second year after inoculation of plants, using a Handy PEA (Hansatech) spectrofluorometer, based on the standard apparatus procedure ( $3 \times 650$  nm LEDs, maximum actinic light intensity  $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The measurements were made twice during the strawberry growing season, for each treatment of the experiment, on the same test dates, and the same leaves on which the other physiological characteristics were determined. Leaves were shaded 20 min before measurement with factory clips (illuminated area with a diameter of 4 mm). The following parameters of chlorophyll fluorescence induction were measured and calculated using the spectrofluorometer:  $F_0$ —initial fluorescence (zero), excitation energy loss index in power antennas;  $F_M$ —maximum fluorescence, after reduction of acceptors in PS II and after dark adaptation;  $F_V = F_M - F_0$ —variable fluorescence, determined after dark adaptation, a parameter dependent on the maximum quantum yield of PS II;  $F_V/F_M$ —the maximum potential photochemical reaction efficiency in PS II determined after dark adaptation and after reduction of acceptors in PS II (Bolhár-Nordenkamp and Öquist 1993); PI—PS II vitality index for the overall viability of this system.

### Yield parameters

During the experiment, the total (cumulative) marketable fruit yield for the whole harvesting period (in the first year, fruits were harvested from June 21 to July 16, in the second year, from June 10 to July 6) and the mean weight of one fruit were determined. Total soluble solids content (TSS) was determined with an Atago Pol 1 refractometer. Titratable acidity (TA) of fruit was determined by titrating an aqueous extract of strawberry homogenate with 0.1 N NaOH to the endpoint of pH 8.1. On the basis of the obtained results, the ratio of extract to total acidity in the fruits was calculated.

## Statistical analysis of results

One-way analysis of variance (ANOVA) and Tukey's test at  $P \leq 0.05$  were used to analyze the experimental results. Prior to analysis, normality was confirmed with the Shapiro-Wilk test and homogeneity of variance verified with the Levene test. If assumptions were violated, the Kruskal-Wallis ANOVA and Dunn's rank-sum test were performed. For the numerical data on the gas exchange parameters, concentration of chlorophyll and carotenoids, and chlorophyll fluorescence parameters, the analysis of variance was performed separately for each measurement date (at the full flowering stage and during fruit ripening). Statistical analyses of data in this study were performed using the STATISTICA v.10 software package.

## Results

A significant increase of the total number of bacteria in samples from the plants treated with Rhizocell C was observed in both years of the study (Table 1). Also, a decrease of spore-forming bacteria in the soil samples from strawberries treated with MYC 800 and Rhizocell C was noticed in 2013 and 2014. All the biopreparations increased the total number of filamentous fungi in the second year of the study.

The biopreparations used in the experiment were found to have a positive influence on the colonization of strawberry plant roots by arbuscular mycorrhizal fungi (Table 2). In the

first year of the study, the biopreparation Mykoflor approximately doubled mycorrhizal frequency ( $F$ ) compared with MYC 800 and was 2.5 times that of the control. The results of microscopic examinations of root specimens showed that in the first year of the study, the inoculation of the roots of "Rumba" strawberry plants with a consortium of mycorrhizal fungi in biopreparation Mykoflor increased the relative intensity of root colonization by mycorrhizal fungi compared with roots of the controls and treated with Rhizocell C. In the second year of the study, all of the applied biopreparations were found to have had beneficial effects on the degree of mycorrhizal association in comparison with the roots of the controls. The roots subjected to the application of the biopreparation MYC 800 were shown higher mycorrhizal association than the roots inoculated with Rhizocell C.

Each of the applied preparations increased the chlorophyll "a" and total chlorophyll concentrations in strawberry leaves, compared with the controls (Table 3). The applied preparations also increased the chlorophyll "b" content during fruit ripening in the first year of the study and during the flowering stage in the second year. In the first year, during the strawberry flowering stage, the applied preparations were found to have no effect on the chlorophyll "b" concentration of the leaves. The highest level of total chlorophyll was found in the plants inoculated with MYC 800. This relationship was demonstrated during the fruit ripening stage in the first year of the study and on both measurement dates in the second year. In the first year of the study during flowering, an increase in the total chlorophyll concentration was observed following the use of

**Table 1** Effect of application of bioproducts containing arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria on the number of isolated microorganisms

Treatment	Year	
	2013	2014
	Total number of isolated bacteria $\text{Log}_{10}$ cfu/g DW <sub>s</sub>	
Control	8.28 ± 0.01b*	7.98 ± 0.00d
Mykoflor	8.25 ± 0.01bc	8.00 ± 0.00b
MYC 800	8.21 ± 0.04c	7.99 ± 0.01c
Rhizocell C	8.37 ± 0.02a	8.07 ± 0.00a
	Total number of isolated spore-forming bacteria $\text{Log}_{10}$ cfu/g DW <sub>s</sub>	
Control	7.50 ± 0.02a	7.53 ± 0.03a
Mykoflor	7.33 ± 0.04c	7.54 ± 0.03a
MYC 800	7.23 ± 0.02d	6.99 ± 0.01c
Rhizocell C	7.38 ± 0.02b	7.07 ± 0.06b
	Total number of isolated filamentous fungi $\text{Log}_{10}$ cfu/g DW <sub>s</sub>	
Control	5.89 ± 0.03a	5.61 ± 0.01c
Mykoflor	5.64 ± 0.04c	5.77 ± 0.02a
MYC 800	5.90 ± 0.04a	5.78 ± 0.01a
Rhizocell C	5.76 ± 0.07b	5.74 ± 0.01b

Means marked with the same letters in each column do not differ significantly at  $P \leq 0.05$ , according to Tukey's test

\*Data are presented as mean ± SD

**Table 2** Effect of application of bioproducts containing arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria on parameters of mycorrhizal colonization in the roots of strawberry plants

Treatment	Year	
	2013	2014
	<i>F</i> (%)—mycorrhizal frequency	
Control	4.44 ± 1.5b* <sup>1</sup>	13.3 ± 5.3c <sup>1</sup>
Mykoflor	11.1 ± 1.9a	26.7 ± 5.3ab
MYC 800	6.7 ± 4.1ab	32.2 ± 3.9a
Rhizocell C	4.4 ± 2.1b	22.2 ± 3.1b
	<i>M</i> (%)—relative mycorrhizal intensity	
Control	0.04 ± 0.02b <sup>2</sup>	0.13 ± 0.05c <sup>1</sup>
Mykoflor	1.24 ± 0.83a	0.27 ± 0.05ab
MYC 800	0.06 ± 0.04ab	0.32 ± 0.04a
Rhizocell C	0.04 ± 0.02b	0.22 ± 0.03b

\*Data are presented as mean ± SD

<sup>1</sup> Means marked with the same letters in each column do not differ significantly at  $P \leq 0.05$ , according to Tukey's test

<sup>2</sup> Means marked with the same letters in each column do not differ significantly at  $P \leq 0.05$ , according to Dunn's test

MYC 800 and Rhizocell C (Table 3). In the first year of the study, during the flowering stage, the leaves of strawberry plants inoculated with Mykoflor showed a level of carotenoids that was the highest and significantly different from that found in control plants and plants inoculated with MYC 800. Each of the applied preparations increased, in comparison with the control, the concentration of carotenoids in the leaves of the tested species, determined during the fruit ripening stage in the first year of the study and during the flowering stage in the second year.

During the flowering stage, the highest CO<sub>2</sub> assimilation rate was shown by the strawberry plants inoculated with MYC 800 and those treated with Rhizocell C and in the fruit ripening stage following the application of Rhizocell C (Table 4). During the flowering stage in the first year of the study and in the fruit ripening stage in the second year, there was an increase in the CO<sub>2</sub> assimilation rate, in comparison with the control plants, under the influence of MYC 800 and Rhizocell C. On the remaining measurement dates, the use of each biopreparation increased the CO<sub>2</sub> assimilation rate, compared with the controls. In the first year of the study, during the flowering stage, the plants treated with the biopreparations had similar transpiration rates, higher than those of the control plants. During the fruit ripening stage, the highest rate of transpiration was found in the plants treated with Rhizocell C (Table 4). In the second year of the study, the highest rate of this process was demonstrated following the use of MYC 800 and Rhizocell C.

In the first year of the study, during the fruit ripening stage, the highest efficiency of water use in photosynthesis was

shown by the controls and plants inoculated with Mykoflor (Table 4). In the second year during the flowering stage, the highest value of the  $\omega_W$  index was obtained for the controls.

During the strawberry flowering stage, in the first year of the study, the highest stomatal conductance was shown by the plants inoculated with Rhizocell C and MYC 800 (Table 4). During the same development stage in the second year of the study, each of the applied biopreparations increased stomatal conductance compared with the controls. During the fruit ripening stage in both years of the study, the biopreparations were found to have no effect on this physiological trait. During the flowering stage, the highest concentration of CO<sub>2</sub> in the intercellular spaces of assimilatory parenchyma was found after inoculation with all biopreparations in the first year and Mykoflor and Rhizocell C in the second year. However, during the fruit ripening period, this effect was observed only in the plants treated with Rhizocell C and MYC 800. On both measurement dates in the first year and on the second date in the second year, there was an increase in the intercellular CO<sub>2</sub> concentration after the application of each biopreparation.

In the first year of the study, the highest relative water content was found in the leaves of plants inoculated with MYC 800 (Table 4). In the second year, during the strawberry flowering stage, an increase in the relative water content (RWC) in the leaves compared with the controls was recorded following the application of the preparation Rhizocell C and the inoculum MYC 800. During the fruiting stage, an increase in RWC was found as a result of using the two mycorrhizal inocula as well as the bacterial preparation.

In the flowering stage of the tested strawberry cultivar, the applied biopreparations were noted to have no effect on the magnitude of the parameter  $F_0$  (Table 5). During the fruit ripening period of the tested strawberry cultivar, the plants inoculated with Mykoflor and MYC 800 were characterized by smaller values of  $F_0$  than were the controls, which may indicate a higher efficiency of excitation energy transfer between pigment molecules in inoculated plants. The application of the bacterial preparation did not change the value of  $F_0$ . On both measurement dates, the lowest values of the  $F_M$  parameter were shown by the control plants. The applied biopreparations increased the value of  $F_M$  determined during both the flowering and the fruiting stage of the strawberry cultivar tested. In both the flowering and the fruit ripening stages of the strawberry cultivar tested relative to the controls, the applied biopreparations increased the  $F_v/F_M$  ratio. The highest values of this parameter were shown on both measurement dates by mycorrhiza-inoculated plants. During the strawberry flowering stage, an increase in the performance index (PI) of PSII was evident for the MYC 800 and Rhizocell C treatments versus controls. In the fruit ripening stage, the applied biopreparations were found to have no effect on the value of the PI index.

**Table 3** Effect of application of bioproducts containing arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria on the concentration of chlorophyll and carotenoids in leaves of strawberry cv. Rumba

Treatment	Year			
	2013		2014	
	Flowering stage	Fruit ripening stage	Flowering stage	Fruit ripening stage
	Chlorophyll “a” (mg g <sup>-1</sup> FW)			
Control	2.124 ± 0.156b*	2.051 ± 0.076c	1.937 ± 0.023d	2.192 ± 0.072c
Mykoflor	2.275 ± 0.022b	2.319 ± 0.024b	2.311 ± 0.030c	2.290 ± 0.015b
MYC 800	2.925 ± 0.057a	2.878 ± 0.057a	2.626 ± 0.045a	2.905 ± 0.013a
Rhizocell C	2.832 ± 0.071a	2.393 ± 0.071b	2.429 ± 0.023b	2.861 ± 0.047a
	Chlorophyll “b” (mg g <sup>-1</sup> FW)			
Control	0.759 ± 0.105a	0.699 ± 0.041b	0.681 ± 0.018c	0.837 ± 0.055bc
Mykoflor	0.858 ± 0.073a	0.862 ± 0.060a	0.920 ± 0.054a	0.880 ± 0.051ab
MYC 800	0.858 ± 0.018a	0.827 ± 0.018a	0.870 ± 0.023b	0.950 ± 0.035a
Rhizocell C	0.780 ± 0.083a	0.805 ± 0.083a	0.805 ± 0.016b	0.759 ± 0.035c
	Total chlorophyll (mg g <sup>-1</sup> FW)			
Control	2.883 ± 0.243b	2.750 ± 0.118c	2.618 ± 0.005c	3.029 ± 0.106d
Mykoflor	3.133 ± 0.096b	3.181 ± 0.048b	3.231 ± 0.084b	3.170 ± 0.037c
MYC 800	3.783 ± 0.075a	3.705 ± 0.076a	3.496 ± 0.054a	3.855 ± 0.025a
Rhizocell C	3.612 ± 0.119a	3.198 ± 0.119b	3.234 ± 0.015b	3.620 ± 0.082b
	Carotenoids (mg g <sup>-1</sup> FW)			
Control	0.373 ± 0.058b	0.286 ± 0.022b	0.307 ± 0.015b	0.399 ± 0.010b
Mykoflor	0.473 ± 0.044a	0.407 ± 0.013a	0.376 ± 0.032a	0.430 ± 0.027ab
MYC 800	0.343 ± 0.062b	0.363 ± 0.062a	0.388 ± 0.019a	0.438 ± 0.017a
Rhizocell C	0.414 ± 0.020ab	0.403 ± 0.020a	0.402 ± 0.014a	0.422 ± 0.011ab

Means marked with the same letters in each column do not differ significantly at  $P \leq 0.05$ , according to Tukey's test

\*Data are presented as mean ± SD

The biopreparation Rhizocell C, in both years of the study, increased the yield and mean weight of strawberry fruit (Table 6). The effect of using the preparation containing *Bacillus amyloliquefaciens* IT45 was particularly evident in the first year of the study in which the obtained yield was higher than for all the other treatments. In the second year of the experiment, inoculation with the mycorrhizal preparations was also found to increase both the yield and the mean fruit weight versus controls.

In the first year of the study, the biopreparations MYC 800 and Rhizocell C reduced the TSS content of strawberry fruit versus controls (Table 6). In the second year, the highest amount of TSS, different from the amounts found in the fruit of the control plants and those treated with Rhizocell C, was found in the fruits of the Mykoflor treatment. The applied preparations did not affect fruit acidity (TA). In the first year of the study, a reduction in the TSS/TA ratio was found in the fruit of plants treated with *Bacillus amyloliquefaciens* IT45 relative to the control plants and those inoculated with

Mykoflor. A similar relationship was not found in the second year of the study (Table 6).

## Discussion

The increase in the total number of isolated bacteria following the use of the biopreparation Rhizocell C, the increase in the total number of isolated fungi resulting from the use of all the biopreparations, and the decrease in the total number of isolated spore-forming bacteria after the application of the biopreparations MYC 800 and Rhizocell C indicate that, apart from environmental factors, the application of microorganisms could affect the microbial community in the soil. Pešaković et al. (2013) showed that the application of microbial inocula increased the number of some groups of microorganisms such as the total number of bacteria and fungi. They also noticed that vegetative seasons had a significant impact on fungi, actinomycetes, or *Azotobacter* populations.

**Table 4** Effect of application of bioproducts containing arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria on the intensity of CO<sub>2</sub> assimilation (*A*) and transpiration (*E*), photosynthetic water use efficiency ( $\omega_w$ ), stomatal conductance for water ( $g_s$ ), CO<sub>2</sub> concentration in chlorenchyma intercellular spaces ( $c_i$ ), and relative water content (RWC) in leaves of strawberry cv. Rumba

Treatment	Year			
	2013		2014	
	Flowering stage	Fruit ripening stage	Flowering stage	Fruit ripening stage
	<i>A</i> —CO <sub>2</sub> assimilation intensity ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )			
Control	6.72 ± 0.607b*	7.95 ± 0.228c	7.69 ± 0.381c	7.89 ± 0.312c
Mykoflor	7.19 ± 0.703b	9.19 ± 0.165b	8.48 ± 0.260b	8.47 ± 0.556c
MYC 800	8.67 ± 0.454a	8.89 ± 0.278b	8.95 ± 0.322a	9.44 ± 0.426b
Rhizocell C	8.70 ± 0.657a	9.80 ± 0.200a	9.10 ± 0.132a	10.14 ± 0.213a
	<i>E</i> —transpiration intensity ( $\text{mmol m}^{-2} \text{s}^{-1}$ )			
Control	1.48 ± 0.075b	1.66 ± 0.078d	1.25 ± 0.135c	1.59 ± 0.093c
Mykoflor	1.82 ± 0.166a	1.87 ± 0.064c	1.68 ± 0.113b	1.77 ± 0.053b
MYC 800	1.84 ± 0.175a	2.02 ± 0.046b	1.92 ± 0.033a	2.04 ± 0.057a
Rhizocell C	2.02 ± 0.152a	2.26 ± 0.166a	2.04 ± 0.087a	2.12 ± 0.016a
	$\omega_w$ —photosynthetic water use efficiency ( $\text{mmol mol}^{-1}$ )			
Control	4.54 ± 0.44ab	4.79 ± 0.29a	6.15 ± 0.364a	4.96 ± 0.451a
Mykoflor	3.95 ± 0.67b	4.91 ± 0.16a	5.05 ± 0.298b	4.78 ± 0.257a
MYC 800	4.71 ± 0.26a	4.40 ± 0.25b	4.66 ± 0.156bc	4.63 ± 0.150a
Rhizocell C	4.31 ± 0.27ab	4.34 ± 0.11b	4.46 ± 0.223c	4.78 ± 0.386a
	$g_s$ —stomatal conductance for water ( $\text{mol m}^{-2} \text{s}^{-1}$ )			
Control	0.059 ± 0.011b	0.080 ± 0.002a	0.100 ± 0.012b	0.196 ± 0.007a
Mykoflor	0.074 ± 0.009b	0.088 ± 0.007a	0.170 ± 0.007a	0.207 ± 0.057a
MYC 800	0.092 ± 0.009a	0.083 ± 0.006a	0.218 ± 0.017a	0.208 ± 0.014a
Rhizocell C	0.089 ± 0.008a	0.087 ± 0.005a	0.215 ± 0.021a	0.215 ± 0.016a
	$c_i$ —CO <sub>2</sub> concentration in chlorenchyma intercellular spaces ( $\mu\text{mol mol}^{-1}$ )			
Control	188.97 ± 3.123b	171.71 ± 4.317c	175.37 ± 4.580b	161.16 ± 3.575c
Mykoflor	200.93 ± 10.857a	179.70 ± 5.606b	186.11 ± 2.803a	171.86 ± 2.047b
MYC 800	204.60 ± 7.583a	192.46 ± 2.826a	175.33 ± 4.823b	183.44 ± 4.468a
Rhizocell C	206.97 ± 6.850a	190.38 ± 1.904a	185.27 ± 7.798a	188.36 ± 6.980a
	RWC—relative water content (%)			
Control	85.67 ± 2.648b	87.04 ± 0.396b	87.59 ± 0.639b	87.00 ± 1.419b
Mykoflor	85.14 ± 3.150b	90.15 ± 1.711ab	90.36 ± 3.007ab	92.11 ± 1.286a
MYC 800	91.20 ± 2.553a	91.81 ± 1.727a	92.11 ± 1.335a	93.56 ± 1.750a
Rhizocell C	84.78 ± 1.506b	89.45 ± 2.797ab	91.33 ± 0.737a	92.78 ± 3.096a

Means marked with the same letters in each column do not differ significantly at  $P \leq 0.05$ , according to Tukey's test

\*Data are presented as mean ± SD

According to Palencia et al. (2015), some rhizospheric bacteria can affect root colonization by AMF. They noticed that strawberry roots from plants inoculated with combined *Bacillus velezensis* and *Rhizophagus intraradices* are more colonized than roots treated with *R. intraradices* only. Application of the plant-beneficial bacterium, *Bacillus amyloliquefaciens* L-S60, significantly increased the presence of beneficial rhizosphere species such as *Bacillus*, *Rhodanobacter*, *Paenibacillus*, *Pseudomonas*, *Nonomuraea*,

and *Agrobacterium* in the bacterial community associated with cucumber seedlings (Qin et al. 2017).

The formation of mycorrhizal structures is associated with the effectiveness of root colonization by various AMF species (Boyer et al. 2015; Sharma et al. 2015). The highest value of mycorrhizal frequency in the first-year Mykoflor treatment is probably related to the high potential of *Funneliformis mosseae* for the formation of mycorrhizas. This species heavily colonized the roots of

**Table 5** Effect of application of bioproducts containing arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria on the fluorescence parameters of chlorophyll “a” in leaves of strawberry cv. Rumba

Treatment	Year	
	2014	
	Flowering stage	Fruit ripening stage
	<i>F</i> <sub>0</sub> —initial fluorescence (zero)	
Control	596.62 ± 11.03a*	602.75 ± 10.81a
Mykoflor	580.62 ± 11.56a	574.00 ± 22.99b
MYC 800	583.94 ± 9.24a	574.50 ± 17.06b
Rhizocell C	595.33 ± 11.67a	594.00 ± 12.11ab
	<i>F</i> <sub>M</sub> —maximum fluorescence	
Control	2759.75 ± 80.61b	2674.25 ± 85.84c
Mykoflor	3252.25 ± 52.70a	3199.00 ± 138.20a
MYC 800	3271.50 ± 48.03a	3248.00 ± 44.99a
Rhizocell C	3166.69 ± 83.40a	3024.50 ± 116.66b
	<i>F</i> <sub>v</sub> / <i>F</i> <sub>M</sub> —maximum potential photochemical reaction efficiency in PS II	
Control	0.783 ± 0.007c	0.774 ± 0.004c
Mykoflor	0.821 ± 0.003a	0.820 ± 0.004a
MYC 800	0.821 ± 0.005a	0.823 ± 0.006a
Rhizocell C	0.812 ± 0.004b	0.803 ± 0.011b
	P I—PS II vitality index	
Control	1.419 ± 0.017c	1.419 ± 0.342a
Mykoflor	1.402 ± 0.016c	1.477 ± 0.155a
MYC 800	1.595 ± 0.014a	1.670 ± 0.073a
Rhizocell C	1.527 ± 0.032b	1.387 ± 0.024a

Means marked with the same letters in each column do not differ significantly at  $P \leq 0.05$ , according to Tukey’s test

\*Data are presented as mean ± SD

strawberry plants, both on its own and in combination with the fungus *Trichoderma viride* (Chauhan et al. 2010). Increased colonization of plant roots by AM fungi is often observed in plants under abiotic stress conditions, e.g., drought (Boyer et al. 2015; Moradtalab et al. 2019), so the near-optimal growing conditions of our study could have been the cause of the low mycorrhizal frequency in the roots of our strawberry plants.

Numerous studies confirm the beneficial effect of AMF inoculation on plant growth (Palencia et al. 2015; Sas-Paszt et al. 2011, 2015; Derkowska et al. 2015a). Boyer et al. (2015) had found that the inoculation of strawberry plants with two species of arbuscular mycorrhizal fungi (*Funneliformis mosseae* BEG25, *F. geosporus* BEG11) had a positive effect on plant growth and on increasing the population of AMF in the roots, in comparison with plants without mycorrhizas. In those studies, it was shown that plant growth was directly related to the degree of root colonization by AMF. Numerous previous studies had also indicated positive effects of beneficial microorganisms on the colonization of roots by arbuscular mycorrhizal fungi (Boyer et al. 2015; Derkowska et al. 2015a; Sas-Paszt et al. 2011, 2015).

Beneficial root-colonizing microorganisms can affect the physiological traits of plants, indirectly affecting their growth and yield. In the present study, an increase, in relation to the control, in the amounts of chlorophyll “a” and total chlorophyll of strawberry leaves was found under the influence of each of the applied preparations. Similar results of studies on the influence of mycorrhizal fungi have been obtained by Wu et al. (2011) and Chen et al. (2017) in cucumber and bermudagrass, respectively. Chen et al. (2017) showed an increase in both chlorophyll “a” and total chlorophyll concentrations due to the action of an inoculum composed of *Claroideoglomus* sp., *Funneliformis* sp., *Diversispora* sp., and *Rhizophagus* sp., and an inoculum composed of *Rhizophagus intraradices*, *Rhizophagus microageregatum* BEG, and *Rhizophagus claroideum* BEG 210, as well as an inoculum containing only one AMF species—*Funneliformis mosseae*. Comparing the preparations containing mycorrhizal fungi used in our study, we found the greatest effect on increasing the amounts of chlorophyll “a” and total chlorophyll for MYC 800, containing *Rhizophagus intraradices*, than in the case of Mykoflor, containing a combination of several AMF strains. For the majority of measurement dates, this



**Table 6** Effect of application of bioproducts containing arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria on yield parameters of strawberry plants

Treatment	Year	
	2013	2014
	Marketable yield (g plant <sup>-1</sup> )	
Control	69.6 ± 9.35b*	455.1 ± 44.3b
Mykoflor	63.1 ± 4.76b	576.2 ± 44.9a
MYC 800	78.2 ± 10.86b	615.1 ± 58.4a
Rhizocell C	108.3 ± 13.31a	631.4 ± 48.1a
	Average mass of 1 fruit (g)	
Control	10.01 ± 1.11b	9.11 ± 0.93c
Mykoflor	10.56 ± 1.23b	11.97 ± 1.04b
MYC 800	10.74 ± 0.95b	12.49 ± 0.51b
Rhizocell C	13.76 ± 1.30a	15.47 ± 1.34a
	TSS (%)	
Control	14.21 ± 0.37a	7.74 ± 0.39b
Mykoflor	13.23 ± 0.47ab	8.80 ± 0.50a
MYC 800	12.24 ± 0.65b	8.36 ± 0.55ab
Rhizocell C	9.87 ± 0.66c	7.91 ± 0.35b
	TA (g citric acid-100 g <sup>-1</sup> F.M.)	
Control	1.00 ± 0.12a	0.60 ± 0.03a
Mykoflor	0.93 ± 0.07a	0.65 ± 0.04a
MYC 800	0.91 ± 0.06a	0.64 ± 0.03a
Rhizocell C	0.86 ± 0.05a	0.58 ± 0.04a
	TSS/TA	
Control	14.2 ± 1.49a	12.9 ± 0.47a
Mykoflor	14.2 ± 1.30a	13.5 ± 0.62a
MYC 800	13.5 ± 1.63ab	13.1 ± 1.04a
Rhizocell C	11.5 ± 0.67b	13.6 ± 1.43a

Means marked with the same letters in each column do not differ significantly at  $P \leq 0.05$ , according to Tukey's test

\*Data are presented as mean ± SD

association was also demonstrated in the case of CO<sub>2</sub> assimilation intensity. This may provide evidence as to the effectiveness of the interaction of *Rhizophagus intraradices* with strawberry plants, because according to Chen et al. (2017), the response of a plant species to AMF varies greatly depending on the AMF species. Seema et al. (2018) showed an increase in the total chlorophyll concentration in the leaves of the strawberry cultivar “Chandler,” to whose root system *Bacillus licheniformis* and *B. subtilis* had been applied, as was the case in the present experiment after applying the bacterial preparation Rhizocell C. Similarly, Stefan et al. (2013) reported that the use of *Bacillus pumilus* and *B. mycoides* increased the chlorophyll concentration in runner bean. According to Samaniego-Gómez et al. (2016), a mixture of *Bacillus subtilis* and *B. amyloliquefaciens* increased the chlorophyll concentration of Habanero pepper plants. Increased

chlorophyll concentration of leaves following the application of the bacterium *Bacillus megaterium* had also been found in cabbage by Turan et al. (2014). The increase in the chlorophyll concentration of strawberry leaves demonstrated in the present study after the application of the biopreparations may have been the result of increased uptake of nutrients such as nitrogen, phosphorus, and magnesium, as indicated by the results of many authors (Smith and Read 2008; Karlidag et al. 2013; Vafadar et al. 2014; Zhu et al. 2014; Fan et al. 2017; Qin et al. 2017).

In the present study, on most measurement dates, there was an increase in the concentration of carotenoids in the leaves after the application of preparations containing either AMF or PGPR. These results agree with previous findings that AMF and PGPR colonization could promote the synthesis of carotenoids (Vafadar et al. 2014; Chen et al. 2017). Elevated amounts of carotenoids may increase the resistance of plants to biotic and abiotic stress factors (Vafadar et al. 2014) through, inter alia, the protection of the photosynthetic apparatus of plants from photodestruction and photoinhibition (Sharma et al. 2015).

The increase in the intensity of CO<sub>2</sub> assimilation and transpiration, as well as CO<sub>2</sub> concentration in the intercellular spaces of the leaves after the application of the biopreparations, especially the preparation containing *Bacillus amyloliquefaciens* IT45, resulted in a greater fruit yield. Similarly, Seema et al. (2018) demonstrated an increase in the rate of assimilation and transpiration in the strawberry cultivar “Chandler” as a result of the application of bacteria of the genus *Bacillus*. According to Samaniego-Gómez et al. (2016), the use of strains of *Bacillus subtilis* and *B. amyloliquefaciens* increased the assimilation rate in *Capsicum chinense*. Porcel et al. (2015) demonstrated an increase in the rate of assimilation, transpiration, and stomatal conductance of rice following the application of *Claroideoglomus etunicatum*. Chen et al. (2017), using mycorrhizal fungi of the genera *Claroideoglomus*, *Funneliformis*, *Diversispora*, and *Rhizophagus* in cucumber cultivation, obtained an increase in the intensity of CO<sub>2</sub> assimilation and stomatal conductance. However, they found no effect of AMF on intercellular CO<sub>2</sub> concentration. The increase in the rate of assimilation demonstrated in our study may have resulted from the beneficial effect of mycorrhizal fungi on Rubisco activity, as indicated by the studies by Porcel et al. (2015) and Chen et al. (2017). Blanke and Cooke (2004) indicate that the reduction in the concentration of CO<sub>2</sub> in the intercellular spaces of assimilatory parenchyma occurs relatively faster in strawberry plants subjected to drought stress than in control or flooded plants. The reduction in CO<sub>2</sub> concentration is associated with the closure of stomata under stress conditions especially because of an increase in the ABA content of cells. In our study, our plants were not subjected to water stress, and there was an increase in the

concentration of CO<sub>2</sub> in the intercellular spaces of assimilatory parenchyma, which may indicate that the applied microorganisms increased the strawberry plant gas exchange.

According to Stefan et al. (2013) and Samaniego-Gómez et al. (2016), the use of preparations containing bacteria of the genus *Bacillus* increased photosynthetic water use efficiency in runner bean and Habanero pepper plants. Many authors point to an increase in  $\omega_w$  under the influence of mycorrhization with species of the genus *Glomus*, especially in plants growing under water deficit conditions (Borde et al. 2012; Zhu et al. 2012). The ambiguous effect of the biopreparations on the value of  $\omega_w$  obtained in our study may have been because our strawberry plants were grown under optimal soil-moisture conditions.

When we compare the AMF-containing preparations, as in the case of leaf chlorophyll concentration and CO<sub>2</sub> assimilation intensity, the most pronounced effect on increasing RWC index was demonstrated for inoculation with *Rhizophagus intraradices*. In the case of the preparation containing *Bacillus amyloliquefaciens*, an increase in relative water content compared with the control was demonstrated only in the second year of the study. These results accord with the results of research conducted by Borkowska (2002), who found an increase in the water content of strawberry leaves of the cultivar “Senga Sengana” as a result of inoculation with *Glomus* sp. Many authors have pointed to the beneficial effect of PGPR on RWC index, which in the case of non-inoculated plants growing under water or salt stress often decreases. This relationship had been noted in strawberry by Karlidag et al. (2011) and Karlidag et al. (2013). According to those authors, and also Zhu et al. (2012), this results from the influence through the synthesis of phytohormones (e.g., IAA) of microorganisms on root longitudinal growth, higher root hydraulic conductivity, and stomatal regulation through hormonal signals. In the case of plants not undergoing abiotic stress as in our study, this effect is not obvious.

The biopreparations we used increased chlorophyll fluorescence parameters such as  $F_M$ —maximum fluorescence ( $F_M$ ) and maximum potential photochemical reaction in PS II ( $F_V/F_M$ ). This is consistent with the results of Gururani et al. (2012) and Samaniego-Gómez et al. (2016) who noted an increase in the  $F_V/F_M$  parameter after the application of bacteria of the genus *Bacillus*. The increase in the maximum potential photochemical reaction efficiency in PS II had also been demonstrated by Zhu et al. (2012) and Chen et al. (2017) after inoculating plants with *Claroideoglossum etunicatum* and *Funneliformis mosseae*. Our results are also supported by the research of Xie et al. (2018), according to whom *Rhizophagus intraradices* and *Bacillus amyloliquefaciens* favorably affected the  $F_V/F_M$  parameter in *Fragaria vesca* growing under optimal substrate moisture conditions. The ratio  $F_V/F_M$  is an indicator defined as a measure of the photochemical activity of the plant photosynthetic apparatus, characterizing the

efficiency with which PSII absorbs light energy. An  $F_V/F_M$  value of around 0.83 is optimal for most plant species (Maxwell and Johnson 2000). Our results, therefore, are evidence that the use of the biopreparations improved the physiological state of the plants, which was reflected in the  $F_V/F_M$  values. In our study, carried out in near-optimal conditions for plant growth, there was no definitive effect of the biopreparations on the PSII vitality index. Gururani et al. (2012) had found an increase in this index after using bacteria of the genus *Bacillus*, but it should be noted that the research by those authors concerned plants growing under conditions of abiotic stresses.

The positive influence, especially in the second year of our study, of the biopreparations on the yield and weight of strawberry fruits finds confirmation in the literature. As reported by many authors (Douds Jr et al. 2008; Cekic and Yilmaz 2011), inoculation of strawberry plants with AMF species such as *Rhizophagus clarus*, *Funneliformis geosporus*, *Claroideoglossum claroideum*, *Claroideoglossum etunicatum*, and *Gigaspora rosea* increased their fruit yield. According to Castellanos-Morales et al. (2010), inoculation with *Rhizophagus intraradices* of strawberry plants growing under conditions of reduced nitrogen fertilization had a positive effect on the weight of fruit. Bona et al. (2015) reported that the use of *Rhizophagus intraradices*, *Rhizophagus aggregatus*, *Septoglossum viscosum*, *Claroideoglossum etunicatum*, and *C. claroideum* with the bacteria *Pseudomonas fluorescens* Pf4 (Pf4) and *Pseudomonas sp.* 5Vm1K positively influenced the productivity of the strawberry cultivar “Selva” grown under conventional conditions as well as under reduced fertilization. A positive effect of the use of preparations containing bacteria (*Bacillus subtilis* GBO3 and *B. amyloliquefaciens* IN937a) on the yield and quality of strawberry fruit was found by Kokalis-Burelle (2003). As reported by Palencia et al. (2015), the effect of mycorrhizal and bacterial preparations on the weight of strawberry fruit depended on many factors, including the variety being cultivated and the time of inoculation. In the opinion of those authors, the largest increase in fruit weight was obtained following the use of bacteria and fungi (*Bacillus velezensis* and *Rhizophagus intraradices*) at the beginning of the strawberry growing cycle. According to Rahman et al. (2018), it is very effective to use bacterial preparations by treating the root system and by foliar application. The cited authors claim that these ways of applying *Bacillus amyloliquefaciens* BChi1 and *Paraburkholderia fungorum* BRRh-4 in the case of the strawberry cultivar “Festival” can increase yields by up to 43% and 48%, respectively. Of a similar opinion are Seema et al. (2018), according to whom the use of *Bacillus licheniformis* CKA 1, *B. subtilis* CB 8 A, *Bacillus sp.* RG1, *Bacillus sp.* S1, and *Bacillus sp.* S2 contributes to increasing the yield and mean weight of strawberry fruit. As reported by Pirlak and Köse (2009), application of *Pseudomonas* BA-8, *Bacillus* OSU-142, and *Bacillus* M-3 to

the roots and leaves of the strawberry cultivar “Selva” considerably increases fruit yield but does not affect the weight of the fruit. Similar results were obtained by Pešaković et al. (2013), according to whom the use of the biopreparations PGPR 1 (*Klebsiella planticola*) and PGPR 2 (*Azotobacter chroococcum*, *A. vinelandi*, *Derxia* sp., *Bacillus megatherium*, *B. licheniformis*, and *B. subtilis*) increases fruit yield without affecting the weight of fruit of the cultivar “Senga Sengana.” The increase in yield and the weight of fruits of the strawberry cultivar tested by us, resulting from the application of AMF and PGPR, were associated with high chlorophyll concentration and CO<sub>2</sub> assimilation intensity as well as with high values of the chlorophyll fluorescence parameters ( $F_M$ ,  $F_V/F_M$ ).

The literature shows that the influence of rhizosphere microorganisms on the total soluble solids content in fruits and their acidity is varied and depends on many factors. Cecatto et al. (2016) found a decrease in TSS and TA values in some strawberry cultivars inoculated with mycorrhizal fungi. However, they did not observe any effect of the fungi on the TSS/TA ratio in the fruit. In a study by Ansari et al. (2018), a marked increase in TSS and TA was observed in mycorrhizal strawberry plants grown under phosphorus deficiency. In the present study, a negative impact of Rhizocell C on the TSS content of strawberry fruit was observed. Palencia et al. (2015) report that the use of *Rhizophagus intraradices* and *Bacillus velezensis* in strawberry cultivation has an ambiguous effect on TSS, TA, and the TSS/TA ratio, and its effect depends on the combination used, the time of inoculation, and the strawberry cultivar. According to Seema et al. (2018), *Bacillus licheniformis* CKA 1, *B. subtilis* CB 8 A, *Bacillus* sp. RG1, *Bacillus* sp. S1, and *Bacillus* sp. S2 applied by soaking roots before planting increase the total soluble solids content in the fruit of the cultivar “Chandler,” decrease the acidity, and increase the TSS/TA ratio. Pešaković et al. (2013) also opine that the use of biopreparations containing *Klebsiella planticola* and the combination of *Azotobacter chroococcum*, *A. vinelandi*, *Derxia* sp., *Bacillus megatherium*, *B. licheniformis*, and *B. subtilis* increases the TSS content in the fruit of the cultivar “Senga Sengana.” However, the biopreparations used by those authors increased the titratable acidity of the fruit and in the first year of their study reduced the TSS/TA ratio. In our study, the TSS/TA ratio in strawberry fruit decreased only in the first year of the study under the influence of inoculation with *Bacillus amyloliquefaciens* IT45. Many authors suggest that changes in sugar content are associated with higher production of assimilates in plants inoculated with AMF and PGPR. This is due to better mineral nutrition of plants and the production of phytohormones (including IAA) by symbiotic microorganisms (Shi et al. 2010; He et al. 2017). Despite the increase in yield caused by more efficient photosynthesis, the increase in assimilation does not have to be clearly positively associated with the concentrations of sugars and acids in the fruit because some of the

assimilates produced by the plant are consumed by symbiotic microorganisms (Porcel et al. 2015).

## Conclusions

The effect of the species of PGPR bacteria and AM fungi used in the experiment on the physiological traits of strawberry plants contributed, especially in the second year of the study, to increasing the yield and mean fruit weight of strawberry fruit. The time-shifted positive effects of mycorrhizal fungi on fruit size and yield indicate the need to inoculate the root system of strawberry plants as early as possible, preferably during the production of seedlings. The results obtained in this study together with those of other authors indicate that the use of microorganisms (AMF and PGPR) does not only reduce the effects of stress but also, by intensifying the photosynthetic activity of plants, increases their yield. Biopreparations can thus be used in horticulture and agriculture to improve the quality and efficiency of crops and at the same time to reduce the fertilization.

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