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EFFECT OF BENEFICIAL MICROORGANISMS ON THE VEGETATIVE GROWTH, YIELDING AND NUTRITIONAL STATUS OF 'ŠAMPION' APPLE TREES

ABSTRACT

The effects of bacterial and fungal inocula on the growth, yielding, and nutritional status of apple trees was evaluated in 3-years experiment (2018 - 2020). The experiment included the following treatments: (i) control (unfertilized soil), (ii) no fertilization + soil application of fungi, (iii) no fertilization + soil application of bacteria. The mixture of beneficial fungi contained two species: Aspergillus niger and Purpureocillium lilacinum. The mixture of beneficial bacteria contained three strains of Bacillus (Bacillus sp., Bacillus amyloliquefaciens and Paenibacillus polymyxa). The application of beneficial microorganisms (especially bacterial strains) to the soil (without additional mineral fertilization) enhanced the growth of the apple trees. In the third year of the study (2020), the trees grown in the plots inoculated with bacteria bloomed the most intensively. Plant nutritional status (expressed as concentrations of elements in leaves) was not affected by the application of the bacterial strains or filamentous fungi. The stronger growth of trees in the plots where the bacteria were used was likely related not so much to the nutritional status of the trees, but to the mitigation of the influence of the negative factors that cause the replant disease.

Keywords: *Malus domestica, fertilization, SPAD, Bacillus, Aspergillus niger, Purpureocillium lilacinum*

INTRODUCTION

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Due to its economic importance, apple cultivation has a significant impact on the development of Polish agriculture. Poland is the number one producer of apples in Europe and the third in the world (GUS, 2017). In 2019, apple orchards accounted for 72.3% of the fruit tree cultivation area, and apples accounted for 78.8% of the fruit harvest in orchards in Poland. The world apple production has increased from 17.05 to 89.33 million tonnes during the period 1961–2016, showing a rapid growth of global apple consumption (FAO, 2018).

In the past years, very intensive fruit-growing systems have been developed in all fruit-growing centres. Such a method of farming requires application of excessive amounts of chemical fertilizers, pesticides and herbicides, which could be harmful to soil microorganisms, human beings, animals and the entire natural environment (Mosa et al., 2016, Garima, 2019).

Due to concerns for food and environmental safety, the use of chemicals and mineral fertilizers must be reduced. In the case of fertilization, the proposed solution is to use natural organic fertilizers; however, their amount might be limited. Another possible solution is to reduce mineral fertilization by increasing its efficiency by enriching the soil with beneficial microorganisms – both fungi and bacteria (Derkowska et al., 2015; Santhos et al., 2018; Sas-Paszt et al., 2019). Mineral fertilizers and beneficial microorganisms can be added to the soil separately or in combination as biofertilizers.

Results of numerous experiments have revealed that biofertilizers enriched with arbuscular mycorrhizal fungi and filamentous fungi produce stimulating effects on the growth and reproduction of several plant species (Sas Paszt et al., 2015, 2019; Santhos et al., 2018). Beneficial microorganisms assist the roots in absorbing minerals from the soil and strengthen plant physiology (Sheraz et al., 2010; Garima, 2019). Microorganisms play a vital role in maintaining long-term soil fertility and sustainability by fixing atmospheric nitrogen, mobilizing fixed macro- and micronutrients, or converting insoluble P in the soil into forms available to plants (Sheraz et al., 2010).

The efficient use of fertilizers in crop production is important to reduce production costs and to minimize possible negative effects on the environment. Prediction of nutrient requirements is necessary for efficient utilization of fertilizers. Improving the efficiency of fertilizer utilization reduces the amount of nutrients that can potentially contaminate soil and water resources. A fertilizer should be applied only after determining that it will be beneficial.

Traditionally, plant nutrient status has been measured using a combination of soil and leaf analyses (Treder et al., 2016). Chemical foliar analysis is considered very practical for determining the nutritional status of a crop and for the diagnosis of nutritional deficiencies (Dezordi et al., 2016). However, although laboratory procedures are accurate, they are cost and time consuming. In recent years, attempts have been made to develop non-destructive methods, based on analyses of leaf blade colour and absorbed or reflected light. These include the use of a leaf colour chart (LCC) (Furuya, 1987) or leaf greenness meters (e.g., SPAD-502, CCM-200) (Zhang et al., 2009; Cerovic et al., 2015). It is also possible to analyse the distribution of colour components of the image of

a single leaf or group of plants and relate them to plant nutritional status (Yuzhu et al., 2011; Wang et al., 2014; Treder et al., 2016). However, application of these methods is limited to the assessment of nitrogen status of leaves only. To apply these methods in agricultural practice, it is necessary to develop mathematical models to describe the relationship between leaf N content and the indices produced by optical meters or distribution of colour components of leaf blade surface. Our research indicates that separate models should be developed and tested for each species or even cultivar to maximize the accuracy of estimating leaf nitrogen content from optical measurements (Treder et al., 2016). Moreover, it is necessary to calculate some threshold values below which nitrogen supply is necessary to avoid deficiency conditions (Gianquinto et al., 2006).

As an alternative, sap analysis might be adopted as a quick tool for determining the nutritional status of a crop. This method is based on the measurement of the concentrations of nutrients in the leaf (petiole) sap (Bityutski et al., 2017). The petioles should be sampled in the field, and the sap can be obtained by using e.g. a hydraulic press, then analyzed immediately to avoid changes in nutrient content (Thompson et al., 2009) using e.g. colorimeters or specific ion electrodes (Hochmuth et al., 2004). Such approach has been used successfully in various crops, such as tomato (Farneselli et al., 2014; Ávila-Juárez and Rodríguez-Ruiz, 2020) and other vegetable species (Ott-Borrelli et al., 2009; Parks et al., 2012).

The aim of the study was to evaluate the effects of using bacterial and fungal inocula on the growth, yielding, and nutritional status of apple trees.

MATERIAL AND METHODS

The experiment was established in the spring of 2018 in the Experimental Orchard of the National Institute of Horticultural Research in Dąbrowice (Central Poland, 51°54'51.1"N 20°06'41.0"E (51.914188, 20.111389), 145 m a.s.l.) and was run for three consecutive years. The subjects of the research were dwarf apple trees of the cultivar 'Šampion' grafted on M. 9 rootstock. They were planted in early April on a podzolic soil underlaid by sandy loam, rated as soil quality class 3b. The study area is characterized by the intensive apple tree cultivation. Despite crop rotation, our earlier study showed occurrence of replant problems in the orchard (Tryngiel-Gać et al., 2015). At planting time, the soil pH was slightly acidic at pH 6.2 (in KCl), and the average humus content of the soil was 1.2%. The levels of minerals in the soil, including macroelements, was as follows: phosphorus (P) – 7.5, potassium (K) – 12.4, magnesium (Mg) – 5.8 mg/100 g, and microelements: boron (B) – 2.4, copper (Cu) – 4.8, iron (Fe) – 862, manganese (Mn) – 75.5, zinc (Zn) – 3.7 mg/1000 g. Soil samples for the analyses were taken from the surface layer (0–20 cm) of herbicide strips, 30 cm from a dripper. The reaction (pH) was measured potentiometrically. The available P and K were determined using a solution of calcium-lactate (at a pH of 3.6) and the available magnesium by means of a solution of 0.0125 M Ca-chloride. Available Fe, Mn, Zn, Cu and B were determined by means of 0.03 M acetic acid. K, Mg, Fe, Mn, Cu, Zn and B were determined by inductively coupled plasma spectroscopy (ICP Perkin-Elmer model Optima 2000 DV, Boston, Massachusetts, USA). Phosphorus was determined using a spectrophotometer (Cintra

916, GBC, Dandenong, Australia) to quantify the molybdenum blue reaction. The details of used soil analysis methods were presented in the paper by Wójcik and Filipczak (2015).

The trees were spaced 4 m between rows and 2 m in the row. The experiment was established in a random block design in four replications. Each experimental combination was represented by 12 trees. The experiment included the following experimental treatments:

- Control (no treatment) – unfertilized soil.
- No fertilization + fungi – beneficial soil fungi in the amount of 5.25 g per plot were applied. The mixture of beneficial soil fungi contained two species: *Aspergillus niger* and *Purpureocillium lilacinum*.
- No fertilization + bacteria – beneficial bacteria in the amount of 3.83 g per plot were applied. The mixture of beneficial bacteria contained three strains of *Bacillus* (*Bacillus* spp., *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa*).

All the treatments were repeated in the second and third years after planting (2019, 2020). In the first year of the experiment, the microorganisms were mixed with the soil in the planting hole. In the second and third years, the microorganisms were mixed with the top soil. The experimental field was fitted with concrete poles reinforced in tree rows, with wires stretched between the poles and bamboo canes tied to the wires and the trees. Drip irrigation was established after planting. The trees were irrigated in a dry season from May to September. In the first year, from spring to August, the soil was kept in clean cultivation with rotating mechanical implements, the alleyways were grassed down and frequently mowed. From the second year, 'Basta' herbicide was applied within tree rows to control weeds. To control pathogens and pests, pesticides were used according to up-to-date recommendations for integrated apple production (Bryk et al., 2018). In the first year after planting, the flower buds that appeared on some trees were removed so that the fruit would not inhibit the growth of the young plants. No flower or fruit thinning was done in the subsequent years. Tree vigour was assessed at the end of the vegetation season by measuring trunk diameter with a caliper and length of annual shoots (using a ruler).

Assessment of leaf mineral nutrition

Samples of leaves from the middle part of shoots were selected at random from each replicate (each consisted of approx. 20 leaves) in early August to measure leaf mineral content. The collected leaves were gently rinsed with 0.01M HCl followed by double-deionized water. Prior to the chemical analyses of the leaves, indirect measurements of chlorophyll content were carried out using a SPAD-502 meter (Konica Minolta Co., Japan).

The concentrations of macroelements in leaf tissue were analyzed by the Chemical Laboratory of the National Institute of Horticultural Research in Skierniewice, Poland. The collected samples of the leaves from each treatment were placed for 48 h in a forced-air dryer at 70°C. After grinding and wet mineralization in acids, the concentrations of macronutrients (P, K, Ca, Mg) were determined using a sequential emission spectrometer with inductively coupled plasma (ICP Perkin-Elmer model Optima 2000 DV, Boston, Massachusetts, USA). The selected elements were determined at their characteristic wavelengths (Boss and Fredeen, 2005). The nitrogen (N) content in plant samples was analyzed using the Kjeldahl method (Latimer, 2012) (the Kjeldahl apparatus Vapodest, Königswinter, Germany). All the nutrients were determined in three repetitions.

Plant sap (leaf petiole) was acquired (after cleaning the leaf surface) by mechanical pressing (using a hydraulic press). The samples were taken from the middle part of long shoots at the beginning of August (the same period as for the SPAD measurements). Sampling was performed in three replicates (each consisted of approx. 20 leaves). The characteristics of the sap (pH, EC, N, K) were determined using ion-selective electrodes (LAQUAtwin, HORIBA, Japan). The electrodes were calibrated using standard solutions provided by the manufacturer.

The results were statistically analyzed using analyses of variance with the Duncan test, $\alpha = 0.05$, using the statistical program Statistica 13.1. Data not significantly different from each other were marked with the same letters. The error bars on the graphs indicate the standard error.

Meteorological data

During the experiment, meteorological data was monitored by the weather station installed in the experimental orchard (iMETOS, Pessl Instruments, Austria; Tab. 1). The average air temperature in the growing season (April–October) during the measurement period was at the level of the 30-year average. The highest average temperatures were recorded in 2018, with the values 1.97°C higher than the multi-year average, while the lowest values were recorded in 2020 (0.52°C lower than the multi-year average). The highest total rainfall was recorded in 2020, 176.8 mm higher than the 30-year average. The lowest rainfall occurred in 2019, 74.2 mm lower than the multi-year average.

Table 1. Average air temperature and total precipitation in Dąbrowice in the 2018–2020 growing seasons in comparison with multi-year averages

	Average air temperature °C							
Year/month	Apr	May	Jun	Jul	Aug	Sep	Oct	Average Apr–Oct
2018	13.2	16.5	18.5	20.3	20.1	15.1	9.6	16.18
2019	9.6	12.5	21.7	18.3	19.8	13.9	10.0	15.11
2020	8.0	11.1	17.7	16.8	18.9	14.0	9.3	13.68

1991–2020*	8.7	13.7	17.2	19.1	18.5	13.9	8.4	14.21

Total precipitation (mm)

Year/month	Apr	May	Jun	Jul	Aug	Sep	Oct	Total Apr–Oct
2018	28.6	51.6	30.0	155.0	55.6	70.6	64.8	456.2
2019	15.6	52.2	36.4	50.8	59.2	82.2	26.4	322.8
2020	9.6	81.6	135.0	63.2	110.4	74.8	99.2	573.8
1991–2020*	40.7	60.3	71.7	75.3	55.6	50.1	43.3	397.0

* 30-year average
Source: own study

RESULTS

The growth of the trees planted in the spring of 2018 was uniform and the diameters of their trunks did not vary significantly. Differences in tree growth vigour were not noticeable until the third year of the study. In the autumn of 2020, marked differences in the average diameters of trees cultivated in the different experimental combinations could be observed. The largest trunk diameters and the largest increases in that parameter in the third year of cultivation were shown by the trees growing in the plots where beneficial bacteria were added to the soil (Fig. 1 and 2). Some positive trends in the impact of the applied fungi and bacteria on tree growth vigour, expressed as annual growth of shoots, had already been observed in the previous year (2019) (Fig. 3). However, not until the year 2020 was it proved that the most vigorously growing shoots were those of the trees in the plots treated with beneficial fungi or bacteria.

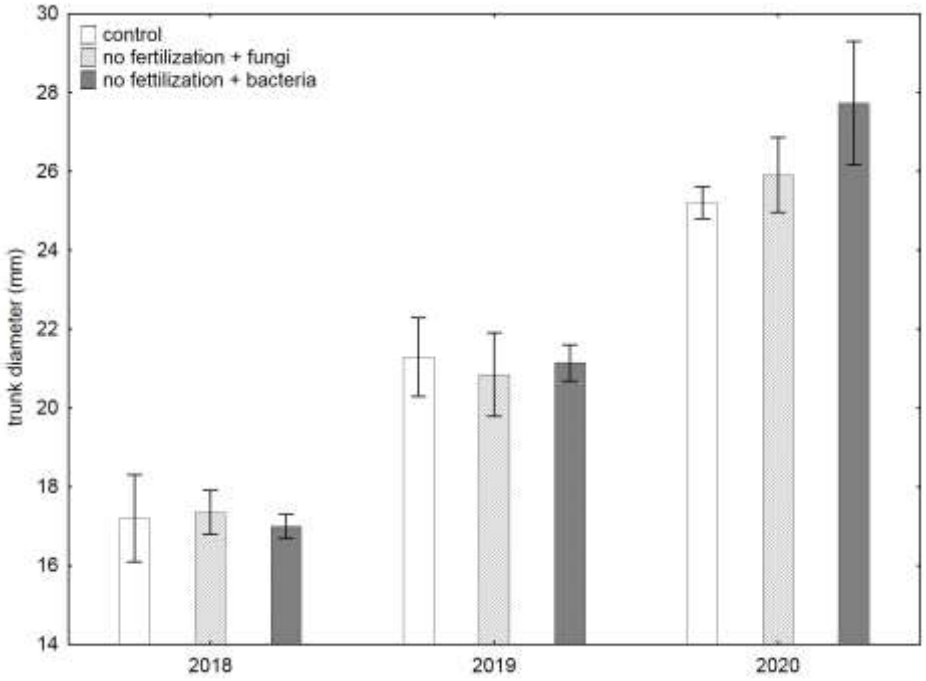

Figure 1. End-of-season tree trunk diameter measured in the autumn of each year

Source: own study

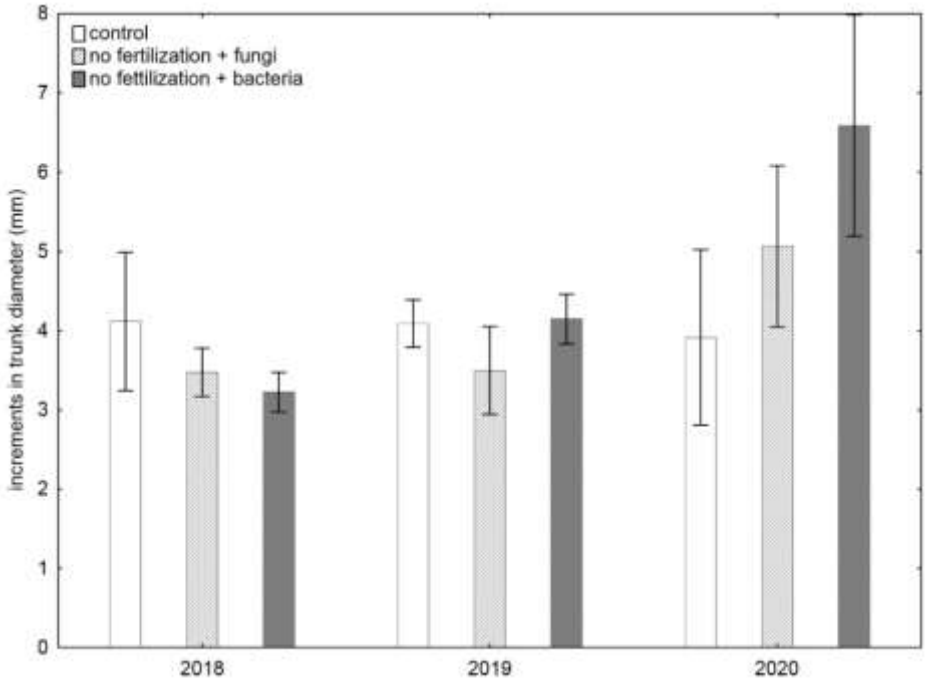

Figure 2. Annual increments in tree trunk diameter

Source: own study

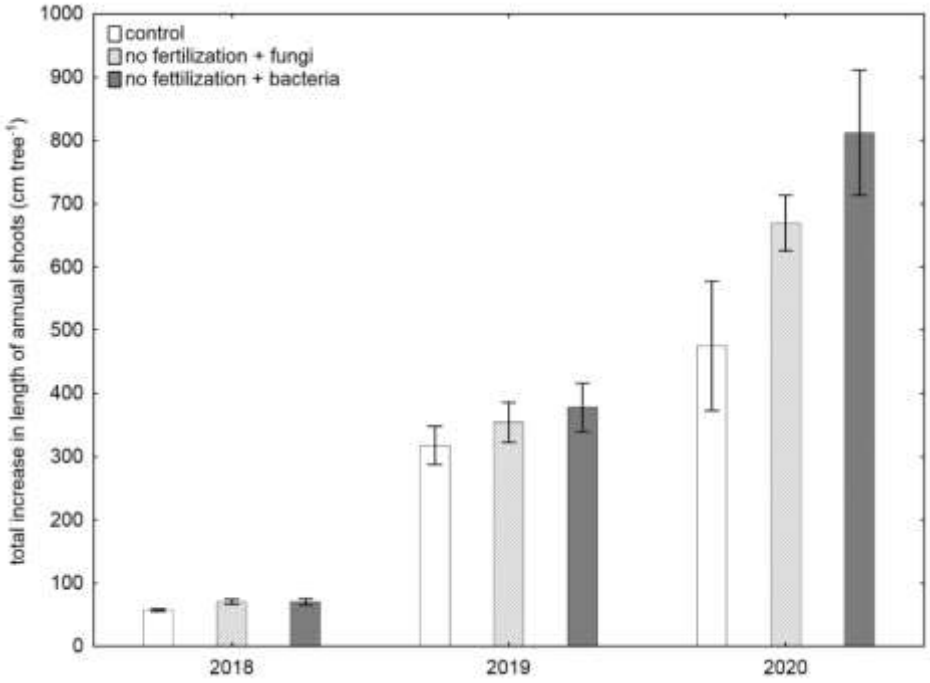

Figure 3. Total increase in length of annual shoots in individual years of the study

Source: own study

The trees bloomed for the first time in the second growing season (2019). The extent of flowering in individual plots was very uniform (Table 2). There were also no differences in the yields that season. In the following year (2020), the trees grown in the plots inoculated with bacteria bloomed the most intensively. Unfortunately, in the spring of that year there were ground frosts, which damaged some of the flowers and caused large variations in the fruiting of individual trees. The consequence of the high variability in fruiting was that the statistical analysis did not show any significant differences between the averages for individual combinations despite the fact that the average yield from the trees grown in the soil enriched with bacteria was much higher than that from the control trees. The different experimental combinations did not significantly affect the quality of the fruit. In both years of fruiting, no significant differences were found between the average values of firmness and refraction for individual combinations. Such differences were observed only in the case of the weight of apples in the first harvest year. Apples harvested from the trees growing in the soil enriched with the fungal preparation had a significantly lower average weight than the control. Such differences were no longer observed in the following year.

Table 2. Flowering and fruiting of apple trees

Parameter	Year	Experimental treatment		
		control	fungi	bacteria
Number of inflorescences (per tree)	2019	23.60 a	26.90 a	26.30 a
	2020	47.25 a	63.75 ab	97.0 b
Yield (kg tree^{-1})	2019	4.33 a	4.42 a	4.42 a
	2020	5.94 a	5.02 a	7.71 a
Average fruit weight (g)	2019	183.5 b	163. 9 a	167.4 ab
	2020	157.75 a	160.75 a	161.75 a
Firmness (kG)	2019	3.75 a	3.74 a	3.59 a
	2020	4.64 a	4.79 a	4.99 a
Total soluble solids content (%)	2019	13.57 a	13.22 a	13.1 a
	2020	15.4 a	14.96 a	14.84 a

Statistical analyses are presented separately for each year. Means marked with the same letters do not differ significantly at $\alpha = 0.05$

Source: own study

Statistical analysis of the results of measurements of green leaf colour intensity (SPAD index) showed differences between the means for individual combinations only in the third year of cultivation. The control trees had the lowest value of the SPAD index (Table 3). However, chemical analyses did not show any differences between the concentrations of nitrogen, phosphorus, calcium, and magnesium in the leaves (Table 3). In the case of potassium, such differences were proved only in the first year of cultivation, in which the control trees had the lowest potassium content.

Table 3. SPAD index and the concentration of macronutrients in apple leaves

Parameter	Year	Experimental treatment		
		control	fungi	bacteria
SPAD	2018	48.9 a	50.9 a	51.1 a
	2019	43.2 a	44.7 a	45.4 a
	2020	39.1 a	42.2 b	43.5 b
N (% dw)	2018	2.40 a	2.46 a	2.50 a
	2019	2.59 a	2.62 a	2.69 a
	2020	2.34 a	2.30 a	2.33 a
P (% dw)	2018	0.14 a	0.15 a	0.15 a
	2019	0.20 a	0.16 a	0.17 a
	2020	0.25 a	0.26 a	0.19 a
K (% dw)	2018	1.95 a	2.14 b	2.16 b
	2019	1.38 a	1.37 a	1.35 a
	2020	1.54 a	1.54 a	1.32 a
Ca (% dw)	2018	0.78 a	0.89 a	0.93 a
	2019	1.62 a	1.34 a	1.62 a
	2020	1.59 a	1.73 a	1.66 a
Mg (% dw)	2018	0.27 a	0.29 a	0.30 a
	2019	0.21 a	0.17 a	0.16 a
	2020	0.22 a	0.21 a	0.21 a

Statistical analyses are presented separately for each year. Means marked with the same letters do not differ significantly at $\alpha = 0.05$

Source: own study

The results for the chemical parameters of the sap collected from apple leaves varied widely in the different years of the study (Table 4). In the case of sap pH, the lowest values in the first year after planting were found in the control trees. But the following year, significantly the lowest pH was shown by the sap of the trees grown in the plots inoculated with beneficial bacteria. In the third year of cultivation, no significant differences were found between the pH values of the cellular sap in the leaves. In the case of electrical conductivity of the sap squeezed from the leaves, significantly the lowest values in the first year of the study were found in the control trees, and in the second and third years in the trees grown in the soil inoculated with fungi. In the first year, the sap of the control leaves had the lowest concentration of nitrate nitrogen. In the following year, the analyses showed them to have the highest $N\text{-}NO_3$ content in the sap. In the third year, no significant differences were found in the nitrate nitrogen content in the sap collected from the leaves. The potassium content in the leaf sap also varied greatly between the years of the study. In the first year, the leaves of the trees in the plots where beneficial bacteria had been inoculated into the soil had significantly the highest potassium content. In the following year, the sap of these leaves contained the lowest concentration of potassium. In the third year, significantly the lowest concentration of potassium was in the leaves of the trees growing in the soil inoculated with beneficial fungi.

Table 4. Analysis of the sap acquired from apple leaves

Parameter	Year	Experimental treatment		
		control	fungi	bacteria
pH	2018	5.96 a	6.19 b	6.26 c
	2019	5.26 b	5.41 c	5.16 a
	2020	6.21 a	5.99 a	6.28 a
EC (mS cm^{-1})	2018	2.00 a	3.10 b	4.60 c
	2019	4.78 b	3.86 a	4.52 b
	2020	2.3 c	1.68 a	2.1 b
$N\text{-}NO_3$ (mg l^{-1})	2018	240.0 a	270.0 b	427.5 b
	2019	204.0 c	57.0 a	171.8 b
	2020	154.13 a	181.25 a	154.25 a
K (mg l^{-1})	2018	466.7 a	506.7 a	723. 0 b
	2019	853.3 ab	893.3 b	580.0 a
	2020	370.0 b	225.0 a	330.0 b

Statistical analyses are presented separately for each year. Means marked with the same letters do not differ significantly at $\alpha = 0.05$

Source: own study

DISCUSSION

In the initial period of cultivation, the microorganisms introduced into the soil did not affect the growth vigour of apple trees. The trees were grown in a soil relatively rich in minerals, therefore, even in the absence of mineral fertilization, the positive effects of the applied fungi or bacteria were delayed and became apparent only after the readily available minerals in the soil had been depleted. The nutritional status of plants during nursery production is not without significance for the root-taking and growth of trees in the first year after planting. In the spring, in the first weeks of cultivation, a large proportion of the necessary minerals come from the so-called reutilization of compounds contained in the wood of plant trunks and branches (Cheng et al., 2004).

This experiment revealed that the beneficial microorganisms applied to the soil without any mineral fertilization enhanced not only the growth of trees but also their yielding (not statistically confirmed). In the study presented here, particularly large increases in the length of annual shoots and in the diameter of tree trunks were found when the soil was treated with bacteria. However, no significant differences were found in tree nutritional status expressed by the concentration of macroelements in the analyzed leaves. Regardless of the experimental combination, the concentration of macronutrients in the leaves was within the optimal or high range. The influence of the applied bacteria on tree growth was likely not related to the nutritional status of the trees, but to the overcoming of the replant disease, which significantly reduces plant growth and yielding. As reported in the literature (Sobiczewski et al., 2009; Tryngiel-Gać et al., 2015), intensive fruit-growing and nursery production in Poland is the reason why the importance of the replant disease is constantly increasing, especially in areas where it is not possible to plant trees on the so-called 'virgin' soil where trees of the same species have not been grown before. We conducted our study in an experimental orchard where apple trees have been grown for at least 60 years. Despite crop rotation, ours was yet another apple-tree planting in this long period of time. Therefore, we are justified in claiming that the stronger growth of trees in the plots where the soil had been inoculated with bacteria was caused by their action of mitigating the effects of the replant disease.

Bacillus species are among the most investigated biocontrol agents, i.e. biopesticides, which contribute to the suppression of plant pathogens by antagonism and/or competition (Mnif and Ghribi, 2015). Inhibition of pathogen growth by *Bacillus* spp. entails the involvement of mechanisms such as competition for nutrients and space, production of antibiotics, hydrolytic enzymes, and siderophores, and/or induction of systemic resistance (Beneduzi et al., 2012). *Bacillus* spp. can also act as biofertilizers or biostimulants, either by facilitating the uptake of certain nutrients from the environment (nitrogen fixation, phosphate solubilization), or by providing the plant with a compound (biosynthesis of plant hormones) (Borriss, 2011). Hence, *Bacillus* spp. represent an alternative to plant growth enhancement agrochemicals, i.e., synthetic pesticides and fertilizers. The beneficial effects of *Bacillus* spp. on plant growth and yielding have been demonstrated also in several agricultural crops including wheat, maize, soybean, sunflower, common bean, tomato, pepper, potato, cucumber, and others (Aloo et al., 2018).

The nutritional status of the apple trees was also monitored by means of leaf colour assessment (SPAD index) and quick field tests for the concentrations of minerals in the sap squeezed from the leaves of the trees. The green colour of the leaves depends on their chlorophyll content. A lighter colour of the leaves of the control trees indicates a lower chlorophyll content and thus a lower potential efficiency of the photosynthetic apparatus. Despite the differences in coloration, no significant differences were found between the laboratory-determined nitrogen concentrations in the leaves. In the case of apple trees, such a relationship has been demonstrated by Treder and Cieśliński (2003) and Treder et al. (2016). Generally, optical methods are recommended for quick and non-invasive assessment of apple tree nutrition with nitrogen. However, readings of optical meters may suffer from interference by environmental conditions and measurement conditions (Treder and Cieśliński, 2003; Naus et al., 2010). The variability observed between different species or even cultivars has been explained by the differences in leaf structure causing differences in light reflection, or by the scattering effect (Richardson et al., 2002). Moreover, if the estimation of the nitrogen content is needed throughout the entire growing period, calibration should be performed several times during the growing season. This is confirmed by the results of Neilsen et al. (1995) and Treder and Cieśliński (2003) showing that SPAD readings depend on the cultivar and time of sampling, and therefore must be standardized for these parameters. The fact that a single prediction equation cannot be applied across a wide range of species/cultivars to determine chlorophyll content is considered a major limitation of indirect methods.

As a quick alternative to soil and plant standard analyses, petiole sap tests have been proposed (Ávila-Juárez and Rodríguez-Ruiz, 2020). Such tests can be carried out directly in the field. This study has confirmed that equipment such as ion-selective electrodes (e.g. LAQUAtwin system) can rapidly and economically measure EC, pH, N and K in petiole extract. However, due to the very high variability of the obtained measurement values (between individual combinations and years of study), the quick tests for the mineral content in the leaves of the apple trees proved to be very difficult to interpret. The results of a plant sap analysis can be influenced by a range of factors. In particular, the plant's development stage (sampling date) should be taken into account while interpreting (comparing) the results during diagnosis of nutrient requirements. Due to limited literature (lack of threshold values for the nutrients sampled using this method) it might also be difficult to interpret the results of sap analysis of crops – to determine whether a nutrient is present in excess or is deficient and thus negatively affecting yield (Ávila-Juárez and Rodríguez-Ruiz, 2020). Further research is needed to develop elements of the methodology for using this type of analysis for assessing the nutritional status of crop plants. This applies in particular to the analysis of the dynamics of the variability in the mineral content of the sap throughout the entire growing season. Critical values (limits) should also be established depending on the nutrition level of the orchard.

CONCLUSIONS

The application of beneficial microorganisms (especially bacterial strains) to the soil enhanced the growth of the apple trees. Also, some differences in the yields were observed. In the 2020, the trees grown in the plots inoculated with bacteria bloomed the most intensively. Unfortunately, spring frost damaged some of the flowers what caused the high variability in fruiting (the statistical analysis did not show significant differences between the combinations). The concentration of macronutrients in the tree leaves was found to be within the optimal range, and was not affected by the application of the bacterial strains or filamentous fungi (except for potassium in the first year of the study). The stronger growth of trees in the plots where the bacteria were used was likely related not to the nutritional status of the trees, but to the mitigation of the influence of the negative factors that cause the replant disease.

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