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# Substrates and IBA concentrations on rooting of herbaceous cuttings of blueberry 'Woodard'

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## ABSTRACT

Brazil offers great perspectives for the expansion of blueberry (*Vaccinium* spp.) cultivation areas, mainly in family agriculture areas. Among the factors that limit the expansion of this culture is the difficulty to propagate some cultivars. Thus, the objective of this work was to evaluate the use of different substrates and concentrations of indolebutyric acid (IBA) for blueberry cuttings cvs. Woodard rooting, to optimize seedlings production system. The experimental design was totally randomized in a 3X3 factorial scheme, with five replications of 10 cuttings, and included the following factors: types of substrate (carbonized rice husk, vermiculite medium granules and coconut fiber) and IBA concentrations (0, 1,000 and 2,000 mg L<sup>-1</sup>). After 150 days, survival percentage was superior for carbonized rice husk, with no influence of IBA concentration. In regards to rooting, the carbonized rice husk and coconut fiber provided better results, being IBA application favorable for this variable. It was concluded that 'Woodard' blueberry can be propagated by herbaceous cuttings, using preferentially carbonized rice husk and application of 1,000 mg L<sup>-1</sup> IBA is recommended for rooting of softwood cuttings.

Key words: Propagation, small fruit, growth regulator, Vaccinium spp.

# INTRODUCTION

The blueberry (*Vaccinium* spp.) is native from North America, the US and Canada, being found in the tropics, in high altitudes, but also in temperate and boreal regions. It belongs to the Ericaceae family and to the *Vaccinium* genus with around 150 to 450 species (Raseira and Antunes 2004).

In Brazil, the cultivation of this fruit is promising, offering great perspectives for income generation in family agriculture areas, due to the intensive need of labor and the low mechanization rate, as well as to its acceptance by the consuming market due to its nutraceutical properties. Among the aspects that limit the expansion of this culture are the climate and the soil, slow plant growth and the difficult propagation of some cultivars, which increases the cost of seedlings (Campos et al., 2005; Fachinello 2008).

Blueberry propagation is carried out through seeds, cuttings, grafting, layering, shoots or micropropagation. Although the use of seeds and tissue culture allows seedlings production in large scale, the first method is used mainly in genetic breeding programs, since the generated plants are different from the stock plant, and the second method demands more investment in adequate and well-equipped laboratories and specialized labor. Therefore, the commercial production of seedlings uses, preferably, semihardwood or herbaceous cuttings (Hoffmann et al., 1995; Beyl and Trigiano 2008; Fachinello 2008; Pelizza et al., 2011).

In the propagation by cuttings must be considered factors such as genetic potential of rooting, physiological condition of stock plant, period of the year, temperature, light, humidity and hormone balance (Fachinello et al., 2005). Another factor that interferes in the rooting of cuttings is the substrate, which should allow retention of sufficient water, to prevent the desiccation of the cutting base, and it have the porous space, which facilitates the supply of oxygen to the roots. Special attention should be given to the pH value, since blueberry cuttings do not root in materials with the pH over 6.5. Therefore, it is fundamental to define the most recommended substrate for each species (Campos et al., 2005; Fachinello et al., 2005; Ristow et al., 2012).

According to Pires and Biasi (2003) and Fachinello et al. (2005), synthetic auxins are used to favor root development, and the indolebutyric acid (IBA) is relatively stable and highly effective to a large number of species. However, studies have shown that the effect of exogenous auxins varies among species, cultivars and concentrations (Fischer et al., 2008; Trevisan et al., 2008; Vignolo et al., 2012).

Thus, the objective of this work was to evaluate the use of different substrates and IBA concentration in the blueberry cv. Woodard cuttings rooting, in order to optimize the seedlings production system.

## **MATERIAL AND METHODS**

The experiment was carried out from April to September 2012, at Universidade Estadual de Londrina (UEL) Agricultural Sciences Center, Fruit Growing Sector (latitude 23°23 S, longitude 51°11 O and an altitude of 566 m), Londrina, Paraná, Brazil. According to the Köppen classification, the region climate is Cfa type (subtropical humid).

It was used herbaceous cuttings of 10-12 cm, taken from the median part of the steam of blueberry (*Vaccinium ashei*) stock plant, being the cutting of the cv. Woodard realized in April.

The experimental design was entirely randomized, in a factorial scheme, with five replications of 10 cuttings. The experiment included the following factors: substrate (carbonized rice husk, vermiculite of medium granulation and coconut fiber 47, Amafibra<sup>®</sup>) and three concentrations of IBA (0, 1,000 and 2,000 mg L<sup>-1</sup>) (Fluka<sup>®</sup>, 99% of purity). Some substrate characteristics such as pH in water, in the 1:1 relation, electrical conductivity ( $\mu$ S cm<sup>-1</sup>), in the beginning and at the end of the experiment, density (kg m<sup>-3</sup>) and substrate water retention capacity (mL L<sup>-1</sup>), at the end of the experiment were determined according to Kämpf et al. (2006) (Table 1).

**Table 1.** pH, electrical conductivity (EC), initial and final, and density and substrates water retention capacity (WRC), 150 days after the installation of the experiment.

Substrates	pH1	pH <sup>2</sup> EC <sup>1</sup>	$EC^2$	Density	wRC	
	_	_	(µS cm <sup>-1</sup> )	(µS cm <sup>-1</sup> )	(kg m <sup>-3</sup> )	(mL L <sup>-1</sup> )
CRH <sup>3</sup>	7.3	6.9	197.8	186.5	171.0	512.0
VERM	7.3	7.1	112.6	138.9	281.0	399.0
COCOF	6.8	6.7	240.0	171.1	134.0	729.0

<sup>1</sup>pH and <sup>1</sup>EC: analyzed in the beginning of the experiment; <sup>2</sup>pH and <sup>2</sup>CE: analyzed 150 days after the installation of the experiment. <sup>3</sup>CRH: Carbonized rice husk; VERM: Vermiculite – medium granules; COCOF: Coconut fiber.

Before collecting the cuttings, an IBA hydroalcoholic solution was prepared, with 0.1 and 0.2 g of IBA and dissolved in 50 mL of alcohol (96°GL). After completely diluted, the IBA volume was completed to 100 mL, with distilled water, reaching concentrations of 1,000 and 2,000 mg L<sup>-1</sup> of IBA (Fachinello et al., 2005).

Cuttings preparation consisted of a bevel cut in the upper part and a straight cut just below the node, with the elimination of the leaves from the basal part, leaving a pair of leaves in the upper part. During preparation, cuttings were disposed in a recipient with water to prevent dehydration. Then, the IBA was applied through immersion of the cuttings base for 10 seconds.

Then, the cuttings were disposed into perforate plastic boxes (44x30x7cm) containing the substrates, and maintained in a mist chamber with controlled intermittent timer and solenoid valve. The valve was programmed to mist during 10 seconds every three minutes. The nozzle nebulizer employed (Model DanSprinklers Mist, Israel) presents flow 35L h<sup>-1</sup>. The mist chamber was maintained in greenhouse covered with transparent polyethylene film and 30% shading.

For fungal diseases control, cuttings were treated weekly with tebuconazol-based fungicide (1mL L<sup>-1</sup>) by spraying. Minimum and maximum temperatures were 15.0 and 28.5 °C during the experiment.

The following variables were evaluated 150 days after the installation of the experiment: cuttings survival (% of live cuttings); foliar retention (% of cuttings that did not lose their leaves); cuttings with shoots (% that emitted at least one shoot); rooted cuttings (% of cuttings that emitted at least one root); number; length (cm) and dry weigh of roots per cutting (g). Root dry weigh was obtained by drying in a oven with forced air circulation at 65°C, for 48 hours.

The data were subjected to analysis of variance, and the means were compared using Tukey's test at 5% probability. Arcsine  $\sqrt{x/100}$  data transformation was performed for the variables expressed in percentages, and the transformation  $\sqrt{x+1}$  was performed for the data derived from counting. Analyzes were conducted through the SISVAR computational program (Ferreira 2011).

## **RESULTS AND DISCUSSION**

There was no significant interaction between types of substrates and IBA concentrations, showing that the factors studied act independently during the rooting process (Table 2 and Table 3). Although there was no significant interaction between the factors studied, the results were shown.

In relation to cuttings survival percentage, there was difference among substrates, with 100% for survival with carbonized rice husk, which presented a result over 10% in relation to that observed for vermiculite and at 24% when compared to coconut fiber (Table 2).

It could be inferred that the lower survival rate of 'Woodard' in coconut fiber may have occurred in function of the high electrical conductivity (240  $\mu$ S cm<sup>-1</sup>) registered in the beginning of the experiment (Table 1), since necrosis symptoms of cuttings in this substrate started in the first weeks after experiment installation. According to Hartmann et al. (2002) and Beyl and Trigiano (2008), materials with high saline concentration may cause growth reduction, burning of leaves or even plant's death, being the values between 80 and 200  $\mu$ S cm<sup>-1</sup> of electrical conductivity considered ideal for commercial propagation of plants. In addition, high water retention capacity of coconut fiber may also have contributed to lower survival rate of cuttings observed in the present work.

	IBA concentrations		Substrates		Mean
	(mg L <sup>-1</sup> )	CRH <sup>1</sup>	VERM	COCOF	
Cuttings survival	0	100.0	88.0	72.0	86.7 ns
(%)	1,000	100.0	94.0	80.0	91.3
	2,000	100.0	88.0	76.0	88.0
Mean		100.0 A <sup>2</sup>	90.0 B	76.0 C	
CV (%)			18.12		
Foliar retention (%)	0	58.0	16.0	42.0	38.7 ns
	1,000	56.0	40.0	46.0	47.3
	2,000	60.0	38.0	46.0	48.0
Mean		58.0 A	31.3 B	44.7 AB	
CV(%)			37.35		
Budding (%)	0	76.0	56.0	38.0	56.7 ns
0	1,000	54.0	58.0	54.0	55.3
	2,000	52.0	28.0	48.0	42.7
Mean		60.7 ns	47.3	46.7	
CV (%)			30.76		

**Table 2.** Cuttings survival, foliar retention and cutting budding of 'Woodard' blueberry (*Vaccinium ashei*) in different substrates and indolebutyric acid (IBA) concentrations, in a nebulization chamber.

<sup>1</sup>CRH: Carbonized rice husk; VERM: Vermiculite – medium granules; COCOF: Coconut fiber.

 $^{2}$ Means not followed by the same uppercase letters in the lines and lowercase letters in the columns differ among them by the Tukey test (p<0.05); ns: non-significant.

**Table 3.** Rooted cuttings, number of roots per cutting, root length and root dry weigh per 'Woodard' blueberry (*Vaccinium ashei*) cutting, in different substrates and concentrations of indolebutyric acid (IBA), in a nebulization chamber.

	IBA concentration	ns	Substrates	Mean	
	(mg L <sup>-1</sup> )	$CRH^1$	VERM	COCOF	
Rooted cuttings (%)	0	58.0	18.0	54.0	43.3 b
	1,000	58.0	42.0	76.0	58.7 a
	2,000	80.0	34.0	70.0	61.3 a
Mean		65.3 A <sup>2</sup>	31.3 B	66.7 A	
<u>CV (%)</u>			32.86		
Number of roots per	0	4.3	2.5	5.5	4.1 b
cutting	1,000	5.4	3.1	8.8	5.7 a
-	2,000	4.9	5.6	11.3	7.2 a
Mean		4.9 AB	3.7 B	8.6 A	
<u>CV (%)</u>			26.81		
Root length (cm)	0	4.0	3.3	8.1	5.0 ns
	1,000	4.6	3.5	6.1	4.7
	2,000	5.4	3.6	7.1	5.4
Mean		4.6 B	3.5 C	7.1 A	
<u>CV (%)</u>			35.98		
Root dry weigh	0	24.6	7.9	73.1	35.2 ns
cutting (mg)	1000	39.6	8.7	69.3	39.1
	2000	43.2	20.3	82.2	48.5
Mean		35.8 B	12.3 C	74.8 A	
CV (%)			63.49		

<sup>1</sup>CRH: Carbonized rice husk; VERM: Vermiculite – medium granules; COCOF: Coconut fiber.

<sup>2</sup>Means not followed by the same uppercase letters in the lines and lowercase letters in the columns differ among them by the Tukey test (p<0.05); ns: non-significant.

In a study carried out by Hoffmann et al. (1995), there was no substrate effect in regards to dead cuttings percentage for 'Climax' and 'Powder Blue' blueberries. However, the increase in water retention by the rice husk ash + vermiculite substrate corresponded to an increase in the percentage of cuttings with necrosis in the base. Pelizza et al. (2011) observed that the substrate Plantmax<sup>®</sup> + carbonized rice husk promoted the highest survival rate.

There was no survival difference in regards to the application of IBA, registering on average 88.7% of live cuttings (Table 2). However, Vignolo et al. (2012) observed an increase in survival percentage of woody cuttings of the

blueberries 'Delite', 'Bluebelle' and 'Brite Blue', with the increase in IBA concentration.

For foliar retention, there was difference only between substrates, among which the carbonized rice husk promoted better results in relation to the vermiculite (Table 2). According to Hartmann et al. (2002), the presence of leaves in the cuttings affects rooting, since the auxin is produced in the new leaves and shoots, being loaded with sugars and other nutritive substances for the inferior part of the plant and piling up at the base of the cut. In an experiment with blueberry cutting, Hoffmann et al. (1995) found out that the permanence of leaves is beneficial to this fruit cuttings rooting.

In regards to shoots number, there was no difference between the studied factors (Table 2). During the evaluation of woody cuttings rooting from different blueberry cultivars, Fischer et al. (2008) observed that the favorable IBA concentration for number of shoots is variable between cultivars, and that the application of 2,000 to 8,000 mg L<sup>-1</sup> in the 'Woodard' promoted greater number of shoots per cutting in relation to the control.

Carbonized rice husk and coconut fiber substrates showed the highest means in regards to rooted cuttings percentage. Regarding to IBA application, its use favored cuttings rooting percentage (Table 3). According to Fachinello et al. (2005), the adequate content of exogenous auxin to stimulate rooting depends on the species and the concentration of auxin in the tissue, indicating that, for this material, the exogenous auxin may promote rooting. However, results obtained by Fischer et al. (2008), in a study on woody cutting rooting of blueberry cultivars submitted to IBA concentrations, reported that, for 'Woodard, the application of IBA had no influence on rooting percentage. Similarly, Trevisan et al. (2008) verified that IBA applications up to 7.500 mg L<sup>-1</sup> had no interference on rooting percentage for blueberry cuttings cultivars such as 'Clímax', 'Bluebelle' and 'Brite Blue'. These results show that, in addition to the cultivar, other factors such as experiment conditions can also influence rooting.

In regards to number of roots per cutting, the coconut fiber favored the development of roots when compared to the vermiculite, showing no difference from the carbonized rice husk. Coconut fiber also promoted greater root length and dry weigh, followed by carbonized rice husk and vermiculite (Table 3). It is important to emphasize that coconut fiber showed a pH lower that other substrates (Table 1), which may have favored the rooting, since blueberry is a species that develops in soils with low pH.

According to Campos et al. (2005) and Fachinello et al. (2005), the substrate pH may affect cutting rooting, and, in the case of blueberry, they do not root in substrates with pH over 6.5. In addition, Ristow et al. (2012) described that the substrate must be porous enough and resistant to compaction, due to the superficial root system of the blueberry, constituted of fine, fibrous and short roots and devoid of root hairs. Thus, the highest density values may represent greater resistance to root expansion in the substrate, which can explain the lower results presented by the vermiculite, which showed greater density compared to other substrates (Table 1).

A study conducted by Hoffmann et al. (1995) with blueberry cuttings showed no difference between substrates for root volume, and mix of sand with the organic compound promoted greater growth followed by sand and ash mixed with vermiculite. Other authors such as Ristow et al. (2012) and Pelizza et al. (2011), observed differences between substrates in blueberry rooting.

In relation to IBA, it influenced positively the number of roots. However, there was no IBA influence on root length and dry weigh (Table 3). An experiment realized by Fischer et al. (2008), showed no IBA concentrations effect for number of 'Woodard' woody cutting roots. However, the application of 2,000 mg  $L^{-1}$  promoted the best result for largest root length.

In the light of the foregoing, carbonized rice husk and coconut fiber provided the best results to rooting characteristics. However, taking into consideration that the survival percentage was superior for carbonized rice husk, the use of this agro-industry residue is recommended in regions where it is available, since, besides being a low cost option, it contribute to the reduction of this material in the environment. In regards to IBA, the application of 1,000mg  $L^{-1}$  was sufficient to favor cuttings rooting process.

### CONCLUSIONS

The 'Woodard' blueberry can be propagated though herbaceous cutting, and carbonized rice husks are the most recommended substrate in regions where this agricultural residue is available. The application of 1,000 mg L<sup>-1</sup> of IBA is recommended for the rooting of herbaceous cuttings of 'Woodard'.

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