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The Influence of Rooting Substrate and Growth Regulators Indol butyric Acid and Naphthalene Acetic Acid in the Number and Length of Adventitious Roots to Hardwood Cuttings in Blueberry cv. 'Bluecrop' (*Vaccinium corymbosum* L.)

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Abstract

The purpose of this research is to determine the impact of the turf-only substrate and turf–perlite in the ratio 2:1 and of growth regulators in the quality of adventive roots (the number and length) of well lignified one-year old branches without fruit buds in the Bluecrop cultivar (*Vaccinium corymbosum* L.) taken at the end of the latent period before budding at the February 15 th during the -2015 growing season. In order to support the increase of the number of roots and their length the hardwood cuttings are treated with different IBA and NAA concentrations (1500, 3000, 4500 ppm), while a part of cuttings were untreated control. The number and the length of roots have increased in relation to the increase of concentration from 1500 to 3000 ppm followed by a decline of these values in concentrations over 3000 ppm. Respectively, the number of roots (8) and the higher values of root length (4.6 cm) are achieved in the turf–perlite substrate, IBA 3000 ppm (compared to the turf-only substrate). The presence of perlite helps the aeration of the substrate and supports biochemical and physiological processes which lead to the inducing of adventive roots. Regarding the number and length of roots an important variation for ($p<0.05$) was observed between different

concentrations of IBA and NAA. In general the effect of IBA was a lot better than the effect of NAA.

Keywords: *Vaccinium corymbosum* L., hard wood cuttings, IBA, NAA, substrate, turf-perlite,number of roots,length of roots.

1. Introduction

The highbush blueberry requires acidic soils with the pH range = 4.0 -5.5 and a high amount of organic matter, Fe and N in the form of $NH4^+$ (Darnell and Hiss, 2006). Humus should be 7-10%. The root system of the highbush blueberry is shallow and does not have fibril roots therefore their absorption capacity is ten times lower compared to field crops ‘ (i.e. wheat). The major part of the root system is at a depth between 10 and 15 cm. (Mišić.P, Nikolić.M 2003).

It is comprised of many thin fibers (strings) therefore its capacity to absorb is significantly lower. In these plants, in the cuttings placed for rooting and in the newly-rooted, the absorption of water and mineral salts is realized through the very thin cell walls of epidermis according to the law of osmosis and diffusion.

Anatomic roots are classified in two main types: Absorption and conductive roots (Mc Cully, 1999). Absorption roots of vaccinium are extraordinary good, with a very short life-span compared to other wood plants (Eissenstat, 1992; Eissenstat et al, 2000.). However continuous changes are observed in the capacity to t water to the roots of upper ranks and continuous changes in absorbing nutrients among the roots of lower ranks. The roots of higher ranks with high tissue density, thick root diameter, the ratio C:N low without mycorrhiza. These root characteristics are related to the permanent half of the root system which functions mainly for the transportation of nutrients and their storage (Wang, et al. 2006).

In the root system of the highbush blueberry the endotrophic mycorrhiza is developed which then helps water absorption and makes the root system more resistant to draught, mycorrhiza, a special group of fungus known for its high enzymatic extracellular activities and the ability to use-absorb organic forms of N and P (insoluble nitrogen matter are converted to soluble matter). (Smith and Read, 1997). The rooting ability of cuttings varies between various stages of plant development (Vakouftis, 2006).

The success of rooting in the cuttings depends on the species and the cultivar, the condition of the hardwood cuttings, the type of the hardwood cutting (string wood, semi-lignified hardwood cuttings, soft wood and herbaceous cuttings), the season when cuttings are taken and many other factors (Hartmann et al, 2002; Daneh-loueipour et al, 2006). Auxin is widely used in hardwood cuttings to accelerate and form adventitious roots (Galavi et al. 2013). Collected runners for rooting were used for their base part taking into account that the basis of hardwood cuttings collects more auxin, phenolic material and nutrients which favor rooting (Rama. P 2013). Adventitious root formation from the stem is known since ancient times and used for the vegetative propagation of elite plants which have been or are selected out of natural populations (De Klerk 2002).

The first adventitious roots occur from the callus and they are the main roots of cuttings. Callus contains a high amount of auxin (Hartman et al. 2002). The rooting capacity for stem cuttings will be determined by the interaction of factors inheriting stem cells and the factors below: the auxin level in the leaves and buds of cuttings, the reserved amount of carbohydrates in cuttings, the stage of plant growth, the position of the cutting in the runner (Rosier et al., 2006). The initiation of adventitious roots and their growth is an intensive metabolic process which is promoted by auxins and other growth regulators that lead to the increase of enzymatic activity for the ARN synthesis and proteins (Hartmann and Kester, 1983;. De Klerk et al, 1999;. Legue et al, 2014).

Organic matter (humus) helps the blueberry root system which is very sensitive by preventing unexpected changes of the pH value, moisture and soil temperature. An important impact on that which makes the nutrients available in the type of soil is made by the pH value which even in its smallest area can fluctuate a lot and have an impact in the processes of mineralizing organic matter. This action than continues on the soil structure and lately in the absorption of ions and their exchange. (Hart, J. D. Horneck, R. Stevens, N. Bell. and C. Cogger. 2003). The best expositions for the highbush blueberry are those of the north and north-west where soil moisture is better preserved. Southern expositions should be removed completely in low altitudes because of the high heating of the soil and the loss of moisture.

The value of soil reaction should be controlled regularly because the changes in the pH value compared to the foreseen conditions are observed very quickly and in the blueberry shrub. The soil depth should be between 30 and 50 cm. If it is deeper than it is positive for the successful cultivation of blueberries. When the soil pH is higher than 6.0 the blueberry plants often become yellow because of the lack of iron (Korcak, 1987). The lack of iron occurs in calcareous soils (lime-rich) where iron is deposited by carbonates or bicarbonates (lime-induced chlorosis). The nitrogen taken in the form of ammonium (NH4 +) from the roots of the blueberry plant leads to the acidification of rhizosphere and will lead to the increase of iron intake (Marschner, 1995).

Likewise, the surplus of manganese (Mn) or other heavy metals may lead to the lack of iron because these ions compete in the suction area. The highbush blueberry does not tolerate a high level of underground waters especially if those waters are closer than 50 cm beneath the soil surface. The water and the water quality have a high importance for the successful cultivation of the highbush blueberry (*vaccinium corymbosum L.*). Along with the provision of water from various sources it is necessary to verify its quality, iron content, electrical conductivity and the total amount of soluble salts, in mg/l (TDS-total dissolved solid) as well as the content of salts such as: carbonates, sulfates and chlorides which also determine the suitability of the water for irrigation purposes, (Ayers and Westcot, 1985). EC values of 1.10 $mS \cdot cm^{-1}$ and 1.45m $\cdot cm^{-1}$ have produced good results in yield as well as in the fruit mass (Jacek Glonek, Andrzej Komosa 2013).

2. Material and Methods

The propagation material mainly used were one-year old branches, well-lignified, without fruit buds with a horizontal position because of the more favorable ratio (C:N). These branches between 6-12 mm thick are shortened at 15 cm length, several mm above the upper bud and several mm under the lower bud. After cutting, the cuttings are prepared, tied in bunches by 40 pcs and their base part is dipped up to 2.5 cm in IBA and NAA solutions with various concentrations of 1500 ppm, 3000 ppm and 4500 ppm for 5-7 seconds, whilst one row per box is not treated (control). Boxes are filled with turf-only and turf-perlite at a ratio 2:1 (substrate depth 25 cm).

At the bottom of boxes a layer of gravel is placed to enable the drainage of excessive water. These treated cuttings were kept for 15 minutes (until they absorbed well the IBA and NAA) and after drying were powdered at their base with captan powder mixed with talk (at a ratio 1:10 – against rotting), then they were placed in boxes for rooting at a distance 10 x 5 cm and depth around ½ of the cutting length leaving at least 2 buds over the substrate where they have stayed for 8 weeks. The experiment was placed in four boxes with 4 repetitions each, 1 repetition = 40 cuttings, 4 x 40 cuttings = 160 cuttings/box, i.e. the experiment has included a total of 640 cuttings.

The rooting of cuttings was achieved with base heating (through pipes with hot water) which has enabled the preservation of the certain temperature at the base part of the cuttings where roots come out. The temperature was 21 c° during the day and 15-16 c° during the night. The air temperature at the upper part of the cuttings was 4 c° lower compared to the air temperature at the base part of the cuttings in order to create a difference between the base of the cutting and the top of the cutting. In this way it temporarily prevented the development of buds located at the terminal part of the cutting whilst the reserves of carbohydrates were oriented towards the base of the cutting which has resulted with the adventitious root formation in a higher number and longer length. Boxes filled with substrate for the rooting of cuttings were placed in a greenhouse where the relative air moisture was 75-80 %.

3. Results and Discussion

The data presented in tables 1, 2, 3 and 4 and in fig. 1 show that the rooting percentage of hardwood cuttings and the number and length of roots is higher in the substrate turf-perlite 2:1 compared to the turf-only substrate. In the torf-perlite substrate 2:1 the rooting percentage has reached 67.5 % compared to the torf-only substrate where the rooting percentage has reached 55 %. Whilst the number of roots in the torf-perlite substrate 2:1 in the variation IBA 3000 ppm reached 8 the length of roots reached 4.6 cm compared to the turf-only substrate, the same variation IBA 3000 ppm where the number of roots reached 6 whilst the length of roots is 3.4 cm.

Table 1. Data averages for hard wood cuttings for the number of roots by repetition

Factor A Substrate	Factor B Growth regulators	Factor C Concentration	Repetition r 1	r 2	r 3	r 4	Mean
Turf	IBA	Control	1.8	2.2	2.4	1.6	2.0
		1500 ppm	4.2	3.8	3.0	5.0	4.0
		3000 ppm	5.0	6.5	6.5	6.0	6.0
		4500 ppm	3.2	4.5	2.8	3.5	3.5
	NAA	Control	2.6	1.8	1.4	2.2	2.0
		1500 ppm	3.5	3.0	2.5	3.0	3.0
		3000 ppm	4.2	4.5	4.8	4.5	4.5
		4500 ppm	3.0	3.2	2.8	3.0	3.0
Turf – Perlite	IBA	Control	3.4	3.0	2.2	3.4	3.0
		1500 ppm	4.5	3.8	4.2	4.3	4.2
		3000 ppm	8.2	7.5	8.3	8.0	8.0
		4500 ppm	4.0	3.8	4.5	3.7	4.0
	NAA	control	2.8	2.5	2.0	2.7	2.5
		1500 ppm	3.5	3.2	4.0	3.3	3.5
		3000 ppm	4.8	5.5	5.0	4.7	5.0
		4500 ppm	3.5	3.0	2.8	3.5	3.2

Table 2. The influence of rooting substrate (turf, turf-perlite) and growth regulators (IBA) Indol butyric Acid and(NAA) Naphthalene Acetic Acid in the number of adventitious roots to hardwood cuttings. (Analysis of the variance three-way)

Factor A - Substrate: Turf	Turf-Perlite	Factor -B Growth regulators	Factor -C Concentration	Average (AB)	Average(A)
2.00	3.00	control	-	2.50	2.38**
2.00	2.50	Control	-	2.25	
Average AC					
2.00	2.75				
4.00	4.20	IBA	1500 ppm	4.10	3.68*
3.00	3.50	NAA	1500 ppm	3.25	
Average AC					
3.50	3.85				
6.00	8.00	IBA	3000 ppm	7.00	5.88**
4.50	5.00	NAA	3000 ppm	4.75	
Average AC					
5.25	6.50				
3.50	4.50	IBA	4500 ppm	4.00	3.55*
3.00	3.20	NAA	4500 ppm	3.10	
Average AC					
3.25	3.85				
Average C					
3.50	4.17				
Average BC				Average B	
2.38**	3.68			3.03	
5.88**	3.55			4.71	

The Factors		A**	B**	C**	AB**	AC	BC	ABC
LSD	1 %	0.54	0.23	0.32	0.60	0.84	0.52	1.64
	5 %	0.39	0.17	0.24	0.41	0.58	0.37	0.99

The statistical analysis ANOVA three way shows that there are significant differences between the rooting substrate turf-only and turf-perlite 2:1. Regarding the number of roots and their length in the turf substrate only the number of roots is 2 in the variation control (without treatment) and reached up to 4.5 in the variation IBA 3000 ppm, whilst in the torf-perlite substrate 2:1 the number of roots is 3 in the variation control thus achieving the highest values in 8 roots/cutting in the variation IBA 3000 ppm (Table 1and 2) Therefore, between the variation control and the variation 3000 ppm there are highly significant differences (P=0.01) in the number of roots as well as in their length whilst between the variation 3000 ppm and 1500 ppm there are significant differences at the safety scale (P =0.05). Variations 1500 ppm and 4500 ppm did not show significant differences among themselves.

Table 3. Data averages for hard wood cuttings for the length of roots by repetition

Factor-A Substrate	Factor-B Growth regulators	Factor-C Concentration	Repetition r1	r 2	r 3	r 4	Mean
Turf	IBA	Control	1.2	1.4	1.5	1.5	1.4
		1500 ppm	2.0	2.2	2.6	2.0	2.2
		3000 ppm	3.2	3.8	3.6	3.0	3.4
		4500 ppm	2.2	2.0	1.8	2.0	2.0
	NAA	Control	1.4	1.2	1.0	1.2	1.2
		1500 ppm	2.3	2.0	1.5	2.2	2.0
		3000 ppm	3.0	2.4	3.2	3.4	3.0
		4500 ppm	1.8	2.0	1.6	1.8	1.8
Turf-Perlite	IBA	Control	2.4	2.0	1.6	1.8	2.0
		1500ppm	2.6	3.2	3.0	3.2	3.0
		3000 ppm	4.5	4.8	4.6	4.5	4.6
		4500 ppm	2.5	3.0	2.8	3.0	2.8
	NAA	Control	1.7	2.0	1.8	2.5	2.0
		1500 ppm	2.5	2.4	2.8	2.7	2.6
		3000 ppm	3.2	3.0	3.4	2.4	3.0
		4500 ppm	2.0	2.4	2.6	1.8	2.2

Table 4. The influence of rooting substrate (turf, turf-perlite) and growth regulators (IBA) Indol butyric Acid and(NAA) Naphthalene Acetic Acid in the length of adventitious roots to hardwood cuttings.(Analysis of the variance three-way)

Factor A - Substrate		Factor -B Growth regulators	Factor -C Concentration	Average (AB)	Average(A)
Turf	Turf-Perlite				
1.40	2.00	control	-	1.70	1.65**
1.20	2.00	Control	-	1.60	
Average AC					
1.30	2.00				
2.20	3.00	IBA	1500 ppm	2.60	2.45
2.00	2.60	NAA	1500 ppm	2.30	
Average AC					
2.10	2.80				
3.40	4.60	IBA	3000 ppm	4.00	3.55**
3.00	3.20	NAA	3000 ppm	3.10	
Average AC					
2.00	2.80	IBA	4500 ppm	2.40	2.20
1.80	2.20	NAA	4500 ppm	2.00	
Average AC					
1.90	2.50				
Average C					
2.07	2.77				
Average BC				Average B	
1.65**	2.45			2.05**	
3.55**	2.20			2.87**	

The Factors		A**	B**	C**	AB**	AC	BC	ABC
LSD	1 %	0.27	0.15	0.19	0.41	0.50	0.31	0.98
	5 %	0.20	0.12	0.14	0.28	0.34	0.26	0.59

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Regarding the concentration of growth bio-regulators IBA and NAA the statistical data show that starting from the concentration at 1500 ppm up to 3000 ppm there is an increase of the number of roots and of their length, followed by a decrease of the number of roots and length of roots in concentrations above 3000 ppm(Table 3 and 4). Among auxins, IBA and NAA are used to induce rooting because these are non-toxic in a wide range of concentrations for a large number of species and chemically stable in contact with the substrate of propagation (Hartmann et al., 1996). Among the growth bio-regulators IBA results as the most successful in terms of inducing adventitious roots as well as in their length in hardwood cuttings.

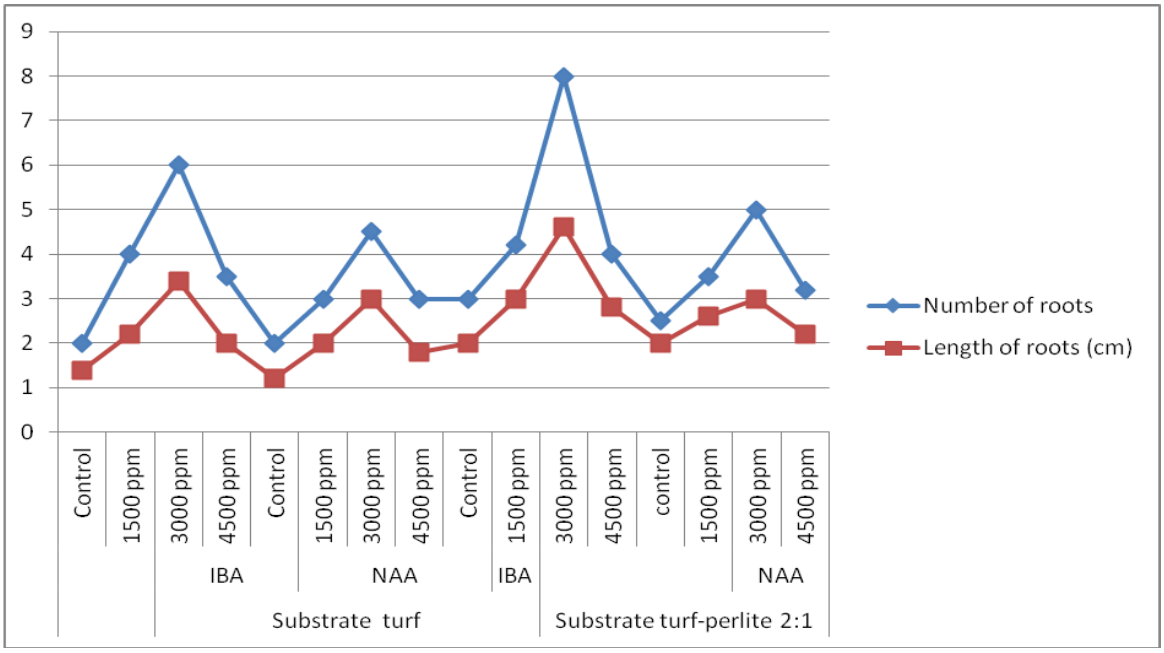

Figure1. The Number, Length of Root for Hardwood Cuttings

4. Conclusions

In this research during the rooting of hardwood cuttings a higher percentage of rooting of hardwood cuttings, a higher number of roots and a higher length were observed in the turf-perlite substrate 2:1 compared to the cuttings placed in the turf-only substrate. The presence of perlite helps a better aeration of the substrate by favoring a higher number of roots and the length of roots in high values since biochemical processes during the induction of adventitious roots are aerobe processes i.e. the presence of oxygen is necessary. Whilst regarding the growth bio-regulators and their concentration in all variations it results that IBA 3000 ppm is more successful in inducing a higher number of roots and in the length of roots compared to NAA in the same concentrations.

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