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EFFECT OF AUXINS AND THE BIOSTIMULATOR ALGAMINOPLANT ON RHIZOGENESIS IN STEM CUTTINGS OF TWO DOGWOOD CULTIVARS (*Cornus alba* 'AUREA' AND 'ELEGANTISSIMA')

Andrzej Pacholczak, Wiesław Szydło, Ewelina Jacygrad, Mariusz Federowicz

Warsaw University of Life Sciences – SGGW

Abstract. The UE-imposed restrictions on the manufacture and application of plant protection chemicals impose on the nurseryman the need to screen for new substances that are environmentally friendly and yet effective in the production of plant material. Biopreparations may constitute such a group as they contain substance little affecting the environment. The aim of the work was to evaluate effects of the biopreparation Algamino-Plant on rooting of stem cuttings in *Cornus alba* 'Aurea' and *C. alba* 'Elegantissima'. Freshly harvested cuttings were sprayed with the 0.2% water solution of the biopreparation. To compare its effectiveness with the commercial rooting powders used routinely in the nursery production part of the cuttings were treated with "Ukorzeniacz AB" (0.3% NAA) or Rhizopon AA (2% IBA). Both powders little affected rhizogenesis. Application of the biopreparation increased percentage and degree of rooting in dogwood as compared to control cuttings what shows its potential in a commercial production. The above treatment increased chlorophyll, carbohydrate contents and free amino acids in leaves of the sprayed cuttings.

Key words: biopreparations, IBA, cuttings, rooting, chlorophyll, carbohydrates

INTRODUCTION

The UE-imposed restrictions on the manufacture and application of plant protection chemicals impose on the nurseryman the need to screen for new substances that are environmentally friendly and yet effective in the production of plant material. Biopreparations may constitute such a group as they contain substance little affecting the environment. Biostimulants induce plant resistance to different stresses and they can also

Corresponding author – Adres do korespondencji: Andrzej Pacholczak Department of Ornamental Plants, Warsaw University of Life Sciences – SGGW, ul. Nowoursynowska 159, 02-787 Warszawa, Poland, e-mail: pacholczak@poczta.onet.pl; andrzej_pacholczak@sggw.pl

positively affect rhizogenesis and the root system development [Pruszyński 2008]. They are either single or multi-compound preparations, containing extracts from sea algas (Eclonia maxima, Laminaria digitata, Fucus vesiculosus or Durvillea potatorum) [Dobrzański et al. 2008]. Such biopreparations as AlgaPlant, Kelpak or AlgaminoPlant may contain low molecular polypeptides and amino acids, vitamins, enzymes, phytohormones, sugars, betains and antioxidants [Basak 2008]. These active compounds may stimulate rhizogenesis by inducing anatomical and morphological changes in plants [Cambri et al. 2008]. Our previous results showed that certain biopreparations not only enhanced rhizogenesis in cuttings of ornamental shrubs but could even replace the rooting powders [Jacygrad and Pacholczak 2010, Pacholczak et al. 2010].

The aim of this work was to compare a biological preparation AlgaminoPlant with two commercial rooting powders containing synthetic auxins, commonly used in nurseries. Its effect was checked on two cultivars of the popular ornamental shrub – dogwood (*Cornus alba* L.). An attempt was also made to detect a relationship between several different biochemical parameters of cuttings, as influenced by treatments, and the subsequent rhizogenesis

MATERIAL AND METHODS

The experiments were carried out in 2009 and 2010 in a commercial nursery of M.M. Kryt in Wola Prażmowska on two cultivars of *Cornus alba* 'Aurea' and 'Elegantissima'. In both years the single node cuttings were prepared from shoots freshly harvested from stock plants on July 1. For the type of cuttings used in the trials this date was found optimal in preliminary experiments carried out earlier. Leaves on cuttings were cut in half to reduce transpiration during rooting. AlgaminoPlant was used as 0.2% water solution. A pressure hand sprayer (2 dm^{-3} volume) was used to spray the cuttings with the biostimulator solution until run-off. The cuttings were sprayed once (July 1), twice (July 1 and 8) or three times (July 1, 8 and 15), depending on a treatment (tab. 1). Auxins were applied in the form of commercially available rooting powders: Ukorzeniacz AB containing 0.3% NAA or Rhizopon AA (2% IBA), directly to the bases of cuttings. Because of the poor results obtained in 2009 with Ukorzeniacz AB and its uncertain legislative situation this rooting powder was omitted from the second-year experiment. The second powder (Rhizopone AA) remained as it registration date expires later than that of Ukorzeniacz and it contains IBA – the auxin of a less drastic action. Spraying cuttings with the water solution of IBA (200 $mg \cdot dm^{-3}$) was introduced instead as another reference treatment to compare the biopreparation efficiency. This treatment allowed to evaluate as well the IBA action applied in a different, more safe way as a foliar application and not as a powder

Cuttings were rooted in styrofoam boxes. They were inserted to a depth of 2 cm into a mixture of peat, perlite and sand (2:1:1), pH 5.0. Rooting took place in plastic tunnels equipped with automatic watering and mist systems as well as with shading devices. Every two weeks, the cuttings were sprayed against *Botrytis* with 0.2% Rovral or Sumilex. The experiment consisted of 8 treatments (tab. 1), each in 3 replications, and each replication containing 20 cuttings. Percentage of rooted cuttings and the degree of

rooting was determined after 6 weeks from the beginning of the rooting experiment, i.e. on August 8. The degree of rooting was evaluated on a 5-point scale describing the development of the root ball (tab. 2). The scores for the degree of rooting represent means of three independent observations by trained personnel.

Table 1. A list of treatments in the experiments
Tabela 1. Sposób traktowania sadzonek w doświadczeniach

No. of treatment Nr kombinacji	Methods of cuttings' treatment Sposób traktowania sadzonek
1*	Control – Kontrola "0" (H_2O)
2*	Rhizopon AA (2% IBA) powder – puder
3	Rhiz. AA (2% IBA) + 3× sprayings with AlgaminoPlant 0.2% Rhiz. AA (2% IBA) + 3× opryskiwanie 0.2% preparatem AlgaminoPlant
4	Ukorzeniacz AB (0.3% NAA) powder – puder
5	Ukorzeniacz AB (0.3% NAA) + 3× sprayings with AlgaminoPlant 0.2% Ukorzeniacz AB (0,3% NAA) + 3× opryskiwanie 0.2% preparatem AlgaminoPlant
6*	1 spraying with AlgaminoPlant 0.2% 1× opryskiwanie 0,2% AlgaminoPlant
7*	2 sprayings with AlgaminoPlant 0.2% 2× opryskiwanie 0,2% AlgaminoPlant
8*	3 sprayings with AlgaminoPlant 0.2% 3× opryskiwanie 0,2% AlgaminoPlant
9*	1 spraying with IBA 200 mg $\cdot dm^{-3}$

2009 – treatments:1–8; 2010 – treatments*1, 2, 6, 7, 8, 9

Table 2. Evaluation scale of the root development
Tabela 2. Skala oceny bonitacyjnej stopnia ukorzenienia sadzonek

No. Nr	Characteristic of the degree of rooting Charakterystyka stopnia ukorzenienia	Score Liczba punktów
1	Cutting without visible roots Sadzonka bez widocznych korzeni.	1
2	A few shorts roots Kilka krótkich korzeni.	2
3	Roots with numerous branched roots, no root ball Korzenie licznie rozgałęzione, brak bryły korzeniowej	3
4	An average root system forming a root ball Średniej wielkości system korzeniowy tworzący bryłę korzeniową	4
5	Well developed, branched root system forming a root ball Dobrze wykształcony, rozgałęziony system korzeniowy, tworzący bryłę korzeniową.	5

In both years samples for biochemical analyses (twenty cuttings per treatment) were collected on July 18 from the control untreated cuttings and those sprayed three times with AlgaminoPlant. They were finely chopped, thoroughly mixed and 0.5–1 g samples

were taken for the measurements of dry matter, total chlorophyll, reducing sugars and free amino acid' contents. Triplicate extracts were prepared for each analysis and three measurements were done for each extract giving nine readings for each data point.

For the dry matter content, 1 g samples were dried at 105°C to constant weight [Strzelecka et al. 1982]. Chlorophyll content was analyzed according to Morgan and Porath [1980] with the modification of Inskeep and Bloom [1985]. Reducing sugars were determined by the colorimetric method of Somogyi in the Nelson's modification [Nelson 1944] and free amino acids were measured according to Rosen [1957]. To compare the means, rooting percentages were transformed according to Bliss [Wójcik and Laudański 1989] while the degree of rooting by root transformation: $y = x^2 + (x + 1)^2$ subjected to ANOVA 1 and tested by the Duncan's test at $\alpha = 0.05$. Results of analyses were subjected to the 2-factorial ANOVA and the means were compared by the Duncan's test at $\alpha = 0.05$.

RESULTS

A single-factorial analysis of variance showed a significant effect of the treatments in 2009 on a root ball development and a percentage of rooted cuttings in both dogwood cultivars: 'Aurea' and 'Elegantissima'.

***C. alba* 'Aurea'**. Less than 50% of the untreated control cuttings produced roots during 6 weeks of the experiment and their root balls were not formed. Application of the commercial powders did not enhanced rhizogenesis, on the contrary, Rhizopon AA significantly decreased a percentage of rooted cuttings. A joint application of the powders and the biopreparation AlgaminoPlant slightly but significantly improved the rooting: percentage of the rooted cuttings in case of Rhizopon AA and the degree of rooting in case of Ukorzeniacz AB. The best effects were observed when the biopreparation was used alone, especially if applied twice on cuttings: this treatment increased the percentage of rooted cuttings to nearly 80%, positively affecting as well the root ball forming. All three treatments with AlgaminoPlant were better than the commercial powders (fig. 1).

In 2010 the positive effect of Algaminoplant on rhizogenesis was confirmed (fig. 1a): the best percentage of rooting was obtained after the twofold spraying with the biopreparation – doubled as compared to the control cuttings. This treatment significantly improved as well the root ball development (expressed as a degree of rooting), similarly as treatments with IBA – either in a rooting powder or by spraying cuttings with the auxin water solutions.

***C. alba* 'Elegantissima'**. In 2009 the control, untreated cuttings of this cultivars rooted in 70% producing several roots but similarly as in 'Aurea' their root balls were not formed during 6 weeks (fig. 2). No significant effects were observed due to the use of rooting powders, neither in percentage of rooted cuttings nor in a degree of rooting. The joint application of powders and the biopreparatrion did not improved the efficiency of powders. Two- and three-fold spraying with AlgaminoPlant enhanced rhizogenesis and significantly increased the percentage of rooted cuttings as compared both to the control cuttings and those treated with the commercial rooting powders. The

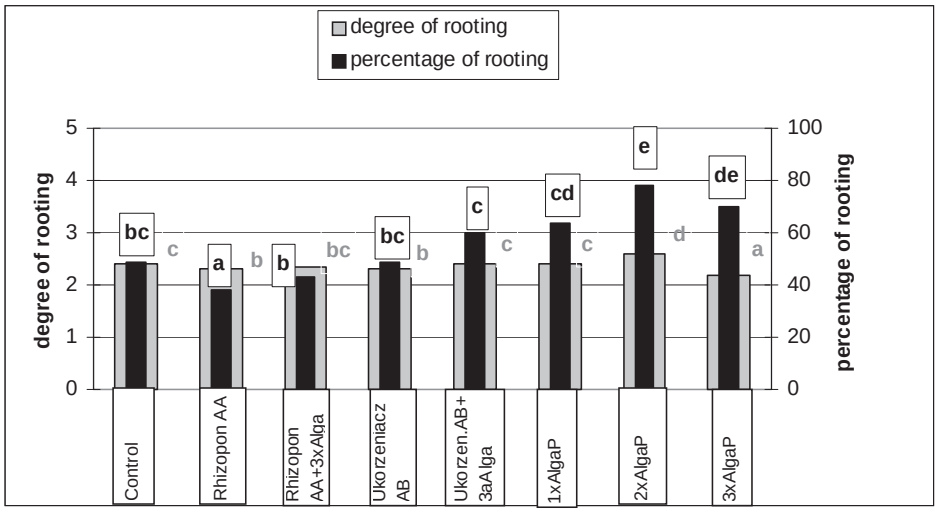

Fig. 1. Effect of biostymulator AlgaminoPlant and two rooting powders on the degree of rooting and percentage of rooted cuttings in *Cornus alba* 'Aurea (2009). The means represented by bars indicated with the same letters do not differ significantly at α = 0.05.

Rys. 1. Wpływ biostymulatora AlgaminoPlant i dwóch ukorzeniaczy na stopień i procent ukorzenienia sadzonek *Cornus alba* 'Aurea' (2009). Średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05.

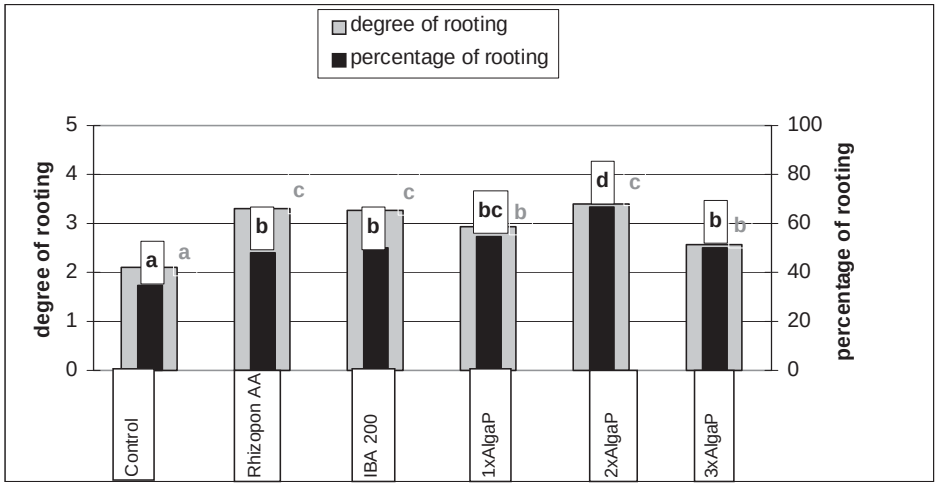

Fig. 1a. Effect of biostymulator AlgaminoPlant and IBA on the degree of rooting and percentage of rooted cuttings in *Cornus alba* 'Aurea (2010). The means represented by bars indicated with the same letters do not differ significantly at α = 0.05.

Rys. 1a. Wpływ biostymulatora AlgaminoPlant i IBA na stopień i procent ukorzenienia sadzonek *Cornus alba* 'Aurea' (2010). Średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05.

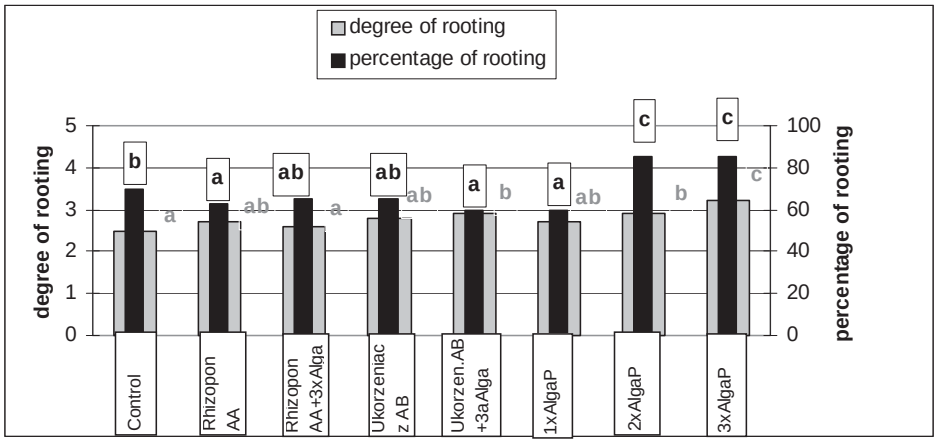

Fig. 2. Effect of biostymulator AlgaminoPlant and two rooting powders on the degree of rooting and percentage of rooted cuttings in *Cornus alba* 'Elegantissima' (2009). The means represented by bars indicated with the same letters do not differ significantly at α = 0.05.

Rys. 2. Wpływ biostymulatora AlgaminoPlant i dwóch ukorzeniaczy na stopień i procent ukorzenienia sadzonek *Cornus alba* 'Elegantissima' (2009). Średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05.

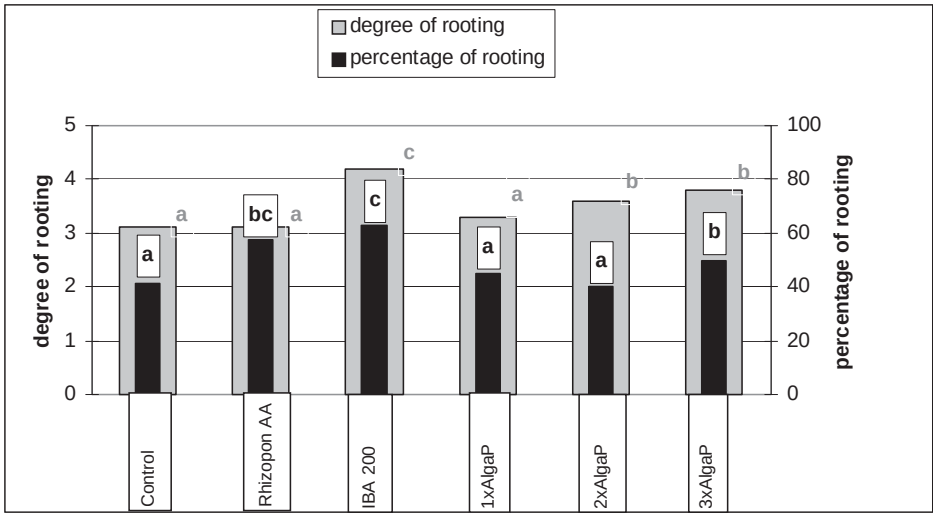

Fig. 2a. Effect of biostymulator AlgaminoPlant and IBA on the degree of rooting and percentage of rooted cuttings in *Cornus alba* 'Elegantissima' (2010). The means represented by bars indicated with the same letters do not differ significantly at α = 0.05.

Rys. 2a. Wpływ biostymulatora AlgaminoPlant i IBA na stopień i procent ukorzenienia sadzonek *Cornus alba* 'Elegantissima' (2010). Średnie oznaczone tą samą literą nie różnią się istotnie przy α = 0,05.

three-fold spraying with the biopreparation resulted as well in the best root development.

In 2010 the positive effect of Algaminoplant on rhizogenesis was confirmed: its two- and threefold application significantly improved degree of rooting as compared to the control cuttings and those treated with the powder while the percentage of rooted cuttings was increased only due to threefold spraying (fig. 2a). In this season also the synthetic auxin gave good results – applied either as a spraying cuttings with the IBA water solution (both paramaters measured) or applying the rooting powder to the cuttings' bases (the percentage of rooting).

Biochemical analyses. Analysis of variance showed significant effects of the biopreparation on contents of several organic compounds determined in leaves of cuttings during rooting (tab. 3). The yellow-leaved cultivar 'Aurea' had a lower initial content of the chlorophyll than the green 'Elegantissima' and the treatment with AlgaminoPlant significantly increased its pigment level while no effect of the biopreparation was seen in the latter cultivar.

Table 3. Effect of biostimulator AlgaminoPlant on content of several organic compounds in cuttings of two cultivars of *Cornus alba* (2009, 2010)

Tabela 3. Wpływ biostymulatora AlgaminoPlant na zawartość związków organicznych w sadzonkach dwóch odmian *Cornus alba* (2009, 2010)

Compound	Species Cultivar	2009		2010	
		Control	AlgaminoPlant	Control	AlgaminoPlant
Chlorophyll mg/g d.w	*C. a.* 'Aurea'	3.7a*	5.7c	2.3a*	5.3b
	C. a. 'Elegantissima'	5.2bc	5.7c	6.3c	6.8cd
Reducing sugars mg/g d.w	*C. a.* 'Aurea'	86.2a	133.3c	45.8a	234.6c
	C. a. 'Elegantissima'	108.7b	156.5d	45.6a	85.9b
Free amino acids µmol/g d.w	*C. a.* 'Aurea'	429.3a	842.9c	135.2a	486.5c
	C. a. 'Elegantissima'	437.2a	722.3b	147.3a	360.9b

means for a given compound followed by the same letter do not differ significantly at the probability level $\alpha = 0.05$

wartości dla podanego związku poprzedzone tą samą literą nie różnią się istotnie na poziomie prawdopodobieństwa $\alpha = 0,05$

Also the initial level of reducing sugars was higher in the green-leaved cultivar as compared to 'Aurea', however both cultivars responded to the treatment with the increases in sugar contents. The range of this increase was slightly higher in 'Aurea' (55%) than in 'Elegantissima' (44%). The treatment with the biopreparation decreased contents of free amino acids in both cultivars.

Generally, leaves of cuttings rooted in 2010 contained less organic compounds than in 2009 but the tendencies in their changes – resulting from the application of AlgaminoPlant – were similar as a year before. The chlorophyll content in the yellowed-leafed

cultivar 'Aurea' more than doubled after the spraying, remaining on the same level in both treatments in the green-leaved 'Elegantissima'. Similarly as in 2009, the increase in reducing sugars occurred in both treatments and was again more pronounced in 'Aurea'. The same phenomenon was observed in contents of free amino acids (tab. 3).

DISCUSSION

Biostimulants contain the biologically active substances which positively affect plant growth and development. They improve plant tolerance to the unfavourable environmental conditions and certain may affect a development of root system [Jankowski and Dubis 2008]. Extract from sea weeds are being used in horticultural production to improve quantity and quality of yielding [Joubert and Lefranc 2008, Matysiak et al. 2008, Rathor et al. 2009]. Recently, there is a growing interest in applying biopreparations made from sea algas to stimulate rooting of cuttings. Studies undertaken by Stirk and Staden [1997] on effect of six preparations made from sea algas confirmed their efficiency in improving rhizogenesis in cuttings. According to the above authors the preparations such as Kelpak or Seamac can deliver to cuttings the sufficient amounts of auxins necessary to induce roots. Presence of auxins in the extracts from sea weeds can qualify them as preparations enhancing rhizogenesis. Crouch and Staden [1993] claim that most of the algas used to produce biopreparations contain auxins or the auxin-like-substances but their concentrations are variable.

Trials on the effect of a seaweed extract on growth of *Vigna sinensis* showed its positive effect on length and mass of roots [Whapham et al. 2006]. Thorsen et al. [2010] tested an effect of the extract from an alga containing brown pigment (*Laminaria digitata*) on germination, rooting and growth of several herbaceous plants and proved its stimulatory effect on rooting of cuttings but a range of response depended on a plant species and a concentration of the preparation. A similar plant response was observed in this trial where AlgaminoPlant significantly improved rhizogenesis in two dogwood cultivars but the effects depended on a number of sprayings.

The application of AlgaminoPlant resulted in an increase in the chlorophyll content in the yellow-leaved cultivar. Similar results were obtained by Whaphamat et al. [2006] in their trial on tomato treated with the extract from *Ascophyllum nodosum*: the increases in the chlorophyll contents were different in different cultivars and depended on a preparation concentration. Results of Blunden et al. [1996] confirm the increase in the green pigment level in several crops treated with the above sea weed extract. Similar increase in the chlorophyll content was observed after application of the extract from *Laminaria digitata* [Joubert and Lafranc 2008].

Sivasankari et al. [2006] report on significant effects of biopreparations on contents of reducing and total soluble sugars in plants. AlgaminoPlant increased the total and reducing sugar levels in carrot roots [Dobrzański at al. 2008]. Similarly, Rathore et al. [2009] observed an elevated level of carbohydrates in plants of *Glycine max* treated with the extract from sea weed *Kappaphycus alvarezii*. Also in both dogwood cultivars under study the contents of reducing sugars increased after spraying with AlgaminoPlant. Thus, the positive effect of the biopreparation on rhizogenesis could have been

related to the improved photosynthesis resulting in an increase in the sugar content in both cultivars and an elevated chlorophyll level in the yellow-leaved taxon. This speculation is supported by the fact that the range of response to the treatment with AlgaminoPlant was larger in the yellow-leaved cultivar, containing less chlorophyll and reducing sugars at the beginning of the experiment.

Amino acids play different roles in plant organisms, serving mainly as building material for synthesis of peptides and proteins. Some of them are storage forms of amine nitrogen, others serve as transportation forms between different plant parts [Kopcewicz and Lewak 2002]. Lesuffleur et al. [2007] proved that an elevated concentration of free amino acids in rhizosphere positively affects rhizogenesis what could be true also in case of *Cornus*.

Differences in absolute values of the above analyzes between two years can be explained by an effect of the environmental conditions affecting stock plants resulting in a different physiological status of cuttings on the date of their harvest. However, both the differences between the cultivars and a range of their response to the treatment are of a comparable magnitude order.

Dogwood cuttings have a relatively high rooting ability (50% and 70% in the cultivars used in this trial) what may explain the lack of response after the application of the routinely used rooting powders containing auxins. Yet, the positive effects of AlgaminoPlant on the percentage of rooted cuttings and the root ball development may in future be translated into economic profits of the growers justifying the use of this biopreparation in a commercial production of dogwood.

CONCLUSIONS

1. The biostymulator AlgaminoPlant positively affected rhizogenesis in dogwood though its effect differed in two cultivars under study.

2. The treatment with the AlgaminoPlant increased contents of chlorophyll a+b, reducing sugars and free amino acids in leaves of cuttings what could explain its positive effect on rhizogenesis.

3. Spraying dogwood cuttings with the AlgaminoPlant solution can be an effective and environmentally friendly method to stimulate rooting.

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WPŁYW AUKSYN I PREPARATU ALGAMINOPLANT NA UKORZENIANIE SADZONEK PĘDOWYCH DWÓCH ODMIAN DERENIA BIAŁEGO (*Cornus alba* 'AUREA' I 'ELEGANTISSIMA')

Streszczenie. Wprowadzane przez UE ograniczenia produkcji i zastosowania środków ochrony roślin wymuszają na szkółkarzach poszukiwania nowych substancji bezpiecznych dla środowiska a skutecznych w produkcji materiału roślinnego. Celem niniejszej pracy było określenie wpływu preparatu z grupy biostymulatorów – AlgaminoPlantu – zastosowanego w formie opryskiwania sadzonek 0.2% roztworem wodnym, na proces ukorzeniania sadzonek pędowych *Cornus alba* 'Aurea' i 'Elegantissima'. Dla porównania użyto stosowane dotąd w praktyce szkółkarskiej ukorzeniacze pudrowe: Ukorzeniacz AB (0.3% NAA) i Rhizopon AA (2% IBA). Zastosowane ukorzeniacze w niewielkim stopniu oddziaływały na rizogenezę u derenia. Wyniki przeprowadzonego doświadczenia wskazują natomiast na możliwość wykorzystania preparatu AlgaminoPlant w procesie ukorzeniania w/w odmian derenia, ponieważ jego zastosowanie zwiększyło procent i stopień ukorzenienia sadzonek w porównaniu z sadzonkami kontrolnymi. Preparat ten wpłynął na wzrost zawartości chlorofilu, węglowodanów zawartość i wolnych aminokwasów w liściach sadzonek.

Słowa kluczowe: biostymulatory, IBA, sadzonki, ukorzenianie, chlorofil, cukry redukujące

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