

THE EFFECT OF ALGAMINOPLANT ON RHIZOGENESIS IN STEM CUTTINGS OF *Physocarpus opulifolius* ‘Dart’s Gold’ AND ‘Red Baron’

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Abstract. The UE-imposed restrictions on the manufacture and use 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 substances that have little environmental impact. This study evaluated biopreparation AlgaminoPlant for its effect on rooting of stem cuttings in *Physocarpus opulifolius* ‘Dart’s Gold’ and ‘Red Baron’ during the vegetative seasons 2010 and 2011. During rooting, cuttings were sprayed once, twice or three times with the water solutions of the biopreparation AlgaminoPlant (0.2%). To evaluate its effectiveness relative to the current treatments routinely used in the nursery production, some cuttings were treated with a rooting powder containing a synthetic auxin IBA or sprayed with a water solution of IBA. The best results were obtained with the IBA solution, however, biopreparation also positively affected rhizogenesis in ninebark. Biopreparation also affected the chlorophyll a+b, reducing sugars, free amino acids and polyphenolic acids contents in leaves of cuttings but its effects varied depending on a cultivar.

Key words: ninebark, IBA, rooting, chlorophyll a+b, reducing sugars, free amino acids, polyphenolic acids

INTRODUCTION

Demand for ornamental shrubs has been increasing lately, due to dynamic development of urban infrastructure. Gardens have become an inseparable element of modern settings. In response to this trend, the ornamental nursery production has been increasing to the point of becoming the most profitable branch of ornamental horticulture in Poland [Olewnicki 2011]. The increasing demand for the ornamental plant material and strong competition among nurseries compels growers to deliver the best material in the

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shortest possible time, and in large quantities. As a consequence, new methods to intensify nursery production are constantly being tested. The UE-imposed restrictions on the manufacture and use of plant protection chemicals create a new production environment. This imposes on the nurseryman a 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. They are either single or multi-compound preparations, containing extracts from sea algae (*Eclonia maxima*, *Laminaria digitata*, *Fucus vesiculosus* or *Durvillea potatorum*) [Dobrzański et al. 2008]. They induce, or are said to induce, plant resistance to different stresses and they can also positively affect rhizogenesis and the root system development [Pruszyński 2008]. Some of these, such as AlgaminoPlant may contain low molecular weight polypeptides and amino acids, vitamins, enzymes, phytohormones, sugars, betains and antioxidants [Basak 2008]. Some of these active compounds may stimulate rhizogenesis by inducing anatomical and morphological changes in plants [Cambri et al. 2008, Khan et al. 2009]. Our previous study has shown that certain biopreparations not only enhance rhizogenesis in cuttings of ornamental shrubs but can even replace the rooting powders [Jacygrad and Pacholczak 2010; Pacholczak et al. 2010].

AlgaminoPlant is a liquid preparation produced on the base of a seaweed extract from *Sargassum*, *Laminaria*, *Ascophyllum* and *Fucus*. It contains three groups of plant hormones: gibberellins, cytokinins and auxins. It is supplemented by potassium salts of amino acids at 10%. The preparation enhances uptake of macro- and microelements and their translocation within plants, increases the respiration rate and root growth, participates in photosynthesis and other metabolic processes. It positively affects plant resistance to stresses, accelerates flowering and fruit set [Bai et al. 2007], improves the soil water-holding capacity and maintains optimal soil pH [Matysiak et al. 2010]. The purpose of this study was to compare the preparation AlgaminoPlant, with a commercial rooting powder containing synthetic auxin that is commonly used in nurseries. The tests were done on two cultivars of the popular ornamental shrub – ninebark – *Physocarpus opulifolius*. An attempt was also made to detect relationships between several different biochemical parameters of cuttings, as influenced by treatments, and the subsequent rhizogenesis.

MATERIAL AND METHODS

The experiments were carried out in 2010 and 2011 in a commercial nursery of M.M. Kryt in Wola Prażmowska on two cultivars of *Physocarpus opulifolius*: ‘Dart’s Gold’ and ‘Red Baron’. The cuttings were sprayed with the water solution of the biopreparation AlgaminoPlant (0.2%) either once, twice or three times during the rooting period. To compare its effects with a commercial rooting powder, a synthetic auxin routinely used in nursery production – β -indolilobutyric acid (IBA) – was included. It was applied either directly to the bases of cuttings in the form of the commercially available rooting powder Rhizopon AA (2% IBA), or by spraying cuttings with the water solution of 200 mg·dm⁻³ IBA. The control cuttings were sprayed with distilled

water. In both years the experiments began at the end of June. Semi lignified two nodal stem cuttings were prepared from shoots harvested from stock plants free from pathogens and diseases. Cuttings were rooted in styrofoam boxes. They were inserted to the depth of 2 cm into a mixture of peat, perlite and sand (2:1:1), pH 5.0. The mixture was thoroughly wetted and pressed, and covered with 0.5 cm layer of coarse sand.

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). Rooting took place in plastic tunnels equipped with automatic watering and mist systems as well as with shading devices. During the first two weeks the cuttings were protected against sun with an opaque foil and a shading cloth. Every two weeks, cuttings were sprayed against *Botrytis* with 0.2% Rovral or Topsin.

Table 1. A list of treatments in the experiment

No. of treatment	Method of cuttings' treatment
1	Control „0” (H ₂ O)
2	Rhizopon AA (2% IBA) powder
3	1 spraying with IBA 200 mg · dm ⁻³
4	1 spraying with AlgaminoPlant 0.2%;
5	2 sprayings with AlgaminoPlant 0.2%
6	3 sprayings with AlgaminoPlant 0.2%

The experiment consisted of six treatments (Tab. 1), each in three replications, each replication containing 20 cuttings. Percentages of rooted cuttings and the degree of rooting were determined after 8 weeks from the start of the experiment. The degree of rooting was evaluated on a 5-point scale rating 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 2. Evaluation scale of the root development

No.	Characteristics of the degree of rooting	No. of points
1	Cutting without visible roots	1
2	A few short roots	2
3	Roots with numerous branched roots, no root ball	3
4	An average root system forming a root ball	4
5	Well developed, branched root system forming a root ball	5

Leaves from 20 cuttings per treatment were collected three weeks after the beginning of the experiment from treated and untreated cuttings. They were finely chopped, mixed and 0.5–1 g samples were taken for the measurements of dry matter and contents of chlorophyll a+b, reducing sugars, free amino acids and polyphenolic acids. Triplicate extracts were prepared for each analysis and three measurements were done for each extract producing 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 (chlorophyll *a* + *b*) 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]. Free amino acids were measured according to Rosen [1957], and polyphenolic acids by the colorimetric method with the Arnow's reagent according to the Polish Norm PN-91/R-87019.

To compare the means, rooting percentages cuttings were transformed according to Bliss [Wójcik and Laudański 1989], while the degree of rooting by root transformation: $y = x^2 + (x + 1)^2$. The above data was subjected to ANOVA 1 and tested by the Duncan's test at $\alpha = 0.05$. Results of biochemical analyses were subjected to the 2-factorial ANOVA and the means were compared by the Duncan's test at $\alpha = 0.05$.

RESULTS

In 2010 the untreated cuttings of the yellow-leaf ninebark cultivar rooted only in 32% (Fig. 1). The commercial rooting powder Rhizopon AA increased this percentage to 40%. Significantly more effective was IBA applied as a water solution sprayed on cuttings (over 50% of rooting). The best results were obtained after triple treatment with AlgaminoPlant which doubled the percentage of rooted cuttings relative to control (64%). A single application of AlgaminoPlant was ineffective while two sprayings gave results comparable to the auxin-based powder.

During eight weeks of the experiment, control (untreated) cuttings developed a few short roots (Fig. 1) and their degree of rooting was scored as 1.5. All the treatments – except the single application of AlgaminoPlant – improved rhizogenesis. The best results were obtained following spraying with IBA. Effects of double application of AlgaminoPlant were comparable to those obtained with Rhizopon AA.

In 2011 the best roots were developed by cuttings sprayed with IBA 200 mg·dm⁻³. Effects of a single or double application of AlgaminoPlant were somewhat weaker but comparable with that obtained due to the application of the rooting powder Rhizopon AA (Fig. 1a). For the rooting percentage the comparable effects resulted from the use of the water solution of IBA and one or two sprayings with AlgaminoPlant.

In the red ninebark cultivar 'Red Baron' the percentage of rooted cuttings in control treatment was 50% in 2010 (Fig. 2). All the treatments with AlgaminoPlant increased the rooting percentage up to 62–67%. Root development proceeded similarly as in the yellow cultivar: a few short roots were formed in untreated cuttings during eight weeks (a degree of rooting 1.5). The highest degree of rooting was in cuttings treated with IBA in both forms: the IBA water solution and the auxin-based powder (Fig. 2). AlgaminoPlant was ineffective as compared to the above treatments.

In 2011 the application of IBA in both forms (Rhizopon AA and IBA water solution) as well as a single and double spraying with AlgaminoPlant resulted in a good development of root balls in ninebark cuttings. Further increase in number of treatments with biostimulant did not improve the degree of rooting. Similar tendencies were observed in the percentage of rooting (Fig. 2a).

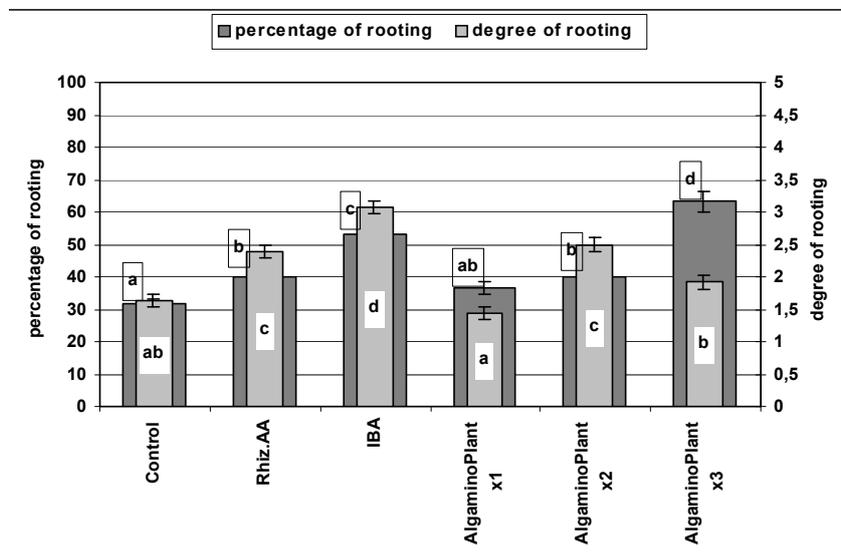


Fig. 1. The effect of biopreparations on the degree of rooting and on percentage of rooted cuttings in *Physocarpus opulifolius* 'Dart's Gold' (2010). The means represented by bars indicated with the same letters do not differ significantly at $\alpha = 0.05$

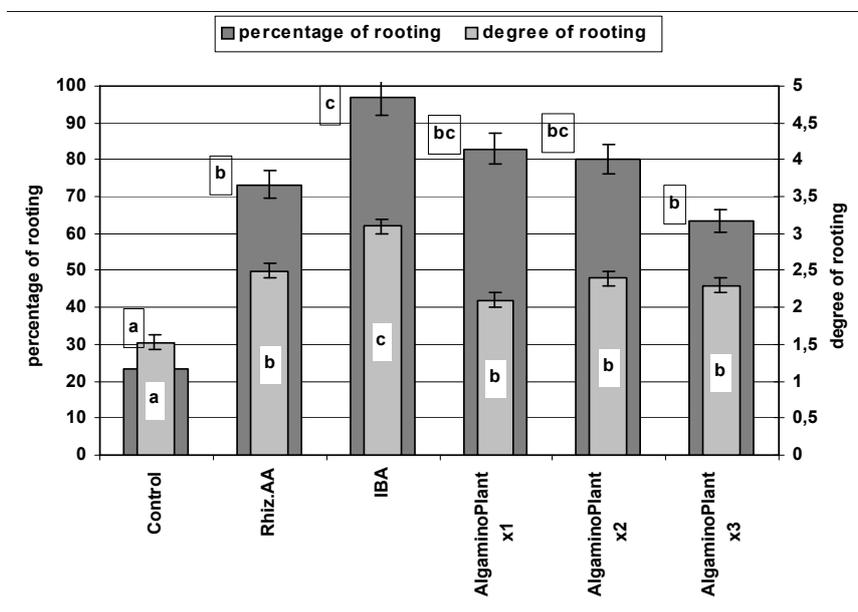


Fig. 1a. The effect of biopreparations on the degree of rooting and on percentage of rooted cuttings in *Physocarpus opulifolius* 'Dart's Gold' (2011). The means represented by bars indicated with the same letters do not differ significantly at $\alpha = 0.05$

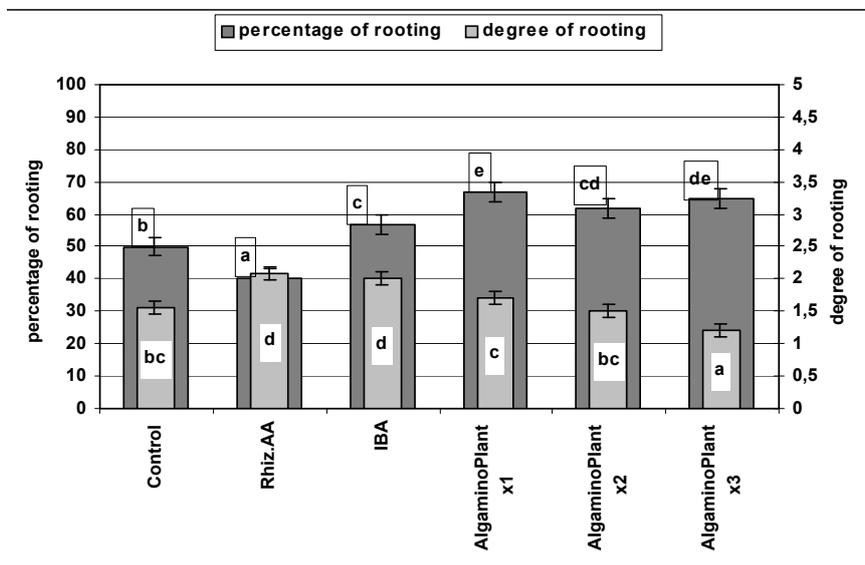


Fig. 2. The effect of biopreparations in *Physocarpus opulifolius* 'Red Baron' on the degree of rooting and on percentage of rooted cuttings (2010). The means represented by bars indicated with the same letters do not differ significantly at $\alpha = 0.05$

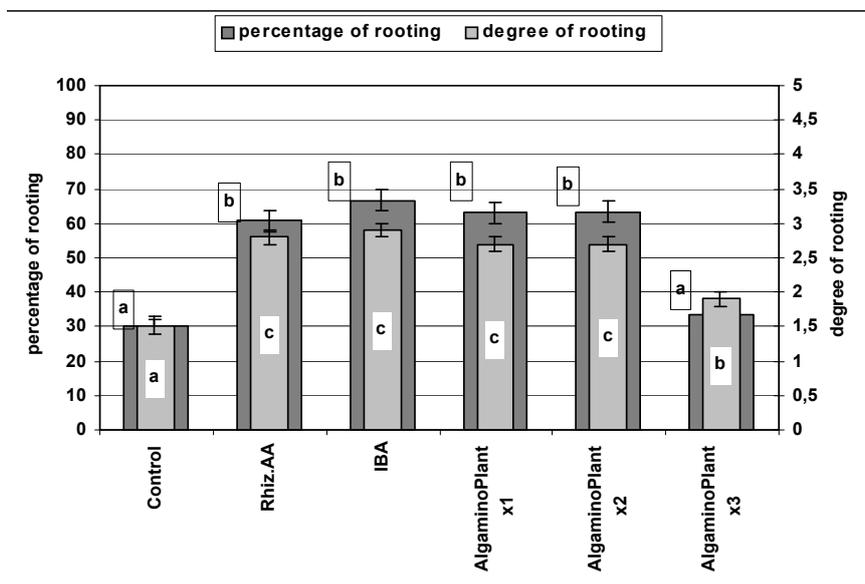


Fig. 2a. The effect of biopreparations in *Physocarpus opulifolius* 'Red Baron' on the degree of rooting and on percentage of rooted cuttings (2011). The means represented by bars indicated with the same letters do not differ significantly at $\alpha = 0.05$

Biochemical analyses. Analysis of variance showed significant effects of the bio-preparation on contents of several organic compounds determined in 2010 in leaves of cuttings during rooting. Ninebark leaves differed considerably in the chlorophyll a + b content: leaves of the control treatment in the yellow cultivar had less than 20% of the pigment content of the red leaved cultivar (Tab. 3). As compared to the control leaves, triple application of AlgaminoPlant increased the chlorophyll content in 'Darts Gold' while decreasing it slightly in 'Red Baron'. Both cultivars had similar amounts of reducing sugars in leaves. The effect of AlgaminoPlant varied in two cultivars: it decreased the reducing sugar level in 'Dart's Gold' (30%) while increasing it in 'Red Baron' (12%). After treatments with AlgaminoPlant, the polyphenolic acid contents dropped by 35% in the yellow cultivar but increased by 7%, in the red one.

The free amino acid content in control cuttings of the yellow cultivar was roughly half of that in the red cultivar. AlgaminoPlant increased the amino acid content in the yellow cultivar by 450% but only by 27% in the red one.

Table 3. Effect of biostimulator AlgaminoPlant on content of several organic compounds in cuttings of two cultivars of *Physocarpus opulifolius* (2010)

Compound	Species/cultivar	Control	AlgaminoPlant
Chlorophyll (mg/g d.w)	<i>P. o.</i> 'Dart's Gold'	2.3a*	4.4b
	<i>P. o.</i> 'Red Baron'	12.2d	11.4c
Reducing sugars (mg/g d.w)	<i>P. o.</i> 'Dart's Gold'	36.2b	25.0a
	<i>P. o.</i> 'Red Baron'	37.5b	42.1c
Polyphenolic acid (mg/g d.w)	<i>P. o.</i> 'Dart's Gold'	8.3b	5.4a
	<i>P. o.</i> 'Red Baron'	12.2c	13.0d
Free amino acids (μmol/g d.w)	<i>P. o.</i> 'Dart's Gold'	140.9a	643.9d
	<i>P. o.</i> 'Red Baron'	271.5b	344.5c

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

Table 3a. Effect of biostimulator AlgaminoPlant on content of several organic compounds in cuttings of two cultivars of *Physocarpus opulifolius* (2011)

Compound	Species/cultivar	Control	AlgaminoPlant
Chlorophyll (mg/g d.w)	<i>P. o.</i> 'Dart's Gold'	2.7a*	3.5b
	<i>P. o.</i> 'Red Baron'	3.0a	8.1c
Reducing sugars (mg/g d.w)	<i>P. o.</i> 'Dart's Gold'	157.7c	49.9a
	<i>P. o.</i> 'Red Baron'	197.4d	76.0b
Polyphenolic acid (mg/g d.w)	<i>P. o.</i> 'Dart's Gold'	7.1a	8.7b
	<i>P. o.</i> 'Red Baron'	30.4d	25.3c
Free amino acids (μmol/g d.w)	<i>P. o.</i> 'Dart's Gold'	312.8b	508.5d
	<i>P. o.</i> 'Red Baron'	193.5a	442.1c

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

In 2011 the AlgaminoPlant application resulted in the increase of the chlorophyll content in both cultivars which more than doubled in leaves of 'Red Baron'. On the contrary, the level of reducing sugars decreased almost threefold (Tab. 3a). Due to the biostimulant application the content of poly phenolic acids increased in 'Dart's Gold' while it dropped in the red-leaved cultivar, contrary to the situation observed in 2010. The contents of free amino acids increased after treatment with AlgaminoPlant by 60% and 30% in 'Dart's Gold' and 'Red Baron', respectively.

DISCUSSION

Two ninebark cultivars used in this experiment differed not only visually but also in their natural rooting capacity, which was higher in the red-leaved cultivar (50% versus 30% in 2010). Of course, there is no reason at this point to associate leaf color with rooting capacity. Positive effects of the treatments during this trial were twice as frequent in 'Dart's Gold' as compared to 'Red Baron'. The synthetic auxin IBA proved effective, serving as a reference point to evaluate the efficiency of biopreparation AlgaminoPlant. Application of synthetic auxins to stimulate rooting, especially in the difficult-to-root taxa, has been a common practice [Khan *et al.* 2009]. Their positive effects on rhizogenesis have been well documented [Czekalski 2005, Korszun 2007], also on ninebark [Pacholczak and Szydło 2008]. Our previous study has shown that certain biopreparations can enhance rhizogenesis in cuttings of ornamental shrubs, comparably to synthetic auxins and they can even replace the rooting powders if those are withdrawn from the market [Jacygrad and Pacholczak 2010; Pacholczak *et al.* 2010]. This possibility was further confirmed by the results of this work.

There are reports that biopreparations obtained from algae increase chlorophyll content in crops [Sivasankari *et al.* 2005]. In 2010, in the yellow-leaved cultivar of *Physocarpus* with low chlorophyll content the pigment level increased after spraying with biostimulator. Also, rhizogenesis was enhanced, more so than in 'Red Baron'. Next year the increase in the green pigment content occurred in both cultivars treated with the biostimulant. Similarly, Jothinayagi and Anbazhagan [2009] showed that seaweed extracts stimulated chlorophyll synthesis in *Abelmoschus esculentus* increasing its level by 20% as compared to control untreated plants. Khan *et al.* [2009] reported that both foliar and soil application of extract from *Ascophyllum nodosum* resulted in an increased chlorophyll level in leaves of *Lycopersicon esculentum*.

Rooting process depends on carbohydrate concentrations in plant tissue [Costa *et al.* 2007]. There is little data on the effects of biopreparations on sugar levels in treated plants and even less so on the carbohydrate balance in cuttings after treatments with biopreparation. In *P. opulifolius* 'Red Baron' spray applications of AlgaminoPlant increased in the reducing sugar contents in cuttings and the percentage of rooting in the first year of trial. However, in 2011 a decrease in reducing sugar contents were observed in 'Red Baron' while in cuttings of the yellow-leaved cultivar the reducing sugar level either decreased or remained unchanged relative to controls yet this did not impede their rooting. Also Jacygrad and Pacholczak [2010] report that the carbohydrate contents in cuttings dropped after the AminoTotal application on two ninebark cultivars, affecting

neither a degree nor a percentage of rooting. According to Sivasankari et al. [2006] contents of total soluble and reducing sugars increase after application of seaweed preparations. As the results of this work are not univocal in regard to the effect of AlgaminoPlant on endogenous sugars the relationship between carbohydrate levels and rhizogenesis following biopreparation applications needs further studies.

Phenolic compounds used together with auxins improve rooting of cuttings [Moore 1984]. They act as auxin co-factors and are active in low concentrations [Jankiewicz 1997]. Their mode of action is not entirely clear but the effects are recognized: they increase a percentage of rooted cuttings and improve root development. An increase in their content following bioprep treatments may be directly related to improved rhizogenesis. However, the levels of polyphenolic acid in both cultivars tested did not appear related to rhizogenesis. In 2010 in the yellow-leaved cultivar, the biopreparation reduced polyphenolic acid levels relative to controls while enhancing rooting; in 'Red Baron' the positive effects of treatments on rhizogenesis were less pronounced while the levels of polyphenolic acids were elevated. The reverse situation was observed in 2011. Such contradictory reports can be found in literature, for example treatments with AminoPlant and Goëmar Goteo resulted in a decrease in contents of polyphenolic acids in basil leaves [Rosłon et al. 2011]. In another trial on ninebark an increase in polyphenolic acids was observed after treatment with biostimulants [Jacygrad and Pacholczak 2010].

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] have shown that an elevated concentration of free amino acids in the rhizosphere positively affects rhizogenesis. This might have been the case for the yellow cultivar of *Physocarpus* with its lower natural rooting ability where both biopreparations increased the free amino acid contents and stimulated root development. In 'Red Baron', the percentage of rooting increased only after treatment with AlgaminoPlant which also elevated the free amino acid content. The increase in free amino acids after application of biostimulants was also recorded by Jacygrad and Pacholczak [2010] in cuttings of two ninebark cultivars and Pacholczak et al. [2012] in two dogwood cultivars.

In easy-to-root plants the use of biopreparations may not be economically justified, however, in some case they may improve rhizogenesis. Generally, in nursery production, rooting powders such as Rhizopon AA, are recommended as effective and cheaper than biostimulators. The efficiency of Rhizopon AA and the water solution of IBA has been confirmed in this work, however, if a pressure to withdraw synthetic auxins grows, there is a chance that certain biopreparations are capable of replacing them, contributing to a more ecology-friendly production methods. In this sense, the results of this study are encouraging. However, varied response of different taxa to treatments with biopreparations indicate that detailed studies are needed to determine the best application regimes and to provide some understanding of their mode of action before any practical recommendations can be formulated.

CONCLUSIONS

1. The synthetic auxin IBA, both as an ingredient of the commercial rooting powder and as a water solution, positively affected rhizogenesis in ninebark, especially in cuttings of the yellow-leaved cultivar with the low natural rooting capacity.

2. Sprayings with biopreparation AlgaminoPlant enhanced rhizogenesis in two ninebark cultivars, to or above the level of the synthetic auxin IBA so this biostimulant may eventually replace the auxin-based commercial rooting powders.

3. No clear relationship was found between values of selected biochemical parameters and the effects of treatments with AlgaminoPlant on rhizogenesis in ninebark.

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**WPLYW PREPARATU ALGAMINOPLANT NA UKORZENIANIE
SADZONEK *Physocarpus opulifolius* ‘Dart’s Gold’ I ‘Red Baron’**

Streszczenie. Celowość badań nad wpływem substancji pochodzenia naturalnego na ukorzenie sadzonek wynika z zapisów prawa unijnego, które nakłada coraz większe ograniczenia w kwestii stosowania syntetycznych substancji w produkcji roślinnej. Założeniem przeprowadzonego w latach 2010–2011 doświadczenia było określenie wpływu biostymulatora AlgaminoPlant na proces rizogenezy w sadzonkach pędowych *Physocarpus opulifolius* ‘Dart’s Gold’ i ‘Red Baron’. Sadzonki opryskiwane były auksyną IBA lub biostymulatorem AlgaminoPlant (0,2%) jedno-, dwu- lub trzykrotnie w odstępach tygodniowych. Na podstawie przeprowadzonych doświadczeń wykazano, że najlepszym preparatem do ukorzenia był roztwór auksyny IBA. Dobre rezultaty uzyskano również dzięki opryskaniu sadzonek AlgaminoPlantem, lecz były one uzależnione od liczby wykonanych zabiegów. Aplikacja biostymulatorów spowodowała również zmianę zawartości w sadzonkach takich związków organicznych jak: chlorofilu, cukrów redukujących, polifenolokwasów i wolnych aminokwasów. Poziom tych związków zależny był od odmiany.

Słowa kluczowe: pęcherznica, auksyna IBA, ukorzenie, chlorofil, cukry redukujące, polifenolokwasy

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