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## Influence of prolonged fertilizers on the subsequent blackberry seedlings growth after in vitro

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### Annotation

*The demand for blackberry planting material (*Rubus Eubatus Focke*) is increasing every year. The purpose of our study is to evaluate the effect of fertilizers on the subsequent blackberry seedlings growing *ex vitro*. The objects of the study were blackberry varieties 'Natchez' and 'Chester Thornless', as well as fertilizers Osmocote Exact Standard High K and OMF (organo-mineral fertilizers) for fruit and berry crops. The seedlings were grown in P9 pots (0.5l). As a result of the research, it was found that the root systems in all variants have a low variation, and the shoots' length varies from 20 to 30%. It was established a significant influence of the factors' interaction on the root system's volume and the roots' number, as well as on the shoots' number and its length. In comparison with OMF, the roots number with Osmocote fertilizers is increased in two times. For its part, OMF significantly affects the roots length's increase.*

**Keywords:** BLACKBERRY, GROWING, PLANTING MATERIAL, FERTILIZERS, CLONAL MICROPROPAGATION, IN VITRO

### Introduction

The trends in the modern domestic nursery farming's development consist in products quality's improving and in the range of planting material for horticultural crops' expanding, [1] as well as in the country's population providing with the own planting material.

Blackberry (*Rubus Eubatus Focke*) is a high-yielding berry crop, which gradually is finding its own niche in the market, while annually the planting material's demand is increasing due to a laying industrial plantations' area increasing [2]. Therefore, the planting material production technology's optimization questions remain open.

Ladyzhenskaya O.V., Aniskina T.S., Kryuchkova V.A.

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«АгроЭкоИнфо»

Blackberries can be propagated by root cuttings, stem cuttings, top cuttings and clonal micropropagation. The in vitro method makes possible to obtain required amount of planting material in a short time [3]. However, the resolving problems of blackberries' adaptation, growing and its development after replanting into pots is of interest [4]. It should be considered that for each genotype require empirically growing conditions selection [5]. It is the reason of our research - studying the effect of prolonged type fertilizers on the ex vitro blackberry seedlings growth and its development.

**Research's objectives:** to evaluate the effectiveness of the prolonged fertilizers' use on the growth and development of ex vitro blackberries seedlings varieties – 'Natchez' and 'Chester Thornless'.

**Study's purpose:**

1. To evaluate the fertilizer application's effect on the root system;
2. To prove the fertilizer application's effectiveness on the shoots' length and its number;
3. To give recommendations in the fertilizers' use growing blackberry seedlings in pots.

**Materials and methods**

The studies were carried out in the Dmitrovsky district (Moscow region), on the territory of the LOVE Berry plant nursery. As research objects were used blackberry cultivars 'Natchez' and 'Chester Thornless', fertilizers: Osmocote Exact Standart High K 5-6 months (N-P-K - 15-9-12+2MgO+ME) - the Netherlands; OMF (organo-mineral fertilizers) for fruit and berry crops (N-P-K - 5.5-9-9+MgO-1.25+S-3.7) - Russia. Recommended fertilizer application rates: Osmocote Exact Standard - 2 g/l; OMF for fruit and berry crops - 3 g/l. Fertilizers were applied once in May, in preparation for planting in the peat substrate Agrobalt-N - Russia.

The plants were propagated in the Microclone laboratory (Moscow region, Pushchino) in May 2021 using the in vitro, adapted in peat substrate in P7 pots. Then we replanted them from P7 pots (0.25l) to P9 pots (0.5l) and put them on an open area for growing to obtain planting material ready for planting in the ground. Plants are presented with five repetitions.

The temperature in June 2021 during the daytime reached critical values (+34°C), which could affect the seedlings' growing and its development. On the territory, where pots were located,

Ladyzhenskaya O.V., Aniskina T.S., Kryuchkova V.A.

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it was installing sprinkler irrigation.

In late September, biometric measurements were taken – roots' volume and its average length, average number of roots, shoots' number and its average length. To measure the volume of the root system, it was cleaned with the water from the entire substrate mass. Next, the root was placed in a volumetric flask filled with water with a volume of 0.5 liters. The volume of the root system was measured by the water displacement method.

Accounting and observations were carried out according to the standard methodology for setting up experiments with fruit crops [6]. Arithmetic means, coefficients of variation and confidence intervals for the arithmetic mean with 95% confidence are given as descriptive statistics. To analyze the experimental data for the normality of the distribution, it was carried out the Kolmogorov-Smirnov test. Next, the samples were compared by the Kruskal-Wallis method for nonparametric samples. Pairwise comparisons were made using the Mann-Whitney method. All calculations were performed in SPSS Statistics 25.

### **Research's results**

A visual inspection of blackberry seedlings showed a variation of the studied traits (Fig. 1). For nurseries, it is important to work with material that is uniform and even, as this directly affects the technological process and the yield of standard plant material.

The root system evenness will make possible to simultaneously seedlings replanting from pots P9 (0.5l) to C3 (3l). In terms of the root system volume, the most even planting material was obtained growing 'Natchez' with using of Osmocote (variation coefficient 8.1%) and OMF (9.2%). 'Chester Thornless' has the largest root system volume ( $22.0 \pm 4.3$  ml) with using Osmocote, but the variation is higher - 15.7% of the sample differs from the average values (Table 1). In terms of the roots number, Osmocote fertilizers show in both varieties the result almost 2 times higher than when using OMF ( $9.2 \pm 1.6$  pcs versus  $5.2 \pm 0.6$  pcs for 'Chester Thornless', and  $9.6 \pm 1, 4$  pcs versus  $5.6 \pm 0.7$  pcs for 'Natchez'). The variability of the average root length in all variants is very low and does not exceed 10%.

Ladyzhenskaya O.V., Aniskina T.S., Kryuchkova V.A.

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Электронный научно-производственный журнал  
«АгроЭкоИнфо»



Fig. 1. Fertilizers influence on the blackberry seedlings root and above-ground systems ('Chester Thornless' and 'Natchez', 09/19/21)

Table 1. Descriptive parameter's statistics

Feature's name	Fertilizers		Osmocote Exact standart High K		OMF for fruit berry crops	
	Statistics	Variety	Chester Thornless	Natchez	Chester Thornless	Natchez
Root system volume, ml	Min-max		17-25	18-22	8-10	15-18
	The coefficient of variation		15,7	8,1	11,9	9,2
	Confidence interval (p=0.05)		22,0±4,3	20,6±2,1	9,2±1,4	16,4±1,9
Number of roots, pcs	Min-max		8-11	8-11	5-6	5-6
	The coefficient of variation		14,2	11,9	8,6	9,8
	Confidence interval (p=0.05)		9,2±1,6	9,6±1,4	5,2±0,6	5,6±0,7
Average root length, cm	Min-max		8,9-9,8	8,6-9,6	9,2-10,5	9,5-11,7
	The coefficient of variation		4,1	4,3	5,1	8,3
	Confidence interval (p=0.05)		9,3±0,5	9,2±0,5	9,8±0,6	10,3±0,7
Number of shoots, pcs	Min-max		2	2	2	1-2
Average shoot length, cm	Min-max		10,5-21,0	20,0-30,0	17,5-23,5	8,7-20,0
	The coefficient of variation		32,2	20,2	10,9	29,6
	Confidence interval (p=0.05)		13,4±5,4	29,6±7,5	20,9±2,8	16,6±6,1

Ladyzhenskaya O.V., Aniskina T.S., Kryuchkova V.A.

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Электронный научно-производственный журнал

«АгроЭкоИнфо»

Almost in all variants of the experiment, there was 100% uniformity by the shoots number - all plants formed 2 shoots. However, in the variety 'Natchez', with the using of OMF, was observed a variation from 1 to 2 shoots per plant, in the bulk of the plants was formed only 1 shoot. The shoot length indicator had an average degree of variability (20-30%) in all variants, except for the OMF\*'Chester Thornless' scheme (10.9%).

To establish the reasons for the variation, we consider the fertilizers and varieties effect on the studied traits:

### 1. The volume of the roots

According to the Kruskal-Wallis analysis results, the hypothesis that all variants in the research do not differ from each other is rejected. The combination of factors "type of fertilizer and variety" influences the variability of root volume. Moreover, there are significant differences between the Osmocote\*'Chester Thornless' and OMF\*'Chester Thornless' combinations. In the case of applying prolonged type's fertilizers, the volume of the root system increases by 2 times (with the first combination the average volume is 22 ml, and with the second - 9.2 ml, so their pairwise difference is 12.8 ml). There are also significant differences between the root volume obtained with the combination of OMF\*'Chester Thornless' (9.2 ml) and Osmocote\*'Natchez' (20.6 ml).

The Mann-Whitney U test, when comparing pairs of varieties, did not reveal significant differences in root volume. However, the difference between the effects of fertilizers is statistically significant, Osmocote fertilizers generally show the best result for the root volume.

### 2. The number of roots

It was found a significant influence of the interaction of factors on the studied trait. The best values were obtained in combinations of Osmocote\*'Natchez' (9.6 roots, pcs) and Osmocote\*'Chester Thornless' (9.2 pcs), which significantly distinguishes them from the data of the OMF\*'Chester Thornless' combination (5.2 pcs).

A paired comparison of varieties did not reveal significant differences, but the differences from the effect of fertilizers on the number of roots are significant - when using Osmocote fertilizers, the number of roots increases almost in 2 times.

### 3. Average length of roots

It was not found the mutual influence of the variety and the type of fertilizer, there are no

Ladyzhenskaya O.V., Aniskina T.S., Kryuchkova V.A.

Influence of prolonged fertilizers on the subsequent blackberry seedlings growth after in vitro

Электронный научно-производственный журнал

«АгроЭкоИнфо»

significant differences between the variants of the experiment. However, a pairwise comparison of the effect of fertilizers showed significant differences between the samples - OMF stimulate the growth of length's roots.

#### 4. Number of shoots

According to the results of the Kruskal-Wallis analysis, an alternative hypothesis is accepted - there are differences between the variants in terms of the mutual influence of factors. It has been established that the use of OMF fertilizers for the 'Natchez' variety leads to a decrease the shoots number, relative to other variants of the study. In the Osmocote\*'Natchez', Osmocote\*'Chester Thornless', OMF\*'Chester Thornless' all plants had average 2 shoots.

A paired comparison of fertilizers did not reveal significant differences, as well as a comparison of varieties.

#### 5. Average shoot length

Significant differences were found between the Osmocote\*'Natchez' variant (average shoot length 29.6 cm) with Osmocote\*'Chester Thornless' (13.4 cm) and OMF\*'Natchez' (16.6 cm).

Differences between samples of different varieties were not found, as well as a pairwise comparison of samples in the effect of fertilizers.

### **Discussion**

It was found that Osmocote Exact Standard High K stimulate an increase of the root system volume and the roots' number in blackberries. Presumably, this is due to the high need of blackberries in potash fertilizers since potassium is the most important element in the cultivation of this crop [7]. This may also be due to the innovative Osmocote Exact Standard High K Double Coated Technology (DCT), which contributes to a more optimal nutrients' release according to the needs of the plants even under difficult growing conditions, which is worth considering, since the temperature in summer was critical high. In the case of the OMF introduction, a longer root system was noted, which is presumably influenced by the composition of the granules (40-50% deoxidized lowland peat and the mineral part). The outer shell of the granule is treated with humate K and microbiological substances. The peat shell does not allow mineral fertilizers to be washed out and mineral substances are released gradually [8]. The influence of the granules' location in the peat substrate around the root system is not excluded. However, this study has not been

Ladyzhenskaya O.V., Aniskina T.S., Kryuchkova V.A.

Influence of prolonged fertilizers on the subsequent blackberry seedlings growth after in vitro

Электронный научно-производственный журнал

«АгроЭкоИнфо»

conducted and this is only a suggestion.

Based on the literature, some scientists have conducted studies on growing seedlings in vitro without fertilization, using only various substrates.

For example, studies conducted at the fruit research station Cluj (Romania) showed a good result when growing ‘Thornless Evergreen’ blackberry seedlings under ex vitro conditions in a peat-perlite substrate (1:1) [9]. Experiments conducted at the Biotechnology Department of the Horticulture Institute RUE showed that the best substrate for the adaptation of ‘Natchez’ and ‘Chester Thornless’ blackberries ex vitro is a mix of Sphagnum L. moss with a surface layer of Dvina peat [10].

It is important to note that growing blackberries in a peat substrate the seedlings dry out rather quickly, so it is recommended to use drip irrigation, using which let stable humidity to affect the release of nutrients from prolonged fertilizers.

Previously, we carried out studies on growing *Viburnum vulgare* in P9 pots (0.5l) using various complex fertilizers, including Osmocote Exact Standart High K and OMF for fruit and berry crops [11]. During the experiment, it was noted a wave of active growth of the aerial part, associated with a temperature’s decrease (from +34°C to +26°C) and a substrate moisture’s increase (from 38% to 75%), which indicates the need to maintain optimal growing conditions.

### **Conclusions**

The variability of blackberry above-ground organs’ traits is higher than that of underground ones. The Osmocote\*‘Chester Thornless’ and Osmocote\*‘Natchez’ trials show the highest root volume and its number in growing blackberries. The use of OMF stimulates the roots length growth in almost 2 times. In all variants of the experiment, 2 shoots were formed in the studied samples, except for Osmocote\*‘Natchez’ (mainly 1 shoot remains). An effect of varieties or fertilizers on the shoots’ length has not been established.

### **Gratitude**

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