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THE IMPACT OF FOLIAR DIKEGULAC AND ASAHI SL SPRAYS ON THE SHOOT PRODUCTION OF HIGHBUSH BLUEBERRY NURSERY PLANTS

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ABSTRACT

The aim of the present study was to assess the usefulness of dikegulac (2,3:4,6-di-O-isopropylidene-α-Lxylo-2-hexulofuranosonic acid) and Asahi SL (sodium ortho- and para-nitrophenolate, sodium 5-nitroguaiacolate) in production of highbush blueberry nursery plants. The experiment was carried out on three cultivars of highbush blueberry: 'Bluecrop', 'Brigitta blue' and 'Darrow'. Pot plants were treated two times with dikegulac (0.1%) or/and Asahi SL (0.2%) foliar sprays in the late spring. The reaction of blueberry clones to the tested chemicals was different. Dikegulac-treated plants developed significantly more shoots making them a good and efficient source of cuttings when using the traditional propagation. Dikegulac limited the shoot elongation of two studied cultivars ('Bluecrop', 'Darrow'). It also reduced the size of leaf blades but generally did not change the fluorescence and the relative content of chlorophyll. The influence of dikegulac in the applied dose was much stronger than the impact of nitrophenolates. The application of Asahi SL, alone or together with dikegulac, did not give any spectacular and advantageous effects.

Key words: branching, nitrophenolates, plant growth stimulators, retardants, *Vaccinium corymbosum*

INTRODUCTION

Highbush blueberry is a crop with high relevance around the world in view of tasty fruits full of health-promoting nutrients. An increase in highbush berries demand and the attractive price are the reasons of the rapidly expanded cultivation area worldwide and the growing need for highbush blueberry plantlets nowadays. The highbush blueberry plants can be propagated conventionally through hard- or softwood cuttings, and by micropropagation. Such methods have both proponents and opponents. Micropropagation is the only way for rapid propagation of plants with improved health status. Thus, micropropagated plants are prevalent in nurseries. However, it should be noted that some controversies about the value of micropropagated plants, among others from *Ericaceae* family, appeared, and although rare, remain until now. They were based on some sporadic but confusing mentions of excessive vegetative growth (too many thin lateral shoots), delayed fruit harvest for some years or smaller berries [Serres et al. 1997, Litwińczuk et al. 2005]. Therefore conventionally propagated highbush blueberry plants still find their recipients. To obtain such plants a hardwood, unleafy cuttings (13–15 cm long) are prepared from strong, healthy shoots that grew in the previous summer. They should be collected in late winter after sufficient, natural chilling. Softwood leafy

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cuttings (about 10 cm long) are collected in late spring from the first flush of spring growth, before initiation second flush. Some nurserymen use with success shorter, 2-node cuttings taken from one year old potted plants or fully acclimatised, micropropagated plantlets [pers. obs.]. Cuttings are rooted in coarse sand and peat mixture in the heated propagation beds with mist control under shade cloth. Apart from gradually deteriorating health status of mother (stock) plants the limited quantity of shoots suitable for rooting is often a 'bottleneck' of the conventional propagation. To avoid such problems, the application of some plant growth regulators, especially retardants, that stimulate shoot proliferation of stock plants, may be considered. One of them is dikegulac which was found to reduce apical dominance, and promote lateral branching in some plants [Sachs et al. 1975, Norcini et al. 1994, Cochran and Fulcher 2013, Sarropoulou et al. 2014, Sun et al. 2015]. It has been introduced on the market under the trade name 'Atrinal', then 'Atrimmec', and 'Augeo' [Rademacher 2015, Whipker and Latimer 2016]. In 2007, any legal use of dikegulac ended in EU member countries [Rademacher 2015]. However, it has been tested especially in micropropagation of woody plants [Thetford and Berry 2000, Pozo et al. 2004, Sansberro et al. 2006, Mendoza-de Gyves et al. 2008, Sarropoulou et al. 2014, Sun et al. 2015, Antonopoulou at al. 2018]. It is also used in USA in the production of ornamental plants, including azaleas (*Ericaceae*) [Whipker and Latimer 2016]. Dikegulac-containing products are used for "chemical pinching" on potted ornamentals, hedges, shrubs, trees, and groundcovers, inhibiting the growth of the terminal bud. As a result, lateral branching is promoted, and plants are denser and fuller looking. Litwińczuk and Prokop [2010] found that dikegulac applied as a foliar spray stimulated branching of highbush blueberry nursery plants (liners), allowing more cuttings to be collected, which also rooted *in vivo* even better than the control ones. However, they carried out experiments on one clone ('Herbert'). Therefore, the aim of the present study was to determine whether the similar effect might be obtained in the case of other highbush blueberry cultivars. On the other hand, Litwińczuk and Prokop [2010] observed also reduction and slight yellowing of 'Herbert' leaves caused by dikegulac. Such phenomenon was also noticed after spraying other plants with products containing dikegulac [Jacyna et al. 1994, Banko and Stefani 1996, Sansberro et al. 2006, Grossman et al. 2013, Whipker and Latimer 2016]. In order to prevent this problem, Asahi SL was tested in some studies, and nitrophenolates-containing products have been reported to delay leaf senescence, stimulate the growth of leaf blades, increase the chlorophyll content and the intensity of photosynthesis [Djanaguiraman et al. 2009, Przybysz et al. 2010, Chen and Dong 2016]. As nitrophenolates stimulate the antioxidant enzyme activity, they could also reduce plant stress. Unlike dikegulac, Asahi SL is approved for use in horticultural crops in Poland, including blueberries [MARD gov.pl 2022]. Therefore, the objective of the presented study was to examine the effects of application of dikegulac and nitrophenolates on highbush blueberry nursery plants and assess the usefulness of those chemicals on the propagation of highbush blueberry cultivars.

MATERIALS AND METHODS

Plant material and treatments. The experiment was carried out on three highbush blueberry cultivars (*Vaccinium corymbosum* hort. non L., syn. *Vaccinium* × *covilleanum* But. *et* Pl.) 'Bluecrop', 'Brigitta blue', and 'Darrow'. One-year old plants were grown in 3 dm^{-3} pots filled with peat moss, sand, and pine bark mixture (3:1:1, *v/v*) in foil tunnel. They were fertilized three times (May-July) with Yara Mila™ Complex (7 g per pot) and twice (September, October) with Intermag K-300 (0.3%, foliar spray). In June (6th and 20th of VI), plants were sprayed twice with dikegulac (diprogulic acid, 2,3:4,6-di-O-isopropylidene-α-L-xylo-2-hexulofuranosonic acid, FLUKA) ('D') 0.1% or/and Asahi SL ('A') 0.2% (14th and 28th of VI). Asahi SL (Arysta LifeScience) product contains sodium 5-nitroguaiacolate ($NaC_7H_6NO_4$) 1.25 g dm^{-3}, sodium ortho-nitrophenolate ($NaC_6H_4NO_3$) 2.5 g dm^{-3} and sodium para-nitrophenolate ($NaC_6H_4NO_3$) 3.75 g dm^{-3} active ingredients. The concentration of dikegulac was chosen according to the previous work of Litwińczuk and Prokop [2010], whereas Asahi SL dose was the same, as used routinely in the nursery. Four treatments were tested: dikegulac 'D+', Asahi SL 'A+', dikegulac and Asahi SL 'D+A+', and control ('D-A-', without those substances).

Observations and measurements. In the middle of July (5 weeks after the first treatment), the number

of shoots (5–10 cm and >10 cm long) and the length of the longest shoot on each plant were determined. The length and width of the second (from the top) full-developed leaf were measured and leaf shape (length/width) ratio and area were calculated. In order to evaluate the physiological state of plants, measurements of the relative chlorophyll content and fluorescence were also conducted. Thus, the leaf greenness index expressed in SPAD units was measured using a portable Chlorophyll Meter SPAD-502 Plus, and the chlorophyll fluorescence analyses were made on dark-adapted leaf material using an IMAGING-PAM M-Series Chlorophyll Fluorimeter a MAXI version manufactured by the Heinz Walz. The initial (F_0) and the maximal fluorescence (F_m), as well as their derivatives (F_v, F_v/F_0, F_v/F_m) were recorded. Similar measurements were made in the middle of October (19 weeks after the first treatment). However, the shoots were categorized into different groups (10–20 cm and >20 cm long) since counting of shorter (<10 cm) shoots was too time-consuming and troublesome. Additionally, the diameter of the longest shoot base on each plant was determined.

Data analyses. Forty-eight plants of each cultivar were treated with each combination of the tested chemicals. However, detailed measurements were made only on eighteen representative plants of each treatment, meaning that about eight untypical (4 weakest and 4 strongest) plants from each treatment were excluded and plants for measurements were chosen randomly from the remaining pot. Collected data were submitted to ANOVA, LSD mean separation test at $P < 0.05$ significance level and cluster analysis according to Ward's method using Statistica 12 computer software.

RESULTS

Shoot growth

Differences in the vegetative growth of shoots of blueberry control plants among tested cultivars were found in summer (Tab. 1). Without any chemical applied, 'Darrow' plants developed significantly more shorter and longer shoots than the other two clones, and thus they were much denser. On the other hand, 'Bluecrop' plants produced longer shoots than the other clones (Tab. 1). Dikegulac activated axillary buds at many leaf axes, promoting the growth of secondary shoots (Fig. 1a, b). The plants of all tested clones developed more shoots after being treated with dikegulac (Tab. 1, Fig. 1c, e, g). However, cultivars responded differently to the tested chemicals what was confirmed by the significant cultivar × treatments interactions (Tab. 1). The reaction of 'Bluecrop' plants to dikegulac was the strongest, especially in the case of proliferation of shorter shoots. Contrary to the other cultivars, 'Brigitta blue' plants developed significantly more longer shoots than the control plants, and the elongation of the main shoots was not significantly reduced (Tab. 1, Fig. 1g). On the other hand, application of Asahi SL did not affect the proliferation of shoots. Only 'Darrow' plants developed significantly longer shoots after being treated with nitrophenolates. Only a few interactions of the tested chemicals were found. Mainly the antagonistic influence of dikegulac and Asahi SL on shoot elongation was ascertained in the case of 'Darrow' and 'Bluecrop' clones (Tab. 1).

The differences in the vegetative growth of shoots of blueberry control plants among tested cultivars remained till autumn. 'Darrow' control plants developed significantly more shorter and longer shoots than the other two clones (Tab. 2). 'Bluecrop' plants developed the longest shoots whereas 'Brigitta blue' ones the shortest. No significant differences in the diameter of shoot base among cultivars were found (Tab. 2). In general, the distinctly different long-term (several months) reaction of the three cultivars to the tested chemicals was noticed, especially in the case of dikegulac (Tab. 2, Fig. 1d, f, h). Dikegulac-treated 'Brigitta blue' and particularly 'Bluecrop' plants developed significantly more shoots. A similar effect was not found in the case of 'Darrow' clone. In the presence of dikegulac, the shoot elongation of 'Darrow' and 'Bluecrop' cultivars was reduced contrary to 'Brigitta blue' (Tab. 2). Dikegulac applied alone did not affect shoot diameter. The application of Asahi SL did not change shoot proliferation but had various impact on shoot elongation. It enhanced the growth (both length and diameter) of 'Darrow' shoots while it did not affect 'Bluecrop' plants, or even limited it ('Brigitta blue'). Some interactions of the tested chemicals were found mainly in the case of 'Darrow' clone. When Asahi SL was applied in combination with dikegulac, they inhibited the proliferation of 'Darrow' shoots whereas when it was used alone it did not influence it. The tested chemicals

Table 1. Vegetative growth of highbush blueberry plants treated with dikegulac 'D' and/or Asahi 'A' (mid-July, about 1 month after application)

Cultivar (Cv)	D	A	No. of shorter shoots (<10 cm)	No. of longer shoots (>10 cm)	Total number of shoots	Length of the longest shoot (cm)
'Darrow'	+	+	45.6 b[1]	4.1 a	49.7 b	15.2 a
	+	–	42.2 b	4.6 a	46.8 b	15.9 a
	–	+	15.4 a	10.2 b	25.6 a	29.1 c
Control	–	–	15.4 a (B)[2]	8.8 b (B)	24.3 a (B)	23.6 b (A)
Interaction (D×A) SL[3]			ns	ns	ns	**
'Bluecrop'	+	+	20.2 b	5.6 b	25.8 b	18.2 a
	+	–	19.6 b	3.6 a	23.2 b	16.9 a
	–	+	3.4 a	5.3 b	8.8	29.2 b
Control	–	–	2.8 a (A)	5.9 b (A)	8.7 a (A)	28.6 b (B)
Interaction (D×A) SL			ns	**	ns	ns
'Brigitta	+	+	12.9 c	8.0 c	20.9 c	22.5 a
blue'	+	–	9.2 b	7.6 bc	16.7 b	22.9 a
	–	+	2.8 a	6.2 ab	9.0 a	26.0 b
Control	–	–	3.1 a (A)	5.2 a (A)	8.3 a (A)	24.2 ab (A)
Interaction (D×A) SL			ns	ns	ns	ns
Interaction (Cv×DA) SL			***	***	***	***

[1] differences among treatments based on $LSD_{0.05}$ within every cultivar separately (small letters)
[2] differences among control plants of studied cultivars based on $LSD_{0.05}$ (uppercase letters)
[3] level of significance: ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

acted antagonistically on shoot growth (elongation, diameter), and the influence of dikegulac was usually stronger (Tab. 2).

Growth of leaves

In summer, the control plants of 'Brigitta blue' developed bigger and relative wider leaves than did the other clones (Tab. 3). Differences in the leaf shape (length/width ratio) among the studied cultivars were not observed. The significant cultivar-specific reaction to the tested chemicals was noticed when the leaf size was considered (Tab. 3). Dikegulac reduced the leaf size of all clones, and its effect was stronger on its width than on its length. As a result, the leaves of dikegulac-treated plants were more slender. The reaction of the cultivars to the application of Asahi SL was not so uniform. It increased significantly the size of leaf blades of 'Bluecrop' plants (Tab. 3). Such an effect was not observed in the other clones. The interaction between the two tested chemicals in the case

a. Activation of lateral buds after dikegulac application

b. Further growth of secondary shoots after dikegulac application

c. 'Darrow' (mid-July, about 1 month after application), from left to right: 'A+D+', 'A-D+', 'A+D-', 'A-D-'

d. 'Darrow' (mid-October, about 4 months after application), from left to right: 'A+D-', 'A-D-', 'A+D+', 'A-D+'

e. 'Bluecrop' (mid-July, about 1 month after application), from left to right: 'A+D+', 'A-D+', 'A+D-', 'A-D-'

f. 'Bluecrop' (mid-October, about 4 months after application), from left to right: 'A+D-', 'A-D-', 'A+D+', 'A-D+'

g. 'Brigitta blue' (mid-July, about 1 month after application), from left to right: 'A+D+', 'A-D+', 'A+D-', 'A-D-'

h. 'Brigitta blue' (mid-October, about 4 months after application), from left to right: 'A+D-', 'A-D-', 'A+D+', 'A-D+'

Fig. 1. Highbush blueberry potted plants after dikegulac 'D+' and/or Asahi SL 'A+' treatment ('A+D+' – Asahi SL and dikegulac; 'A-D+' – dikegulac alone; 'A+D-' – Asahi SL alone; 'A-D-' – control plants)

Table 2. Vegetative growth of highbush blueberry plants treated with dikegulac 'D' and/or Asahi 'A' (mid-October, about 4 months after application)

Cultivar (Cv)	D	A	No. of shorter shoots (10–20 cm)	No. of longer shoots (>20 cm)	Total number of shoots	Length of the longest shoot (cm)	Diameter of the longest shoot at base (mm)
'Darrow'	+	+	7.4 a[1]	4.4 a	11.8 a	38.1 a	4.3 a
	+	–	14.9 b	4.6 a	19.5 b	37.9 a	4.5 ab
	–	+	15.8 b	6.4 b	22.2 b	58.2 c	6.4 c
Control	–	–	15.3 b (B)[2]	5.4 ab (B)	20.7 b (B)	47.7 b (B)	5.2 b (A)
Interaction (D×A) SL[3]			***	ns	***	**	**
'Bluecrop'	+	+	12.3 b	5.0 b	17.3 b	52.4 a	5.5 a
	+	–	11.6 b	5.7 b	17.3 b	49.8 a	5.0 a
	–	+	3.9 a	5.4 b	9.3 a	62.7 b	5.2 a
Control	–	–	5.7 a (A)	4.1 a (A)	9.7 a (A)	61.3 b (C)	5.2 a (A)
Interaction (D×A) SL			ns	**	ns	ns	ns
'Brigitta blue'	+	+	9.3 b	4.6 a	13.9 b	38.8 ab	5.7 a
	+	–	8.6 b	5.0 a	13.6 b	39.2 b	6.3 a
	–	+	5.7 a	4.7 a	10.4 a	35.6 a	5.6 a
Control	–	–	6.5 a (A)	4.3 a (A)	10.8 a (A)	40.8 b (A)	5.8 a (A)
Interaction (D×A) SL			ns	ns	ns	*	ns
Interaction (Cv×DA) SL			***	**	***	***	***

[1] differences among treatments based on $LSD_{0.05}$ within every cultivar separately (small letters)
[2] differences among control plants of studied cultivars based on $LSD_{0.05}$ (uppercase letters)
[3] level of significance: ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

of 'Bluecrop' plants, showed that they act antagonistically and the effect of dikegulac was stronger than the Asahi's SL one (Tab. 3).

Differences in leaf size among control plants of the studied cultivars were not reported in autumn (Tab. 4). However, 'Bluecrop' leaves were relative broader than the ones of the other clones (lower leaf length/width ratio). The long-term influence of the tested chemicals on the leaf vegetative traits were not ascertained in the case of two clones ('Brigitta blue', 'Bluecrop') – Table 4. Dikegulac reduced the leaf length of 'Darrow' plants whereas did not influence its width. As a result the leaves of dikegulac-treated plants were relative broader. Asahi SL had no proven effect on leaf traits. However, when applied in combination with dikegulac, it significantly increased the leaf width and neutralized the negative impact of dikegulac on leaf length (Tab. 4).

Chosen aspects of photosynthesis

In summer, significant differences among control plants of the studied blueberry cultivars regarding the relative chlorophyll content (RChC) and the photosynthesis yield (F_v/F_0, F_v/F_m) were not ascertained (Tab. 5). However, the value of initial chlorophyll fluorescence (F_0) recorded for 'Darrow' was significantly higher than for 'Brigitta blue' whereas was intermediate for 'Bluecrop' clone. The maxi-

mal fluorescence (F_m) measured for 'Darrow' control plants were significantly higher than for other clones (Tab. 5). The tested chemicals did not change distinctly the values of any fluorescence parameters recorded for all studied clones. The obtained values varied up to 4% from the appropriate controls and such differences were not statistically significant (Tab. 5). The only one clone-specific difference was found in the case of the RChC. Dikegulac significantly reduced the RChC of 'Bluecrop' leaves, while it did not affect the other two cultivars. When Asahi SL was applied alone it did not influence the RChC, whereas when it was combined with dikegulac, it significantly increased for 'Brigitta blue' plants and decreased it for the rest of the tested cultivars (Tab. 5).

Significant differences among control plants of the studied blueberry cultivars were also observed in autumn. Generally, the values of relative chlorophyll content and fluorescence recorded for 'Bluecrop' control plants were significantly lower than the corresponding values of the other two cultivars (Tab. 6). The tested chemicals had various impacts on the studied clones regarding the relative chlorophyll content (RChC) – Table 6. In the presence of dikegulac the RChC was reduced in the leaves of 'Bluecrop' whereas such an effect was not confirmed for the other cultivars. Asahi SL did not change the values of this factor. The higher effect was reported when the two formulations were combined, as they increased RChC in 'Brigitta blue' leaves and decreased it in the leaves of the other two

Table 3. Leaf growth of highbush blueberry plants treated with dikegulac 'D' and/or Asahi 'A' (mid-July, about 1 month after application)

Cultivar (Cv)	D	A	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Leaf length/ width ratio
'Darrow'	+	+	4.8 a[1]	2.8 a	10.7 a	1.8 a
	+	–	5.1 a	2.9 a	11.6 a	1.8 a
	–	+	8.0 b	4.6 b	29.6 b	1.7 a
Control	–	–	7.4 b (A)[2]	4.4 b (A)	26.2 b (A)	1.7 a (A)
Interaction (D×A) SL			ns	ns	ns	ns
'Bluecrop'	+	+	6.6 b	3.5 b	18.3 ab	1.9 b
	+	–	6.0 a	3.1 a	14.7 a	2.0 b
	–	+	9.2 c	5.4 d	39.5 c	1.7 a
Control	–	–	7.0 b (A)	4.0 c (A)	22.3 b (A)	1.8 a (A)
Interaction (D×A) SL			***	**	***	ns
'Brigitta blue'	+	+	7.7 a	3.8 a	23.1 a	2.1 b
	+	–	8.1 a	3.9 a	25.4 a	2.1 b
	–	+	9.9 b	5.3 b	41.3 b	1.9 a
Control	–	–	10.2 b (B)	5.5 b (B)	44.1 b (B)	1.9 a (B)
Interaction (D×A) SL			ns	ns	ns	ns
Interaction (Cv×DA) SL			***	***	***	ns

[1] differences among treatments based on $LSD_{0.05}$ within every cultivar separately (small letters)
[2] differences among control plants of studied cultivars based on $LSD_{0.05}$ (uppercase letters)
[3] level of significance: ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4. Leaf growth of highbush blueberry plants treated with dikegulac 'D' and/or Asahi 'A' (mid-October, about 4 months after application)

Cultivar (Cv)	D	A	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Leaf length/ width ratio
'Darrow'	+	+	10.5 c[1]	6.1 b	51.0 b	1.7 ab
	+	–	9.3 a	5.7 ab	42.6 ab	1.6 a
	–	+	9.5 ab	5.3 a	39.8 a	1.8 bc
control	–	–	10.3 bc (A)[2]	5.5 a (A)	45.3 ab (A)	1.9 c (B)
Interaction (D×A) SL[3]			**	ns	*	*
'Bluecrop'	+	+	9.5 a	5.4 a	40.6 a	1.8 a
	+	–	9.7 a	5.6 a	43.5 a	1.7 a
	–	+	9.9 a	5.7 a	44.8 a	1.8 a
Control	–	–	9.7 a (A)	5.5 a (A)	42.8 a (A)	1.8 a (A)
Interaction (D×A) SL			ns	ns	ns	ns
'Brigitta blue'	+	+	10.2 a	5.6 a	46.1 a	1.9 a
	+	–	10.1 a	5.3 a	43.0 a	1.9 a
	–	+	10.6 a	5.6 a	47.1 a	1.9 a
Control	–	–	10.6 a (A)	5.5 a (A)	47.1 a (A)	1.9 a (B)
Interaction (D×A) SL			ns	ns	ns	ns
Interaction (Cv×DA) SL			ns	ns	ns	*

[1] differences among treatments based on $LSD_{0.05}$ within every cultivar separately (small letters)
[2] differences among control plants of studied cultivars based on $LSD_{0.05}$ (uppercase letters)
[3] level of significance: ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

clones. The applied chemicals did not alter the values of the basic fluorescence parameters (F_0, F_m) of 'Bluecrop' and 'Brigitta blue' leaves. However, some of them (F_v/F_0 and F_v/F_m) were changed in the case of 'Darrow' plants. The application of dikegulac and/or Asahi SL significantly increased their values compared to the control plants (Tab. 6).

Similarity of cultivar reaction

Cluster analysis allowed to distinguish two main categories of nursery plants: treated and untreated ones with dikegulac (Fig. 2a–c). Such analyses were consistent with the visual observation of plants (Fig. 1c–h). The influence of Asahi SL in the applied dose was definitely weaker in all tested cultivars.

DISCUSSION

The aim of the present study was to examine the effect of the foliar application of dikegulac and Asahi SL on blueberry propagation, and to assess their usefulness in the shoot production of highbush blueberry nursery plants. Dikegulac is a by-product in the synthesis of vitamin C [Rademacher 2015]. It is a growth retardant which was found to reduce apical dominance and promote lateral branching in some species [Sachs et al. 1975, Norcini et al. 1994, Jacyna et al. 1994, Sansberro et al. 2006, Litwińczuk and Prokop 2010, Cochran and Fulcher 2013, Sarropoulou et al. 2014, Sun et al. 2015]. Although dikegulac has been known since 1975 [Rademacher 2015] the mechanism of

Table 5. The relative chlorophyll content (RChC) and fluorescence of leaves of highbush blueberry plants treated with dikegulac 'D' and/or Asahi 'A' (mid-July, about 1 month after application)

Cultivar (CV)	D	A	RChC (SPAD)	F_0 (r.u.)	F_m (r.u.)	F_v/F_0 (r.u.)	F_v/F_m (r.u.)
'Darrow'	+	+	31.7 a[1]	0.164 a	0.570 a	2.5 a	0.710 a
	+	–	33.0 ab	0.164 a	0.582 a	2.6 a	0.717 a
	–	+	35.8 b	0.162 a	0.579 a	2.6 a	0.719 a
Control	–	–	35.7 b (A)[2]	0.164 a (B)	0.581 a (B)	2.6 a (A)	0.715 a (A)
Interaction (D×A) SL[3]			ns	ns	ns	ns	ns
'Bluecrop'	+	+	32.3 a	0.152 a	0.521 a	2.4 a	0.706 a
	+	–	32.3 a	0.151 a	0.531 a	2.5 a	0.713 a
	–	+	36.6 b	0.149 a	0.528 a	2.6 *a*	0.716 a
Control	–	–	36.7 b (A)	0.152 a (AB)	0.522 a (A)	2.4 a (A)	0.705 a (A)
Interaction (D×A) SL			ns	ns	ns	ns	ns
'Brigitta blue'	+	+	40.9 c	0.143 a	0.499 a	2.5 a	0.710 a
	+	–	35.9 a	0.141 a	0.490 a	2.5 a	0.711 a
	–	+	39.9 bc	0.148 a	0.526 a	2.6 a	0.716 a
Control	–	–	37.0 ab (A)	0.144 a (A)	0.503 a (A)	2.5 a (A)	0.712 a (A)
Interaction (D×A) SL			ns	ns	ns	ns	ns
Interaction (Cv×DA) SL			**	ns	ns	ns	ns

[1] differences among treatments based on $LSD_{0.05}$ within every cultivar separately (small letters)
[2] differences among control plants of studied cultivars based on $LSD_{0.05}$ (uppercase letters)
[3] level of significance: ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

its action is still unclear. There are some hypotheses about it. According to Cline [1996, 1997], dikegulac can act as an anti-auxin, like 2,3,5-triiodobenzoic acid (TIBA), which blocks auxin translocation and reduces the apical dominance. Dikegulac most probably inhibits gibberellin biosynthesis by inhibiting the oxidation of ent-kaurene to ent-kaurenic acid [Thetford and Berry 2000]. Such a hypothesis is supported by the fact that its action is counteracted by GA_3 [Bocion and de Silva 1977]. On the other hand, Mendoza-De Gyves et al. [2008] propose that dikegulac at 16.9 to 100.5 μM probably enhances cytokinin action on olive (*Olea europaea* L.) *in vitro* shoot formation whereas at higher concentrations (133.4 μM) it inhibits it. Unlike EU member countries, dikegulac is still used as a chemical pinching agent in many ornamental species in USA [Whipker and Latimer 2016]. Dikegulac is reported to be a relatively non-phytotoxic plant growth regulator [Pozo et al. 2004]. However, it caused high leaf loss, floral abscission and fruit peel damage in citrus [Pozo et al. 2004]. Other adverse effect of the use of dikegulac is the reduction of the size of leaf blades and the slight chlorosis observed occasionally in several plant species [Jacyna et al. 1994, Banko and Stefani 1996, Sansberro et al. 2006, Litwińczuk and Prokop 2010, Grossman et al. 2013, Whipker and Latimer 2016]. Some of the effects of nitrophenolates, such as the stimulation of leaf growth, the increase of leaf chlorophyll content [Djanaguiraman et al. 2009, Przybysz et al. 2010, Chen and Dong 2016], and the enhancement of shoot elongation [Djanaguiraman et al. 2004, 2005a, b], are opposite to the negative effects of

Table 6. The relative chlorophyll content (RChC) and fluorescence of leaves of highbush blueberry plants treated with dikegulac 'D' and/or Asahi 'A' (mid-October, about 4 months after application)

Cultivar (CV)	D	A	RChC (SPAD)	F_0 (r.u.)	F_m (r.u.)	F_v/F_0 (r.u.)	F_v/F_m (r.u.)
'Darrow'	+	+	49.0 a[1]	0.121 a	0.445 a	2.7 b	0.726 b
	+	–	49.1 a	0.124 a	0.468 a	2.8 b	0.731 b
	–	+	45.7 a	0.118 a	0.435 a	2.7 b	0.726 b
Control	–	–	49.5 a (B)[2]	0.135 a (B)	0.452 a (B)	2.3 a (B)	0.694 a (B)
Interaction (D×A) SL[3]			ns	ns	ns	*	**
'Bluecrop'	+	+	42.0 a	0.107 a	0.335 a	2.1 a	0.674 a
	+	–	39.3 a	0.108 a	0.360 a	2.3 a	0.690 a
	–	+	38.6 a	0.108 a	0.343 a	2.2 a	0.676 a
Control	–	–	36.8 a (A)	0.101 a (A)	0.300 a (A)	1.9 a (A)	0.648 a (A)
Interaction (D×A) SL			ns	ns	ns	ns	ns
'Brigitta blue'	+	+	46.8 a	0.117 a	0.393 a	2.4 a	0.698 a
	+	–	47.9 a	0.112 a	0.398 a	2.5 a	0.712 a
	–	+	49.1 a	0.104 a	0.374 a	2.6 a	0.716 a
Control	–	–	52.2 a (B)	0.102 a (A)	0.354 a (A)	2.5 a (B)	0.705 a (B)
Interaction (D×A) SL			ns	ns	ns	ns	ns
Interaction (Cv×DA) SL			*	ns	ns	ns	ns

[1] differences among treatments based on $LSD_{0.05}$ within every cultivar separately (small letters)
[2] differences among control plants of studied cultivars based on $LSD_{0.05}$ (uppercase letters)
[3] level of significance: ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

dikegulac. Stutte and Clark [1990] explained last phenomenon (i.e. the stronger shoot growth) by the presence of higher concentration and/or activity of auxins, as a result of the higher inhibition of IAA oxidase and/or the higher number of high-affinity binding sites of IAA [Libbenga and Mennes 1987]. As mentioned above, dikegulac could act as TIBA [Cline 1996, 1997] whereas the effect of TIBA on the morphological characteristics had been reverted by nitrophenolates [Djanaguiraman et al. 2005b]. Therefore it was interesting to check whether nitrophenolates like Asahi SL could counteract the negative effects of dikegulac (leaf reduction and chlorosis) in highbush blueberry plants.

In the present study, the control plants of all highbush blueberry cultivars differed in terms of shoot and leaf growth, which seems to be a common phenomenon in many species, including highbush blueberry. All clones were more or less susceptible to the tested chemicals as indicated by the significant interactions of cultivar × treatments. Nonetheless, the influence of dikegulac in the applied dose was generally much stronger than the impact of Asahi SL. As expected, dikegulac significantly stimulated branching of the blueberry potted plants. It seems that dikegulac-treated plants might be a good and efficient source of softwood cuttings used in the traditional propagation of blueberries. However, it should be mention that rooting of shoots obtained in such way was not checked in the present study, unfortunately. Nevertheless, in a previous study none negative effect of dikegulac on shoot rooting were found [Litwińczuk and Prokop 2010]. Thus, it is possible, its application to facilitate

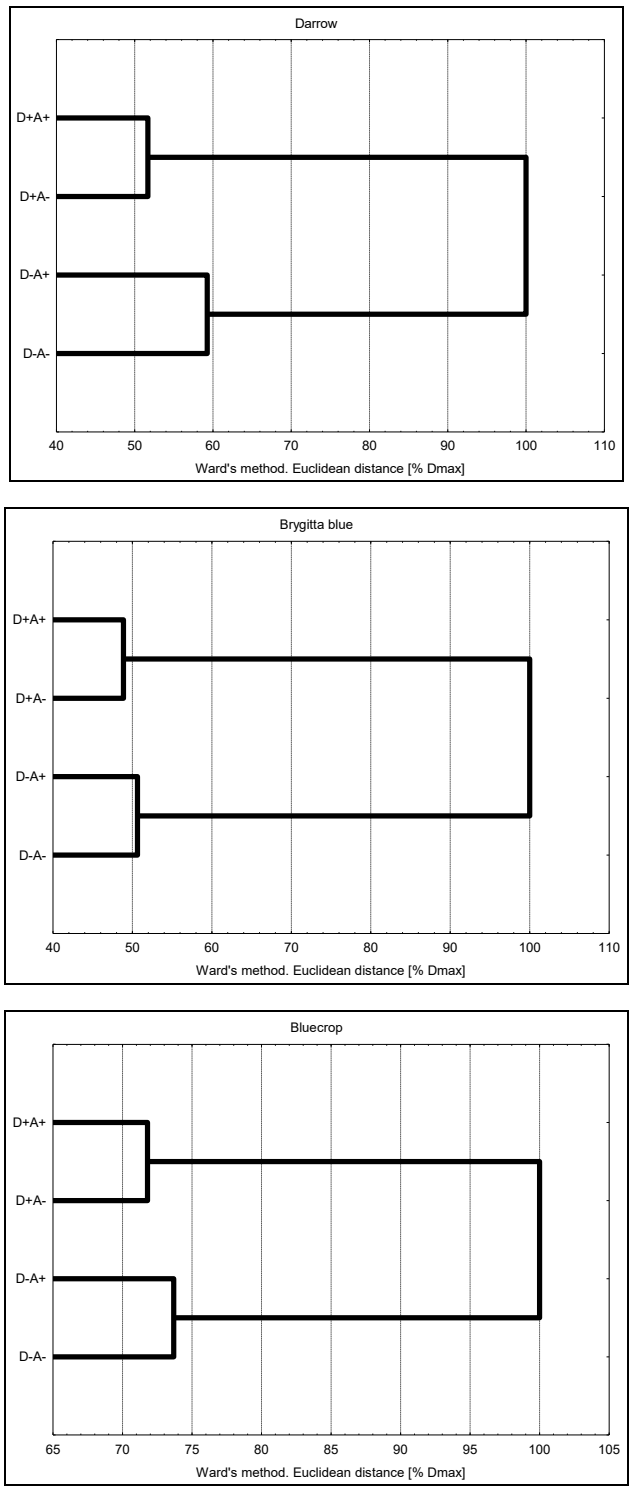

Fig. 2. Similarity of growth of highbush blueberry under the influence of dikegulac 'D+' and Asahi SL 'A+' ('A+D+' – Asahi SL and dikegulac, 'A-D+' – dikegulac alone, 'A+D-' – Asahi SL alone, 'A-D-' – control plants)

the combination of biotechnological and conventional propagation methods, as it takes place in the case of strawberry. For 'Bluecrop' and 'Darrow' cultivars, dikegulac limited shoot elongation, which is a well-known impact of growth retardants on ericaceous ornamentals [Banko and Stefani 1995, Marosz and Matysiak 2005, Litwińczuk and Prokop 2010]. To a certain extent, it might be considered positively as it facilitates nursing more plants at a smaller area. However, such a phenomenon was not observed in the case of 'Brigitta blue' clone. The influence of dikegulac on this cultivar was also most short-termed as treated plants resembled the control ones in autumn, in contrast to the other clones. In accordance with previous reports [Jacyna et al. 1994, Banko and Stefani 1996, Sansberro et al. 2006, Litwińczuk and Prokop, 2010, Grossman et al. 2013, Whipker and Latimer 2016], when dikegulac was applied alone it reduced the size of leaf blades of all clones in summer. Moreover, it relatively slendered the leaves of 'Bluecrop' and 'Brigitta Blue' clones. The leaf width depends more on the avaiability of saccharides than leaf length. It seems that narrowing of 'Bluecrop' and 'Brigitta Blue' leaves were caused by enhanced proliferation of shoots which required more assimilates, thus by modified distribution of assimilates. To a certain extent it may be also explained by worsened photosynthesis because of lower chlorophyll content. However, it seems that dikegulac did not generate plant stress as both basic chlorophyll fluorescence parameters (F_0, F_m) and the photosynthesis yield (F_v/F_m, F_v/F_0) remained unchanged while compared with control. The changes of leaves were not observed in autumn (4 months after application), with the exception of 'Darrow' plants treated previously with dikegulac, which leaves were relative wider than the control ones and presented improved efficiency of PS II (F_v/F_m, F_v/F_0). They developed also shorter shoots. Probably they had produced an excess of assimilates what became visible in the form of wider leaves. In comparison with dikegulac the results of the application of nitrophenolates were less intense. Generally, Asahi SL alone did not stimulate plant branching. Its influence on shoot elongation was clone-dependent. In summer, only 'Darrow' plants developed stronger shoots than the control ones. This effect was also preserved in autumn. On the other hand, 'Brigitta Blue' plants treated previously with Asahi SL had shorter main (skeletal) shoots than the control ones in autMK et al. 2015]. Generally, only a few interactions of the tested chemicals were confirmed, and mainly in such cases the antagonistic influence of dikegulac and Asahi SL on the growth of shoots and leaves was found. Only in one case, dikegulac and Asahi SL acted synergistically and inhibited shoot proliferation of 'Darrow' plants in late summer and autumn.

CONCLUSIONS

Summarizing, the nursery plants of the studied highbush blueberry cultivars differed in their reaction to the tested chemicals (foliar sprays of dikegulac 0.1% and/or Asahi SL 0.2%,), concerning the growth of shoots and leaves. The genotype-specific reaction to several treatments is a well-known fact in horticulture. However, it may obstruct their application in the nursery of blueberry plants. Dikegulac stimulated significantly branching of the blueberry pot plants. It reduced the shoot elongation of 'Bluecrop' and 'Darrow' plants. Moreover, its application limited the size of leaves but generally did not influence the chlorophyll fluorescence and relative content. Therefore, it seems that dikegulac applied as a foliar spray can be helpful in conventional propagation of highbush blueberry plants especially by softwood cuttings since it promotes the development of new shoots. After such treatment potted mother plants become a efficient source of cuttings. Thus, the application of dikegulac may solve the problem of shortage of plant material for propagation. The influence of dikegulac in the applied dose was much stronger than the impact of Asahi SL, which alone or together with dikegulac, did not give any advantageous results from a practical point of view. Thus, it seems its usage in highbush blueberry nurseries is unnecessary.

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