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Effect of mycorrhization treatment on the yield and quality of highbush blueberries in conditions of replantation

Zofia Zydlik[1], Piotr Zydlik[1*], Dariusz Kayzer[2] and Robert Wieczorek[1]

[1]*Poznań University of Life Sciences, Faculty of Horticulture and Landscape Architecture, 60-594 Poznań, Poland*
[2]*Poznań University of Life Sciences, Faculty of Agronomy and Bioengineering, 60-637 Poznan, Poland*
[*]*Corresponding author:* piotr.zydlik@up.poznan.pl

Abstract

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In the years 2010 – 2016, the effect of mycorrhization treatment in the cultivation of the Highbush blueberry (*Vaccinium corymbosum* L. „Bluecrop") on the yield and fruit quality was examined. The bushes were growing in replanted soil (after 30 years of blueberry cultivation) and in so-called virgin soil – not previously used for orchards. The mycorrhizal vaccine was used one-time during the growing season at the point when the plants were planted permanently. It was shown that the mycorrhizal treatment had a positive effect on the yield of the Highbush blueberry bushes and on improving the quality of the fruit, in particular under stress conditions for the plants (replanted soil). The yield and quality of the blueberry fruit growing in the soil after the previous 30-year-old cultivation of this species has usually not deteriorated.

Keywords: arbuscular fungi; replant disease; *Vaccinium corymbosum;* yielding; fruit quality

Introduction

In areas with intensive cultivation of fruit, so-called soil fatigue is becoming more common. It is the result of many years of growing and planting of the same species. Fatigue soil has worse production parameters, i.e. reduced activity of soil microorganisms, inferior structure and a lower nutrient content (Zydlik, 2010). The phenomenon known as soil fatigue leads to the formation of replantative disease. Among the most frequently mentioned perpetrators are soil microorganisms: fungi of genera *Cylindrocarpon* and *Rhizoctonia, Oomycetes Phytophthora* and *Pythium* (Kelderer et al., 2012), nematodes *Pratylenchus penetrans* (Mazzola & Manici, 2012), bacteria belonging to the *Actinomycetes,* and to the genus *Bacillus* and *Pseudomonas* (Mazzola & Manici, 2012). Plant species susceptible to replantative disease are poorly nourished (Zydlik, 2013), and their growth and yield are inferior to plants growing in optimal conditions (Pacholak, 2009; Manici et al., 2013). Studies on the number of nematodes inhabiting the zone of the blueberry root system conducted in recent years show that the *Paratrichodorus renifer* and *Mesocriconema ornatum* species are the most important, which are considered to be the main causes of blueberry replant disease. A much smaller number of crop species exhibited *Pratylenchus penetrans*, mentioned as a frequent culprit of the disease of replantation species of the family *Rosacea* (Mazzola & Manici, 2012).

There is a growing interest in the cultivation of the Highbush blueberry around the world. The global area of cultivation of this species has increased by 53% in the last 10 years (Brazelton, 2015; FAO). Due to the intensification of production and frequent planting changes, it is a species potentially exposed to the effects of replantative disease.

The elimination of the consequences of a replantative disease, depending on its severity, may last from a few to a dozen or so years. There are various ways to mitigate the effects of this phenomenon. One of them is the activation of the microbial environment of the soil replanted by means of a suitably selected mycorrhizal vaccine. It increases the phenomenon of mycorrhiza – i.e. the beneficial interaction between the majority of higher plants and mycorrhizal fungi. A number of previous works point out that mycorrhizal fungi increase plant resistance to stress caused by biotic and abiotic factors (Porras-Soriano et al., 2009), facilitate the transport of nutrients from the soil (Asrar et al., 2012; Gamal et al., 2014; Chatzistathis et al., 2013), and increase the yield of some plant species (Hu et al., 2014).

The aim of the study was to determine the effect of mycorrhizal vaccine on the yield and quality of Highbush blueberry fruit (*Vaccinium corymbosum* L. „Bluecrop") growing on replanted soil.

Material and Methods

The study was carried out in the years 2010 – 2016 at the Agricultural-Orchard Holding Farm in Przybroda (52°31' north latitude and16°38' east latitude). The experiment used potted blueberry cuttings of the highbush variety Bluecrop. The plants were planted at a spacing of 2.5 x 1.5 m in two soil types: replanted – on which blueberries were grown for 30 years, and so-called virgin soil – not used in an orchard. The replanted soil used in the experiment had worse biological parameters than the new: a lower activity of soil enzymes (proteases and dehydrogenases), smaller number of proteolytic bacteria and significantly lower respiratory activity (Zydlik et al., 2016).

Immediately prior to being planted permanently, the root balls of the plants were soaked once in a mycorrhizal suspension (*Vaccinium myrtillus* L.*, V. vitis-idaea* L., and *Arctostaphylos uva-ursi* L.). The vaccine was prepared in the Mycoflor laboratory in Puławy, based on fungi isolated from roots of *Vaccinium myrtillus* L.*, V. vitis-idaea* L., and *Arctostaphylos uva-ursi* L. that originated from Polish ecosystems. Distilled water was added to the vaccine containing live mycelium (500 ml), and then mixed for 15 seconds. Next 1000 ml of suspension was obtained, in which the blueberry seedlings were inoculated. The methodology for its preparation and application (concentration and method of application) was carried out in accordance with the recommendations of its manufacturer.

The soil used in the experiment was strong loamy sand with a 20% content of the floatable fraction and a reaction (in 1 M KCl) in the pH range 3.8 – 5.4. The chemical composition of the soil was as follows (mg 100 g^{-1} d. m.): replantation: P 5.66; K 9.10; Mg 3.10; pH 3.98; virgin: P 5.77; K 10.90; Mg 3.78; pH 5.45. The course of the average monthly temperatures and the amount of precipitation during the growing season during the research period are shown in Table 1.

The following combinations were tested: 1 – replanted soil; 2 – replanted soil using the mycorrhizal vaccine; 3 – virgin soil; 4 – virgin soil with the mycorrhizal vaccine. The experiment was carried out in 4 replications, where one replica was 20 bushes (4 combinations x 20 bushes x 4 replications = 320 shrubs).

Table 1. The course of climatic conditions in the years 2010-2016

Years	Months						Sum
	IV	V	VI	VII	VIII	IX	
Precipitation (mm)							
2010	19.0	110.1	13.0	111.4	124.1	72.4	450.0
2011	9.2	32.8	56.2	182.4	32.4	27.8	340.8
2012	9.8	57.0	127.8	121.8	39.0	24.6	380.0
2013	85.2	99.4	46.0	37.6	38.2	81.0	387.4
2014	41.8	65.2	33.0	48.4	93.6	36.6	318.6
2015	18.0	28.8	58.6	78.6	15.2	31.2	230.4
2016	35.4	16.4	121.2	120.8	37.4	18.6	349.8
1978-2007	30.3	45.5	64.5	77.9	59.6	45.2	323.0
Air temperature (°C)							Mean
2010	8.7	11.6	17.0	22.0	18.8	12.7	15.1
2011	12.6	16.5	21.3	18.6	20.6	16.2	17.6
2012	9.8	15.4	16.0	19.1	18.3	14.2	15.5
2013	9.5	14.4	17.4	19.7	18.7	12.6	15.4
2014	10.6	13.3	16.1	21.6	17.4	15.2	15.7
2015	8.5	12.9	15.7	19.1	22.0	14.2	15.4
2016	8.9	15.2	18.2	18.9	17.5	16.3	15.8
1978-2007	8.6	14.3	17.1	19.1	18.6	13.9	15.3

During the experiment, analyses and measurements were made such as: the amount of yield (kg shrub^{-1}), fruit size (height, width), fruit firmness and their extract content, leaf area.

In each year of research, the fruit harvest and its qualitative analysis were carried out five times during the growing season: from the first half of July to the second half of August (320 shrubs x 5 dates x 8 years = 12 800 measurements). In the assessment of yield, the unit yield from a bush (kg shrub^{-1}) was taken into account. Firmness was tested on 50 randomly chosen fruits from each replication using a Fruit Pressure Tester 327. The measurement value is expressed in kG. The extract content was evaluated with the aid of an Abbe refractometer. The measurement value was expressed in % °Brix.

The leaf surface was measured annually. After the second fruit harvest, 5 leaves were collected from each bush under controlled conditions (100 leaves from each combination). The collected material was scanned, and then the leaf surface was calculated using the DigeShop program. The results are given in cm^2.

The results of the Highbush blueberry yield were analysed using profile analysis and canonical variate analysis (Kayzer et al., 2015). Canonical variate analysis is a method which enables a graphical presentation of the multidimensional results. This method consists in the transformation of the original set of variables into a set of new variables, which carry similar information, but are distributed in a multivariate Euclidean space (Lejeune & Caliński, 2000; Kayzer et al., 2009). In this case, canonical variate analysis is based on the matrix include the differences between mean values of annual yield for the considered stand types and the general annual means. Due to correlations between parameters describing the quality of the fruit is sufficient to use tools of multivariate analysis for determination the differences between analysed soil types (Budka et al., 2011).

Results and Discussion

The mycorrhization applied in the experiment influenced the yield of the Highbush blueberry in different ways. Table 2 contains the values representing the difference between the average yields in combinations in particular years and the average annual yield. In 2010, the yield of the bushes growing in the replanted soil after a single application of the mycorrhizal vaccine was significantly higher compared to the yields obtained in the remaining combinations. A year later, the mycorrhized Highbush blueberries from the same site yielded the least (Table 2). One of the reasons could be their high yield in the previous year, as a result of which the phenomenon of alternating fruiting could occur.

The yield of the Highbush blueberries varied depending on the type of soil on which the bushes grew. During the seven-year research period, in five years (2012 – 2016) the yield of the bushes growing in the replanted soil was significantly higher than for the virgin one. Average yield differences fluctuated from 1.1 kg bush^{-1} in 2014 to 3.1 kg bush^{-1} in 2016 (Table 2). Only in 2011 was there a reverse tendency. Then, the yield from the blueberry bushes growing in the virgin soil was almost 2.5 times higher than on the replanted stand.

The differences in the yield of bushes growing at various sites throughout the research period are graphically represented in the first two canonical variables (Figure 1). Points characterising the yield of bushes growing under replantation conditions are located on one side of the axis, and growing in the virgin soil – on the opposite side. It results unambiguously from the high variability of the yield of the bushes at the surveyed stands in particular years of the duration of the experiment.

Some literature sources indicate a positive effect of mycorrhizal fungi on the growth, development and yielding of such fruit species as apple, cherry (Grzyb et al., 2015) and pear (Świerczyński et al., 2015). The results of the use of the mycorrhizal vaccine in the cultivation of highbush blueberry on the replanted stand and on the virgin are depicted in Figures 2 and 3. Their analysis indicates a greater variation in the yield of blueberry bushes growing in the replanted soil than in the virgin one. While on the virgin stand the differences in the yield of bushes treated with the mycorrhizal vaccine and bushes lacking this treatment ranged from 0.04 to

Table 2. The difference between the Highbush blueberry yield (kg bush^{-1}) in combinations and the average annual yield

Sites	Myccorhization	Years						
		2010	2011	2012	2013	2014	2015	2016
Replanted soil	–	-0.093[1]	-0.074*	0.729**	0.679**	0.737**	0.471*	2.424**
	+	0.243**	-0.151	0.997**	0.385**	0.393**	1.835**	0.682**
Virgin soli	–	-0.038	0.043**	-0.842**	-0.601**	-0.539**	-0.836**	-1.464**
	+	-0.111	0.182**	-0.884**	-0.464**	-0.591**	-1.469**	-1.641**
Mean	0.381	0.271	1.614	1.542	1.535	2.485	3.322	

[1] – The difference between values in particular combinations and the average annual value for the experiment; * significance level α = 0.05; ** significance level α = 0.01

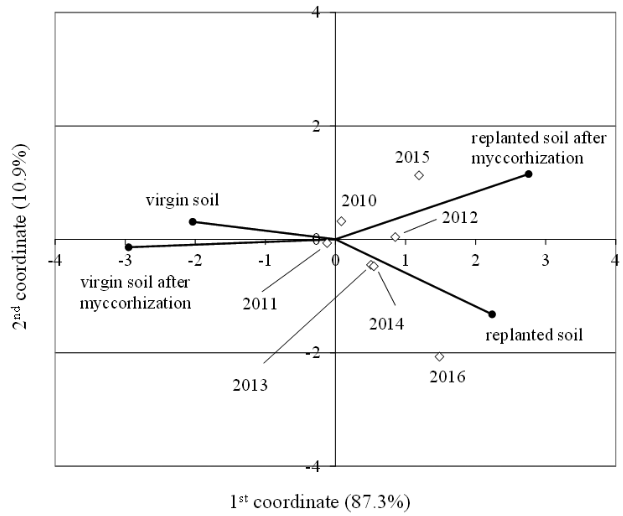

Fig. 1. Positions of analyzed soil types differing in the American blueberry yield in the space of the first two canonical variates and spacing of the investigation years in the dual space of canonical variates (values of dual canonical coordinates multiplied by 2.5)

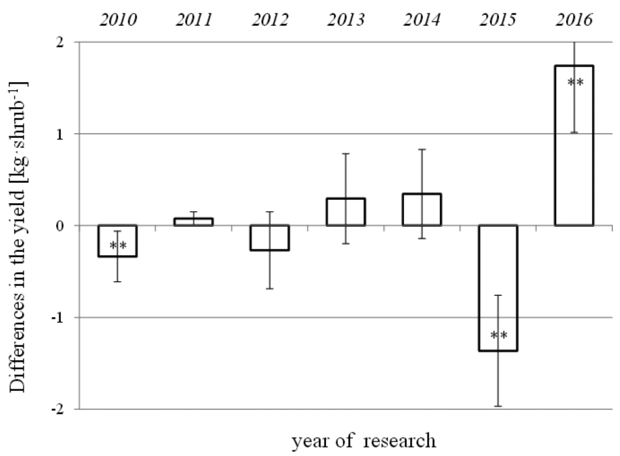

Fig. 2. Differences in the yield of the American blueberry bushes on the replanted stand after mycorrhization and without this treatment in the years 2010 – 2016 (±confidence intervals for difference between two means)

0.63 kg shrub^{-1}, on the replanted stand they varied from 0.07 to 1.7 kg.

On the basis of the analysis of blueberry bush yield differences on the replanted site after mycorrhization and without this treatment, it cannot be unequivocally stated that the vaccine significantly influenced the yield increase. Such an increase occurred once – in 2016, on the replanted site. It should be noted that on the same type of substrate, in the years 2011 – 2014 an increase in the yield of the blueberry bushes after the mycorrhizal vaccine was also recorded. However, this increase was small (Figure 2). In 2012 and 2015, the yielding of the mycorrhized blueberry bushes was even lower than that of the bushes without this treatment. The drop in yield in 2015 may have resulted from the unfavourable climatic conditions during this period; it was an exceptionally dry year. Precipitation during the growing season was more than 100 mm lower compared to the average rainfall over several years (1978 – 2007) (Table 1). In addition, in 2015 the average temperatures in May and June, at the time of establishment and intensive fruit growth, were higher on average by 1.4°C than the corresponding values over several years.

The differences in the yield of mycorrhized and non-mycorrhized blueberry bushes growing in the virgin soil were small (Figure 3). In 2015 year the yielding of shrubs growing in the virgin soil after a mycorrhization treatment was higher by 0.63 kg bush^{-1} compared to the yield without this treatment. Considering the particularly unfavourable meteorological conditions for the growth and yield of the blueberry bushes in this year, one can infer a high effectiveness of the mycorrhizal vaccine used under stressful conditions for the plants. A number of research authors confirm the thesis about the increase in the effectiveness of mycorrhization performed under biotic stress (lower enzyme activity) or abiotic (nutrient deficiency, drought, salinity) (Shi et al., 2002; Swarty et al., 2004; Porras-Soriano et al., 2009; Wu et al., 2013).

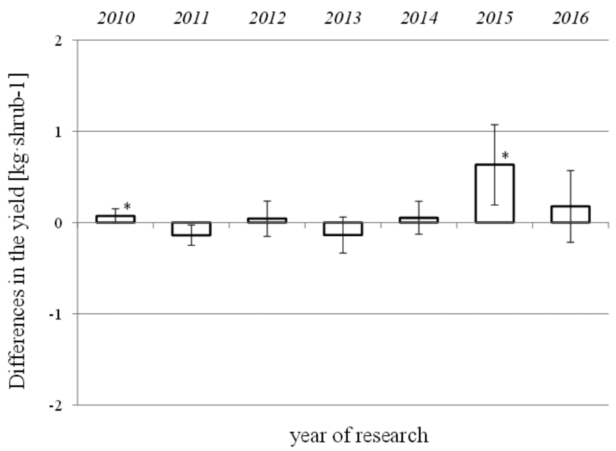

Fig. 3. Differences in the yield of the American blueberry bushes on the virgin stand after mycorrhization and without this treatment in the years 2010 – 2016 (±confidence intervals for difference between two means)

The increase in the Highbush blueberry yield growing in the replanted soil may result from the positive effect of the applied mycorrhizal vaccine on improving the improvement of soil production properties, in particular its biological activity. In previous experiments of the authors' research, the mycorrhization procedure performed in the conditions of replantative disease increased the soil respiratory activity and increased the activity of some soil enzymes (Zydlik et al., 2016), improving the nutrition of the blueberries with some macronutrients (Zydlik et al., 2019). Mycorrhized plants generally have a better developed root system (Aka-Kacar et al., 2010) that easily absorbs nutrients from the soil (Asrar et al., 2012), and their transport to above-ground parts is facilitated (Gamal et al., 2014).

In summary, the type of stand on which the bushes grew had a greater impact on the yield of the Highbush blueberry than the mycorrhization treatment. The analysis of the results of the discussed experiment does not allow for concluding on the clear positive effect of the mycorrhizal vaccine on the increase in the blueberry yield. In the replanted soil, the crop was more diversified than in the virgin one. This may suggest that performing mycorrhization treatment will be the most justified in the case of predicting the occurrence of stress conditions for plants.

The assessment of the impact of the mycorrhizal vaccine on the quality of the blueberry fruit is presented in Table 3 and Figure 4. Among the analyzed parameters, fruit firmness was the most varied. As can be seen from Figure 4, the point that characterises this feature is located at a considerable distance from the points characterising other qualitative features. Moreover, its distance from the beginning of the canonical variable coordinate system, associated with the average values of the qualitative parameters, is greater than the distance of the points characterising other features. The points characterising the width and height of fruit and the content of the extract in them are located in their immediate vicinity. This means that the tendencies for these traits are similar to each other, and at the same time dissimilar to the trends associated with fruit firmness (Figure 4).

The factors affecting fruit firmness were both the previous manner of soil use and the mycorrhization procedure. From the bushes growing in the replanted soil, after their

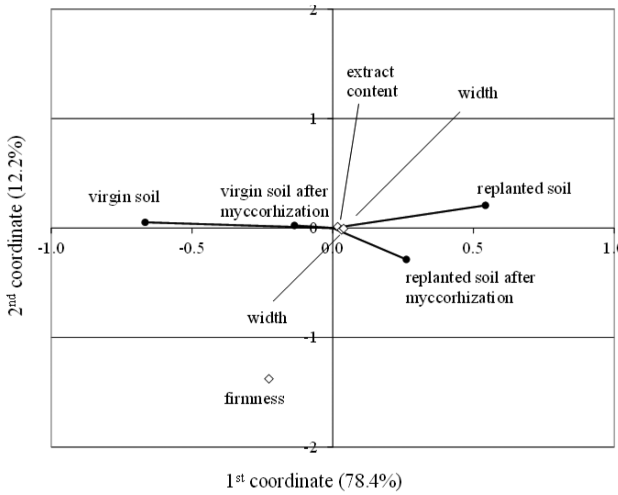

Fig. 4. Positions of analysed soil types differing in the quality of the fruit in the space of the first two canonical variates and spacing of the investigation parameters in the dual space of canonical variates (values of dual canonical coordinates multiplied by 0.05)

mycorrhization, fruit with higher firmness (14.7 kG) were harvested compared to the fruit harvested in this soil without such treatment (Table 3). With regard to the bushes growing in the virgin soil, the mycorrhizal treatment did not improve the firmness of the blueberry fruit.

Another qualitative feature of the fruit showing variation was the extract content. The factor determining this feature was the previous manner of soil use. The extract content in the fruit from bushes growing on the replanted soil was significantly higher than on the virgin one (Table 3). Irrespective of the stand analysed, the one-off use of the mycorrhizal vaccine did not significantly affect the increase in the extract content in the fruit.

Another factor affecting the content of the extract in the fruit could be the temperature during the growing season. During the whole research period it was close to the average value over several years (Table 1). In addition, it should be noted that the size of the fruit (height, width) can affect the content of the extract in them. The fruit collected from

Table 3. Effect of mycorrhization treatment on the quality of the Highbush blueberry fruit in the years 2010-2016

Sites	Myccorhization	Firmness	Extract content	Height	Width
Replanted soil	–	-7.379**[1]	0.287**	0.260**	0.362**
	+	7.307**	0.066	0.252**	0.199**
Virgin soli	–	3.079*	-0.147**	-0.478**	-0.605**
	+	-3.007*	-0.206**	-0.034	0.044

[1] – Remark as to Table 2

Table 4. The quality of the Highbush blueberry fruit depending on the date of harvest in the years 2010-2016

Collections	Firmness	Extract content	Height	Width
1st	12.70**[1]	0.171**	0.732**	1.807**
2nd	-4.814**	-0.041	0.409**	1.037**
3rd	-3.082	-0.182**	-0.149**	-0.621**
4th	-2.011	0.143**	-0.193**	-0.732**
5th	-2.796	-0.091*	-0.800**	-1.491**
Mean	206.7	12.71	10.53	14.38

[1] – Remark as to Table 2

bushes growing in the replanted soil were larger compared to fruit growing in the virgin one (Table 3).

There was a difference in the quality of blueberry fruit at different harvest dates. As can be seen from Table 4 and Figure 5, the fruit harvested in the first period was of the highest quality. They were the largest, and their firmness and extract content was the highest. The quality of the fruit harvested in subsequent dates usually decreased. The only exception in this respect were the height and width of fruit harvested in the second harvest period (Table 4). It could be related to a relatively large amount of rainfall in July – during the second term for harvesting fruit (Table 1). In four out of seven years of the research, precipitation during this period significantly exceeded the average value over several years. The smallest diversity of qualitative parameters occupies a nice place in the third and fourth term of fruit harvest. The fruits collected in the last period (end of August) were the smallest (Figure 5).

A positive effect of a one-off mycorrhization treatment on the surface of the highbush blueberry leaves was found. With few exceptions, the mycorrhized plants, growing both in the replanted soil and in the virgin, had a larger leaf area than those without this treatment (Table 5). On the virgin stand, more effects of using the mycorrhizal vaccine were reported. Differences in the surface of the leaves of mycorrhized and non-mycorrhized blueberries growing at this site ranged from 25 to 45%, whereas on the replanted soil they did not exceed a dozen or so percent (Table 5). The most obvious reason for the increase in the area of blueberry leaves after mycorrhizal treatment is their better nutrition. The positive effect of mycorrhization treatment on the strength of

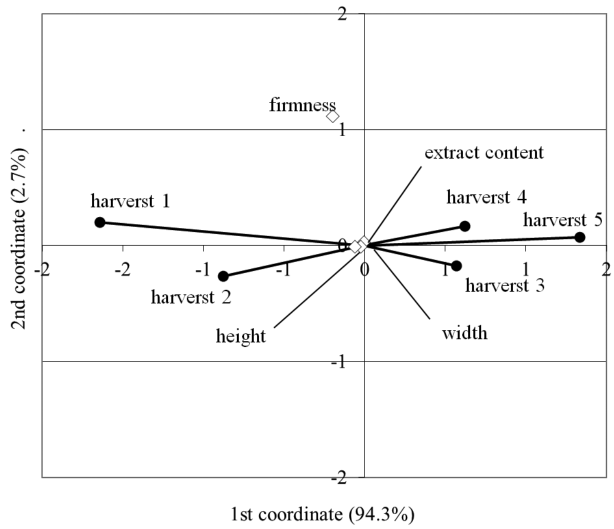

Fig. 5. Positions of analysed soil types differing in the fruit harverst dates in the space of the first two canonical variates and spacing of the investigation parameters in the dual space of canonical variates

plant growth was also concluded in studies of Aka-Kacar et al. (2010) and Gamal et al. (2014).

In the discussed experiment, both the yield of the Highbush blueberry and the quality of its fruit harvested from the bushes growing on the site after previous long-term cultivation of bilberries (replanted soil) have not deteriorated. This may indicate that the Highbush blueberry is a species that can cope relatively well in conditions of replantation. For proper growth and development, this species requires acidic

Table 5. The surface of the highbush blueberry leaves (cm^2) in years 2010-2016

Sites	Mycorrhyzation	Years						
		2010	2011	2012	2013	2014	2015	2016
Replanted soil	–	14.43 c[1]	11.61 b	15.24 c	15.28 c	14.89 c	10.18 b	9.82 b
	+	13.71 c	12.92 c	15.02 c	16.42 d	17.53 d	11.35 c	11.46 c
Virgin soil	–	8.07 a	9.80 a	8.73 a	8.38 a	8.21 a	7.46 a	7.23 a
	+	10.36 b	10.21 a	11.26 b	10.96 b	11.28 b	10.82 bc	10.32 b

[1] – Statistical analyses were made separately for each year

soil reaction. In such an environment, the effects of replantative disease are generally less pronounced. Such dependence is demonstrated by a number of studies by other authors (Sewell et al., 1992; Strączyński, 1999). The results of many years of research obtained by the authors give the basis for stating that even a single application of the mycorrhizal vaccine on Highbush blueberry plantations may contribute to the improvement of the amount and quality of the yields obtained, especially in stressful conditions, with reduced soil fertility.

Conclusions

The type of stand has a greater impact on the yield of blueberries than the one-time use of the mycorrhizal vaccine. More fruit was collected from the bushes growing on the replanted soil than from the ones growing on the virgin one.

The Highbush blueberry is a species with relatively low sensitivity to the effects of replantative disease.

Various effects of the mycorrhizal vaccine were achieved depending on the type of soil on which the bushes grew. The effectiveness of its use in increasing the yield and improvement of fruit quality (firmness and extract content) was higher in the replanted soil than in the virgin one. It is advisable to use the mycorrhizal vaccine in blueberry crops under stress conditions (on replanted soil).

The climatic conditions and harvest time had a significant impact on the yield of the Highbush blueberries and the quality of the fruit. The highest quality fruit was collected during the first and second term (July), and the lowest – during the last term (end of August).

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