**The effect of two biopreparations on rhizogenesis  
in stem cuttings of smoke tree (*Cotinus coggygria* Scop.)**

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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 two biopreparations for their effect on rooting of stem cuttings of two cultivars of smoke tree (*Cotinus coggygria*). During rooting, cuttings were sprayed once, twice or three times with aqueous solutions of 0.2% AlgaminoPlant or 0.1% Route. To evaluate their effectiveness relative to the treatments routinely used in the nursery production, some cuttings were treated with a rooting powder Rhizopon AA containing a synthetic auxin IBA (2%) or sprayed with a water solution of IBA (200 mg dm-3). The biopreparations enhanced rhizogenesis in both cultivars. They increased the photosynthetic efficiency and chlorophyll contents, endogenous indolile acids, free amino acids and phenolic compounds in the leaves of cuttings as well as they stimulated the activities of polyphenol oxidase and catalase, while lowering the content of hydrogen peroxide and the endogenous ethylene production. Given the performance of the two tested preparations it appears that they can be recommended to growers as a replacement for synthetic IBA in nursery production.

**Key words:** *Cotinus coggygria*, rooting, biopreparations, auxin, hydrogen peroxide, enzymes

**Introduction**

Production of plant material in EU is controlled by numerous environmental, economic, juridical and social regulations [Szydło and Pacholczak 2004]. In the name of environmental protection, restrictions in application of commonly used rooting powders containing synthetic auxins and fungicides are being imposed on the nurserymen [Ludwig-Muller 2000, Szydło and Pacholczak 2004]. It is therefore necessary to seek other preparations which are more environmentally friendly yet still effective in rhizogenesis and in successful acclimation of plants to different conditions in successive steps of plant production, while maintaining high quality level of the nursery plant material [Wojdyła 2004, Pacholczak et al. 2012].

Biopreparations may constitute such a group of preparations as they contain natural substances with little, if any, predicted environmental effect. They have been viewed as biological substances that stimulate certain physiological or biochemical processes within plants. They are single- or multi-compound preparations containing extracts from plants and/or animals. Their effect is in influence on plant metabolism (Basak, 2008).They are used to obtain the highest possible and the best quality yields, especially under environmental conditions unfavorable for plant growth and development (Przybysz et al. 2008).

The potential of biopreparations to enhance rooting of stem cuttings has been reported previously (Pacholczak et al. 2010; 2012). They positively affect quality of the plant material and its resistance or tolerance to stresses in every phase of nursery production. These preparations affect some processes in plant cells enhancing plant vitality which in turn appears to lead to a more intense growth and vigor, both of which are important for potential consumers [Gawrońska 2008, Pacholczak and Szydło 2010]. Biopreparations enable plants to better exploit the growth and development potential of the environment in which they grow. Their effects are more pronounced under less favorable environmental conditions as they strengthen the plant natural abilities to cope with stresses [Jankowski and Dubis 2008, Pruszyński 2008, Pacholczak and Szydło 2010].

One of biopreparations – 'AlgaminoPlant' (produced by Varichem, Poland) - is a liquid preparation produced on the base of a seaweed extract (18%) from *Sargassum*, *Laminaria*, *Ascophyllum* and *Fuscus*.It containsthree 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 (Matysiak et al. 2010), increases the respiration rate and root growth, promotes photosynthesis and other metabolic processes. It positively affects plant resistance to stresses, accelerates flowering and fruit set (Anyszka and Pałczyński 2006, Dobrzański et al. 2008). It also improves the water-holding capacity of the soil and maintains optimal soil pH [Matysiak et al. 2010].

'Route' (Dalgety, Poland) is a formulation of zinc ammonium acetate (ZAA) composed of acetic acid, water, ammonium and zinc oxide. Zinc helps plants overcome environmental stresses and enhances their productivity through a better development of root system and a more efficient use of irradiance (Fraser and Percival 2003). 'Route' reinforces (about 18%) cell walls, thus reducing their porosity and indirectly decreasing of mineral nutrient losses from cells (Horne and Leitch 2006). ZAA action in rhizogenesis is associated with stimulation of endogenous biosynthesis of auxins, which changes the auxin to cytokinin ratio. This in turn results in a more abundant production of auxiliary roots.

Vegetative propagation of smoke tree and its cultivars by conventional methods is slow and problematic [Jacygrad et al. 2012]. Acceleration of this process by biopreparations which enhance the regeneration of the root system in cuttings appears promising. For these reasons, trials were undertaken to evaluate the efficiency of 'AlgaminoPlant' and 'Route' in propagation of two smoke tree cultivars. An attempt was also made to detect relationships between several biochemical parameters and two parameters of gas exchange in cuttings, as influenced by treatments, and the subsequent rhizogenesis.

**Materials and methods**

The experiments were carried out in 2011 in a commercial nursery of M.M. Kryt in Wola Prażmowska, on two cultivars of smoke tree (*C. coggygria*): 'Royal Purple' and 'Young Lady'. 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. Two biopreparations were used, in aqueous solutions: AlgaminoPlant (0.2%) and Route (0.1%). Cuttings were sprayed with either solution once, twice or three times during the rooting period. The effects of the two preparations were compared to those of a commercial rooting powder, a synthetic auxin (β-indolilobutyric acid, IBA) routinely used in nursery production. IBA 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 aqueous solution of 200 mg dm-3 IBA. Control cuttings were sprayed with distilled water. The experiment began on June 28 and ended on August 29, 2011.

Water and the solutions tested were applied with a hand pressure sprayer (volume 1 dm-3) on the start date of the experiment (June 28), and repeated twice at one week intervals, on July 5 and July 12. 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 Bravo. Why not state “as previously described”

The experiment consisted of nine treatments (Tab. 1), each in three replications, each replication containing 20 cuttings. Percentages of rooted cuttings and the degree of rooting were determined 8 weeks after 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) provided by Pacholczak and Szydło [2010]. The scores for the degree of rooting represent means of three independent observations by trained personnel. Percent of rooted cuttings was also calculated - only the cuttings with root system within the scale range of 2-5 were regarded as rooted and counted. As previously described.

**Table 1.** A list of treatments in the experiment.

|  |  |
| --- | --- |
| **No. of treatment** | **Methods of cuttings treatment** |
| 1 | Control '0' 1 spraying with distilled water (H2O) |
| 2 | Rhizopon AA (2% IBA) powder |
| 3 | 1 spraying with IBA 200mg ·dm-3 |
| 4 | 1 spraying with AlgaminoPlant 0.2% |
| 5 | 2 sprayings with AlgaminoPlant 0.2% |
| 6 | 3 sprayings with AlgaminoPlant 0.2% |
| 7 | 1 spraying with Route 0.1% |
| 8 | 2 sprayings with Route 0.1% |
| 9 | 3 sprayings with Route 0.1% |

**Table 2.** Evaluation scale of the root development.

|  |  |
| --- | --- |
| **Characteristic of the degree of rooting** | **Score** |
| Cutting without visible roots | 1 |
| A few (1-3) short roots | 2 |
| 4 - 5 roots, some of them branched, no root ball formed | 3 |
| Medium sized root system composed of 6 – 10 branched roots forming a root ball | 4 |
| Well developed, branched root system forming a root ball (over 10 roots) | 5 |

On July 12 the following measurements of the gas exchange parameters were done: Rd – respiration rate (µ·mol CO2·m-2·s-1) and Pn – net photosynthetic rate(µ·mol CO2·m-2·s-1). The parameters of gas exchanged were measured by the CIRAS-2 gas analyzer (PP System Inc., Amesbury, MA, USA). The measurements were done in triplicate, at noon, under natural irradiance of 1000-1400 µmol PAR·m-2·s-1 at 25oC, RH 50-60% and CO2 concentration 300-350 µmol mol-1 air. Conditions under tunnel where cuttings for measurements were grown and sampled were: 30-39oC, RH 90-100%. The respiration rate was determined after shading the measurement chamber and photosynthesis extinction. Measured with an infra red gas analyzer, negative photosynthetic rate is equal to the respiration rate.

For biochemical analyses, 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 g samples were used for the measurements of dry matter and contents of chlorophyll (*a* + *b*), endogenous indolyl acids, reducing and total sugars, free amino acids, soluble proteins, polyphenolic acids, hydrogen peroxide as well as activities of polyphenolic oxydase, catalase and peroxydase. 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 105oC to constant weight [Strzelecka et al. 1982]. Chlorophyll content (chlorophyll *a* + *b*) was analyzed according to Lichtenthaler and Wellburn [1983]. Endogenous indolyl acid contents were measured according to Donate-Correa et al. [2004]. Reducing sugars were determined by the colorimetric method of Somogyi in the Nelson’s modification [Nelson 1944], and total soluble sugars were determined according to Dubois et al. [1956]. Free amino acids were measured by the method of Rosen et al. [1957], soluble proteins according to Lowry et al. [1951], and polyphenolic acids by the colorimetric method with the Arnow’s reagent according to the Polish Norm PN-91/R-87019. Polyphenolic oxidase activity (PPO) was determined according to Jariteh et al. [2011]. The hydrogen peroxide content was measured according to Siedlecka [2010]. The catalase activity was analyzed according to Goth [1991], and the peroxidase activity - according to Toczko and Grzelińska [2001]. The endogenous ethylene production was determined with a Hewlett Packard gas chromatograph Model 5890 II.

**Statistical analyses**

To compare the means, percentages of rooted cuttings were transformed according to Bliss [Wójcik and Landański 1989], while the degree of rooting by root transformation: y=x2+(x+1)2, subjected to ANOVA 1 and tested by the Duncan’s test at α=0.05. Results of the gas exchange analyses were subjected to the 2-factorial ANOVA and the means were compared by the Duncan’s test at α=0.05.

**RESULTS**

**The degree of rooting and rooting percentages in cuttings of smoke tree 'Royal Purple'**

**\*** The means represented by bars indicated with the same letters do not differ significantly at α = 0,05.

**Figure 1.** Effect of auxin and biopreparations applications on the rooting degree and rooting percentage of the cuttings of smoke tree 'Royal Purple'.

Less than 20% of the untreated control cuttings rooted, and their degree of rooting was below 1.5 (Fig.1). Synthetic auxin more than doubled these values with no significant differences between the rooting powder and sprayed water solution of IBA. Foliar application of AlgaminoPlant did not improve rooting relative to the control. Neither did spraying with Route with the exception of a single application which doubled the percentage of rooting and the degree of rooting relative to the untreated control.

**The degree of rooting and rooting percentages in cuttings of cv*.* 'Young Lady'**

The effects of treatments were also significant in 'Young Lady' (Fig. 2). While the commercial powder proved inefficient, sprayed water solution of IBA nearly tripled the percentage of rooted cuttings and doubled the value of rooting degree relative to control treatment. AlgaminoPlant sprayed on cuttings once or twice markedly improved the root development and doubled the rooting percentage relative to control. However, third application did not increase the rooting percentage and arrested root development. 'Route' more than doubled the parameters of rhizogenesis when applied two or three times while the single spraying proved inefficient as compared to untreated cuttings

**\*** The means represented by bars indicated with the same letters do not differ significantly at α = 0,05.

**Figure 2.** Effect of auxin and biopreparations applications on the rooting degree and rooting percentage of the cuttings of smoke tree 'Young Lady'.

**Respiration efficiency**

The treatments significantly affected the mean respiration efficiency while the effect of cultivar was insignificant (Table 3). In the cultivar 'Royal Purple' respiration did not differ among the treatments while in 'Young Lady' two or three sprayings with Route doubled respiration efficiency relative to control (Table 3).

**Table 3.** Effect of biostimulators AlgaminoPlant and Route on respiration efficiency (µmol CO2 · m-2 · s-1) in cuttings of smoke tree 'Royal Purple' and 'Young Lady'.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Taxon** | | | | **Means**  (for treatments) | | |
| *C. coggygria* 'Royal Purple' | | *C. coggygria* 'Young Lady' | |
| **Control** | 1,04 | **ab\*** | 0,96 | **a** | 1,00 | **a** | |
| **Rhizopon AA** | 1,01 | **ab** | 0,89 | **a** | 0,95 | **a** | |
| **IBA** | 1,03 | **ab** | 0,93 | **a** | 0,98 | **a** | |
| **AlgaminoPlant × 1** | 1,64 | **abc** | 0,98 | **ab** | 1,31 | **ab** | |
| **AlgaminoPlant × 2** | 1,69 | **abc** | 0,81 | **a** | 1,25 | **ab** | |
| **AlgaminoPlant × 3** | 1,70 | **bc** | 1,30 | **abc** | 1,5 | **ab** | |
| **Route × 1** | 1,04 | **abc** | 1,11 | **ab** | 1,08 | **a** | |
| **Route × 2** | 1,03 | **ab** | 1,94 | **c** | 1,49 | **ab** | |
| **Route × 3** | 1,64 | **abc** | 1,84 | **c** | 1,74 | **b** | |
| **Means**  (for cultivars) | 1,31 | **a** | 1,19 | **a** |  | |  | |
| \* Means followed by the same letter do not differ significantly at α = 0,05. | | | | | | | |

**Photosynthesis efficiency**

**Table 4.** Effect of biostimulators AlgaminoPlant and Route on photosynthesis rate (µmol CO2 · m-2 · s-1) in cuttings of smoke tree 'Royal Purple' and 'Young Lady'.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Taxon** | | | | **Means**  (for treatments) | | |
| *C. coggygria* 'Royal Purple' | | *C. coggygria* 'Young Lady' | |
| **Control** | 1,16 | **a\*** | 1,17 | **a** | 1,17 | **a** | |
| **Rhizopon AA** | 2,53 | **bc** | 3,22 | **bcd** | 2,88 | **b** | |
| **IBA** | 2,61 | **bc** | 3,59 | **de** | 3,10 | **b** | |
| **AlgaminoPlant × 1** | 4,46 | **fg** | 2,94 | **bcd** | 3,70 | **b** | |
| **AlgaminoPlant × 2** | 3,74 | **def** | 3,52 | **de** | 3,65 | **b** | |
| **AlgaminoPlant × 3** | 3,92 | **efg** | 3,31 | **cde** | 3,63 | **b** | |
| **Route × 1** | 2,53 | **bc** | 3,66 | **def** | 3,10 | **b** | |
| **Route × 2** | 5,57 | **h** | 2,20 | **b** | 3,88 | **b** | |
| **Route × 3** | 4,76 | **gh** | 2,65 | **bc** | 3,71 | **b** | |
| **Means**  (for cultivars) | 3,48 | **b** | 2,92 | **a** |  | |  | |
| \* Means followed by the same letter do not differ significantly at α = 0,05. | | | | | | | |

ANOVA 2 showed significant effects of both treatments and cultivars on the photosynthetic rate in cuttings (Table 4). In the red-leaved 'Royal Purple' the average photosynthetic rate was 20% higher than in green-leaved 'Young Lady'. In both cultivars all treatments stimulated photosynthesis relative to controls. Auxin applications resulted in a two- and threefold increase in photosynthesis in 'Royal Purple' and 'Young Lady', respectively. Generally, cuttings of 'Royal Purple' treated with biopreparations had higher photosynthetic rates those treated with the auxin (3.2 – 4.8 times higher than in control) while in 'Young Lady' no significant differences were observed between treatments (Table 4).

**Total chlorophyll**

Treatments and cultivars significantly affected the levels of chlorophyll a+b in leaves of cuttings (Table 5 and 6). 'Royal Purple' contained more chlorophyll, and its levels were not affected by biopreparations. In 'Young Lady', sprayed AlgaminoPlant and Route increased the chlorophyll amounts by 9% and 35%, respectively (Table 7).

**Endogenous indolyl acids**

The treatments but not cultivars affected the IA contents in cuttings (Table 5 and 6). The average IA content in cuttings sprayed with 'Route' was twice as high as in untreated cuttings or those sprayed with AlgaminoPlant (Table 7). In 'Royal Purple', 'Route' did not affect the IA content relative to control but increased it by 2/3 relative to AlgaminoPlant. In 'Young Lady' , the effect of Route was more pronounced as it increased the IA level to 403% and 262% of control and the AlgaminoPlant treatment, respectively (Table 7).

**Reducing sugars**

There was no effect of the biostimulator treatments on the reducing sugars contents, however, the cultivar effect was significant (Table 5 and 6). Leaves of the purple cultivar contained more reducing sugars than had the green leaves of 'Young Lady', by 10%, 50% and 49% in control, AlgaminoPlant- treated and Route-treated cuttings, respectively (Table 7). In the cuttings of 'Royal Purple' treated with AlgaminoPlant, the reducing sugar level increased by 14% relative to control while the increase caused by Route was insignificant. In 'Young Lady', both preparations produced a decrease in reducing sugars in the leaves (Table 7).

**Table 5.** Significance ofcultivar effects on mean contents of analyzed compounds and activity of enzymes in cuttings of smoke tree (*C. coggygria*)'Royal Purple' and 'Young Lady' (means for cultivars of ANOVA 2).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Concentration / activity of** | ***C. coggygria* 'Royal Purple'** | | ***C. coggygria* 'Young Lady'** | |
| **total chlorophyll** (mg · g d.m.-1) | 6,08 | **b\*** | 3,57 | **a** |
| **endogenous indolyl acids** (µg of IAA · g f.w.-1) | 3,93 | **a\*** | 5,52 | **a** |
| **reducing sugars** (mg of glucose · g d.m.-1) | 143,73 | **b\*** | 106,76 | **a** |
| **total soluble sugars** (mg of glucose · g d.m.-1) | 132,16 | **a\*** | 145,01 | **a** |
| **free amino acids** (µg of leucine · g d.m.-1) | 460,54 | **b\*** | 333,11 | **a** |
| **soluble proteins** (mg · g d.m.-1) | 142,07 | **b\*** | 85,77 | **a** |
| **polyphenolic acids** (mg of caffeic acid · g d.m.-1) | 19,57 | **b\*** | 12,21 | **a** |
| **polyphenolic oxydase (PPO)** (nkat · g d.m.-1) | 7,53 | **a\*** | 14,48 | **b** |
| **hydrogen peroxide (H2O2)** (µg ·g d.m.-1) | 20,98 | **a\*** | 68,24 | **b** |
| **catalase** (mkat · g d.m.-1) | 1448,43 | **b\*** | 819,13 | **a** |
| **peroxidase** (mkat · g d.m.-1) | 0,747 | **a\*** | 0,686 | **a** |

\* Means followed by the same letter do not differ significantly at α = 0,05.

**Table 6.** Significance oftreatment effects on mean contents of analyzed compounds and activity of enzymes in cuttings of smoke tree (*C. coggygria*)(means for treatments of ANOVA 2).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Concentration / activity of** | **Control** | | **AlgaminoPlant** | | **Route** | |
| **total chlorophyll** (mg · g d.m.-1) | 4,60 | **a\*** | 4,80 | **ab** | 5,08 | **b** |
| **endogenous indolyl acids** (µg of IAA · g f.w.-1) | 3,28 | **a\*** | 3,37 | **a** | 7,52 | **b** |
| **reducing sugars** (mg of glucose · g d.m.-1) | 128,70 | **a\*** | 127,72 | **a** | 119,30 | **a** |
| **total soluble sugars** (mg of glucose · g d.m.-1) | 167,17 | **b\*** | 119,30 | **a** | 129,28 | **a** |
| **free amino acids** (µg of leucine · g d.m.-1) | 292,68 | **a\*** | 404,08 | **b** | 493,72 | **c** |
| **soluble proteins** (mg · g d.m.-1) | 104,34 | **a\*** | 118,55 | **a** | 118,86 | **a** |
| **polyphenolic acids** (mg of caffeic acid · g d.m.-1) | 11,07 | **a\*** | 18,02 | **b** | 18,58 | **b** |
| **polyphenolic oxydase (PPO)** (nkat · g d.m.-1) | 4,54 | **a\*** | 14,94 | **b** | 13,54 | **b** |
| **hydrogen peroxide (H2O2)** (µg ·g d.m.-1) | 60,93 | **b\*** | 34,18 | **a** | 38,72 | **a** |
| **catalase** (mkat · g d.m.-1) | 434,75 | **a\*** | 1676,66 | **b** | 1289,92 | **b** |
| **peroxidase** (mkat · g d.m.-1) | 0,780 | **b\*** | 0,700 | **a** | 0,671 | **a** |

\* Means followed by the same letter do not differ significantly at α = 0,05.

**Table 7.** Effect of AlgaminoPlant and Route on contents of analyzed compounds and activity of enzymes in cuttings of smoke tree (*C. coggygria*)'Royal Purple' and 'Young Lady'.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration / activity of** | ***C. coggygria* cv.** | **Control** | | **AlgaminoPlant** | | **Route** | |
| **total chlorophyll** (mg · g d.m.-1) | 'Royal Purple' | 6,08 | **de\*** | 6,21 | **e** | 5,96 | **d** |
| 'Young Lady' | 3,11 | **a** | 3,39 | **b** | 4,21 | **c** |
| **endogenous indolyl acids** (µg of IAA · g f.w.-1) | 'Royal Purple' | 4,03 | **ab\*** | 2,95 | **a** | 4,80 | **b** |
| 'Young Lady' | 3,11 | **a** | 3,39 | **b** | 4,21 | **c** |
| **reducing sugars** (mg of glucose · g d.m.-1) | 'Royal Purple' | 134,88 | **c\*** | 153,49 | **d** | 142,81 | **cd** |
| 'Young Lady' | 122,53 | **b** | 101,95 | **a** | 95,79 | **a** |
| **total soluble sugars** (mg of glucose · g d.m.-1) | 'Royal Purple' | 171,43 | **d\*** | 109,06 | **a** | 116,00 | **ab** |
| 'Young Lady' | 162,91 | **d** | 129,55 | **bc** | 142,57 | **c** |
| **free amino acids** (µg of leucine · g d.m.-1) | 'Royal Purple' | 344,39 | **b\*** | 479,79 | **d** | 557,45 | **e** |
| 'Young Lady' | 240,97 | **a** | 328,36 | **b** | 430,00 | **c** |
| **soluble proteins** (mg · g d.m.-1) | 'Royal Purple' | 111,92 | **c\*** | 159,64 | **d** | 154,63 | **d** |
| 'Young Lady' | 96,75 | **b** | 77,46 | **a** | 83,09 | **a** |
| **polyphenolic acids** (mg of caffeic acid · g d.m.-1) | 'Royal Purple' | 13,06 | **c\*** | 24,18 | **f** | 21,48 | **e** |
| 'Young Lady' | 9,08 | **a** | 11,85 | **b** | 15,69 | **d** |
| **polyphenolic oxydase (PPO)** (nkat · g d.m.-1) | 'Royal Purple' | 3,94 | **a\*** | 9,47 | **b** | 9,17 | **b** |
| 'Young Lady' | 5,14 | **a** | 20,41 | **d** | 17,90 | **c** |
| **hydrogen peroxide (H2O2)** (µg ·g d.m.-1) | 'Royal Purple' | 31,54 | **b\*** | 15,28 | **a** | 16,11 | **a** |
| 'Young Lady' | 90,32 | **e** | 53,08 | **c** | 61,33 | **d** |
| **catalase** (mkat · g d.m.-1) | 'Royal Purple' | 439,02 | **a\*** | 2226,78 | **c** | 1679,49 | **bc** |
| 'Young Lady' | 430,49 | **a** | 1126,54 | **b** | 900,347 | **a** |
| **peroxidase** (mkat · g d.m.-1) | 'Royal Purple' | 0,836 | **c\*** | 0,672 | **ab** | 0,733 | **b** |
| 'Young Lady' | 0,724 | **b** | 0,727 | **b** | 0,608 | **a** |

\*Means followed by the same letter do not differ significantly at α = 0,05.

**Total soluble sugars**

The effect of treatments on total soluble sugars content was significant while that of cultivar was not (Table 5 and 6). Biopreparations sprayed on cuttings reduced carbohydrate levels in both cultivars as compared to respective controls, by as much as 1/3 in 'Royal Purple' sprayed with AlgaminoPlant (Table 7).

**Free amino acids**

Both, treatments and cultivars significantly affected the free amino acid contents in the leaves (Table 5 and 6). The red-leaved cultivar contained more free amino acids than the 'Young Lady' (Table 5). Biopreparations increased their contents in both cultivars relative to respective controls (Table 7). Route was more effective than AlgaminoPlant in increasing the amino acid contents, by 62% and 78% in 'Royal Purple' and 'Young Lady', respectively (Table 7).

**Soluble proteins**

Leaves of control cuttings of 'Royal Purple' contained more soluble proteins than did the leaves of 'Young Lady' (Table 5); and spraying them with both preparations significantly increased their contents, on the average by 40% (Table 7). An opposite effect was observed in 'Young Lady' where treatments with both preparations reduced the levels of soluble proteins, by 14-20% as compared to the control cuttings (Table7).

**Polyphenolic acids**

Royal Purple had a significantly higher level of polyphenolic acids (Table 5). In both cultivars the levels were increased by treatments with biopreparations (Table 6): in 'Royal Purple' AlgaminoPlant was more effective increasing their content by 85% relative to the control while in 'Young Lady' Route caused the 72% increase in the content of polyphenolic acids (Table 7).

**Polyphenolic oxydase activity (PPO)**

Both, the cultivars and the treatments significantly affected PPO activity in cuttings (Table 5 and 6). In both cultivars the enzyme activity was increased due to the application of both biopreparations (Table 7). There was less activity in 'Royal Purple' and the increase was 2.3–2.4-fold after spraying with Route and AlgaminoPlant, respectively. In 'Young Lady', the increase in the PPO activity relative to control was 3,5- to 4-fold after Route and AlgaminoPlant applications, respectively (Table 7).

**Hydrogen peroxide (H2O2)**

Treatments and cultivars significantly affected the hydrogen peroxide contents (Table 5 and 6). In both cultivars the levels dropped after applications of either biopreparation, on the average by half relative to respective controls (Table 7). In control cuttings of 'Young Lady' the amount of H2O2 was three times higher than in 'Royal Purple' and decreases resulting from biopreparation use were somewhat smaller in this cultivar as compared to the red-leaved 'Royal Purple' (35-43%) (Table 7).

**Catalase activity**

The treatments and the cultivars significantly affected the catalase activity in leaves (Table 5 and 6). Though the enzyme activities in untreated controls were similar in both cultivars, the average activity in 'Royal Purple' was almost double of that found in 'Young Lady' (Table 7). In, 'Royal Purple' both biopreparations increased the catalase activity - 4-5 times relative to control (Table 7). In 'Young Lady' such an increase occurred only in the AlgaminoPlant-treated cuttings - to 260% of the control – while the doubling of the activity by Route proved statistically insignificant (Table 7).

**Peroxidase activity**

The peroxidase activity was not affected by the cultivar while the effects of treatment were significant (Table 5 and 6). In control cuttings, the peroxidase activity was higher in 'Royal Purple' than in 'Young Lady' (Table 7). Both treatments with biopreparations decreased this activity in the red-leaved cultivar (20% and 13% for AlgaminoPlant and Route, respectively) while in 'Young Lady' only Route showed similar effect lowering the enzyme activity by 16% relative to control (Table 7).

**Endogenous ethylene production**

**Table 8.** Effect of AlgaminoPlant and Route on endogenous ethylene production efficiency (µl · l-1 · kg-1 · h-1) in cuttings of smoke tree 'Royal Purple' and 'Young Lady'.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Taxon** | | | | **Means**  (for treatments) | | |
| *C. coggygria* 'Royal Purple' | | *C. coggygria* 'Young Lady' | |
| **Control** | 152,85 | **g\*** | 68,32 | **b** | 110,85 | **b** | |
| **Rhizopon AA** | 165,96 | **h** | 99,75 | **de** | 132,85 | **c** | |
| **IBA** | 177,55 | **i** | 165,63 | **h** | 171,59 | **d** | |
| **AlgaminoPlant × 1** | 91,22 | **cd** | 56,98 | **a** | 74,10 | **a** | |
| **AlgaminoPlant × 2** | 71,68 | **b** | 65,06 | **b** | 68,37 | **a** | |
| **AlgaminoPlant × 3** | 70,37 | **b** | 71,85 | **b** | 71,11 | **a** | |
| **Route × 1** | 112,74 | **f** | 90,29 | **c** | 101,52 | **b** | |
| **Route × 2** | 103,46 | **e** | 92,59 | **cd** | 98,03 | **b** | |
| **Route × 3** | 117,26 | **f** | 91,22 | **cd** | 104,24 | **b** | |
| **Means**  (for cultivars) | 118,12 | **b** | 89,08 | **a** |  | |  | |
| **\*** Means followed by the same letter do not differ significantly at α = 0,05. | | | | | | | |

Both, the cultivars and the treatments significantly affected the ethylene production (Table 8). Its rate was higher in 'Royal Purple' than 'Young Lady' . In both cultivars treatments with auxins significantly increased ethylene biosynthesis – less so in 'Royal Purple' while in 'Young Lady' the sprayed water IBA solution more than doubled the amount of gas emission. In 'Royal Purple' all the treatments with biopreparations significantly reduced ethylene biosynthesis relative to control (Table 8).

**Discussion**

The process of forming a root ball by a stem cutting depends on numerous factors, both internal and external [Buraczyk and Zakrzewski 1990, Bojarczuk 1997, Spethmann 2001]. Rooting powders containing auxins have been routinely used to enhance rhizogenesis with β-indolilobutyric acid (IBA) being the most effective. Pacholczak and Szydło [2008] reported positive effects of Rhizopon AA (containing IBA) on rooting of ninebark cuttings. In this study, in two cultivars of smoke tree, both methods of IBA application (rooting powder Rhizopon AA and sprayed water solution of IBA) stimulated rhizogenesis by increasing the percentage and the degree of rooting.

Biopreparations are relatively new tools created to assist plants in adjustment to stressful conditions, such as detaching cuttings from stock plants. They also enable plants to make the best use of the rooting conditions provided by nurserymen [Schmidt et al. 2003]. Khan et al. [2009] reports that seaweed extracts promote growth of roots in *Pinus pinea* seedlings, increasing their dry mass content. In the trials on maize, biopreparations applied in early stages of plant development produced plants with well developed root systems [Jeannin et al. 1991]. Nedumaran and Peruman [2009] after trials on *Rhizophora mucronata* suggested that the best growth of shoots and roots could be obtained with frequent applications of seaweed extracts in low concentrations. Using a seaweed preparation, Thorsen et al. [2010] enhanced root development in *Plantago lanceolata* though not in *Vigna radiata* where the auxin IAA was effective in promoting rooting. AlgaminoPlant tested here on smoke tree stimulated rhizoogenesis in dogweed (*Cornus alba* 'Aurea' and 'Elegantissima'), but its efficiency depended on a number of treatments [Pacholczak et al. 2012].

Zinc ammonium acetate (ZAA) is an active ingredient of the preparation Route [Cattanach 1992, Pacholczak and Szydło 2008]. As shown in ninebark, its foliar application can improve the percentage and the degree of rooting [Pacholczak and Szydło 2008]. In this work it enhanced rhizogenesis in both smoke tree cultivars tested while AlgaminoPlant was effective only in one.

The efficiency of the photosynthetic apparatus in cuttings remains low during rooting until the first roots appear. Therefore little energetic substrates are available in the early stages of rhizogenesis. They are indispensable for respiration which provides energy for rhizogenesis , a process demanding a high energy input [Couvillon 1988, Spethmann 2001]. In this study, no effect of biopreparations on respiration was observed (excepting triple application of Route) but both preparations increased the activity of the photosynthetic system. Positive effects of biostimulators on the photosynthetic rate were reported in *Arabidopsis thaliana* [Przybysz et al. 2010, Borowski and Blamowski 2009].

The efficiency of the photosynthetic apparatus depends on the chlorophyll contents [Couvillon 1988]. Both biopreparations tested enhanced chlorophyll synthesis. Jacygrad and Pacholczak [2010] reported an increase in green pigment contents in nine bark cuttings treated with biopreparation Amino Total which – similarly to AlgaminoPlant used here– contains a mixture of amino acids. Jothinayagi and Anbazhagan [2009] observed that in *Abelmoschus esculentus* the chlorophyll content increased by 20% after applications of low doses of sea weed extracts while high doses limited pigment synthesis. According to Khan et al. [2009] both soil and foliar applications of an extract from *Ascophylum nodosum* in low concentration increased chlorophyll contents in tomato leaves. Matysiak et al. [3011] recorded the 40% increase in green pigment content in plants of *Zea mays* after a double foliar application of AlgaminoPlant*.* Also Pacholczak et al. [2012] observed an elevated chlorophyll level in dogwood cuttings treated with AlgaminoPlant. However, Kumar and Mohan [1997] reported a negative effect of sea weeds on chlorophyll content in *Vigna mungo* var. *mungo.*

An increase in the chlorophyll contents was also observed after zinc application on plants [Nahedet al. 2007], however, a fall in pigment level occurred after a treatment with a high zinc dose. Reports on the positive effects of zinc on chlorophyll synthesis also come from some work on cypress (*Cupressus sempervirens*) [Farahat et al. 2007] and pea (*Pisum sativum*) [Massoud et al. 2005]

Auxins participate in numerous processes of plant growth and development, however, from the point of view of woody plant propagation the most important aspect of their action is in stimulation of rhizogenesis [Bojarczuk 1997]. Blakesley et al. [1991] studied changes in the auxin contents in smoke tree cuttings harvested from stock plants of different age and on two dates - in June and July. Rooting was better in June and the results were correlated with higher levels of free auxins. In cuttings harvested in July, the levels of free IAA were lower while those of conjugated IAA elevated as compared to cuttings from June. The authors speculate that in smoke tree the elevated IAA level may be indispensable for root initiation while its subsequent decrease enables the root to elongate. There was a positive effect of Route on the content of endogenous indolil acids in both cultivars while AlgaminoPlant was ineffective. Probably, zinc from the preparation participated in auxin synthesis.

Carbohydrate accumulation in plant tissues is crucial for successful propagation by cuttings as rhizogenesis demands a high energy input, especially in the first stages of root initiation. Sugars are substrates for respiration that provide energy necessary for organ differentiation [Couvillon 1988, Costa et al. 2007]*.* According to Sivasankari et al. [2006] the levels of reducing and total soluble sugars increase in plants of *Vigna sinensis* after seaweed extract applications. Rathore et al. [2009] reported an increase in the carbohydrate contents in plants of *Glycine max* treated with a biopreparation based on an extract from *Kappaphycus alvarezii.* However, Jacygrad and Pacholczaka [2010] observed a drop in the sugar contents in cuttings of two ninebark cultivars treated with biopreparation Amino Total although this drop did not affect the rooting ability. In dogwood, the carbohydrate levels in cuttings increased after AlgaminoPlant [Pacholczak et al. 2012]. Zinc applications also resulted in increases in sugar contents in *Ocimum basilicum* [Bedour at al.1994] and radish [Nabila et al.2003] although no such changes were observed in *Salvia farinacea* [Nahed et al.2007]. In the present work, no significant changes occurred in reducing sugars but the amounts of total soluble sugars dropped in both cultivars tested.

Amino acids are used for protein synthesis and their binding into macromolecules is a process requiring a high energy input. Plants are able to synthesize all amino acids needed for peptide and protein synthesis but supplying them with an mixture of exogenous amino acids may save them energy and improve growth, especially in the critical developmental stages when large amounts of proteins or their precursors are needed [Bojarczuk 1997]. A positive correlation between an elevated level of free amino acids and rhizogenesis in six species (*Trifolium repens*, *Lolium perenne*, *Zea mays*, *Brassica napus*, *Lycopersicon esculentum*, *Medicago sativa*) was reported by Lessufleur et al. [2007]. In ninebark, the amino acid contents increased during rooting [Jacygrad and Pacholczak 2010]. A similar increase was observed in dogwood cuttings after application of AlgaminoPlant [Pacholczak et al. 2012].

Proteins play multiple functions in plants: structural, metabolic, transport and storage. As enzymes, they participate in multiple reactions and are responsible for a dynamic balance within a cell [Bojarczuk 1997]. Higher contents of soluble proteins were reported in *Hosta* sp. and *Bergenia cordifolia* treated with biopreparations [Krajewska and Latkowska 2008]. Increases in free amino acids and soluble proteins after zinc applications were reported by Tarraf and al.[1999], Nahed et al.[2007] and Farahat et al. [2007], the latter on *Cupressus sempervirens.* In both smoke tree cultivars tested here, AlgaminoPlant and Route increased the free amino acids contents. However, the cultivars differed with the levels of soluble proteins: it increased in 'Royal Purple' and fell in 'Young Lady' after treatments.

Phenolic compounds improve plant metabolism by improving rthe espiration efficiency. They also increase the auxin contents and the number of their receptors [Gawrońska 2008]. They are supposed to protect auxins against oxidation by inhibiting the IAA oxidase with which they may form the phenol-IAA complexes considered as co-factors of auxin metabolism and as rooting.co-factors [Bhattacharya 1988, Fu i in. 2011]. Polyphenolic acids also affect the activity of certain enzymes participating in rhizogenesis and take part in forming lignins covering cell walls [Gorter 1969, Przybysz et al. 2010]. Predictably, an increase in phenolic compounds was observed here after treatments with each of the tested compounds. Similarly, in trials on ninebark, the phenolic compounds increased after foliar application of biostimulators [Jacygrad and Pacholczak 2010]. On the contrary, phenol levels dropped in basil after watering plants with solutions of biopreparations Aminoplant and Goëmar [Rosłon et al. 2011].

Reactive oxygen species (ROS) participate in multiple metabolic processes in cells. Their presence is often related to stress conditions such as drought or wounding, when their synthesis and accumulation take place after normal cell activities are disturbed [Hodges [2001]. They can affect the structure and properties of cell organelles and disturb their functions by oxidizing various compounds and this in turn may lead to degradation of membranes, nucleic acids or even cause mutations [Starck and Chołuj 1995]. Hydrogen peroxide (H2O2) is one of ROS and it may accumulate in damaged tissues, vessels, stomata and neighbouring cells. It appears to be produced by transformation of the superoxide anion in a spontaneous reaction of dismutation or in a reaction catalyzed by the superoxide dismutase [Dietz et al. 2006]. Both, AlgaminoPlant and Route reduced the hydrogen peroxide contents in cuttings. It appears that both biopreparations mitigated the effects of the oxidative stress thus creating conditions for better root regeneration.

Oxidoreductases are specific enzymes catalyzing redox reactions. They transport electrons and hydrogen atoms between the reductant and the oxidant. Such reactions maintain homeostasis between reduced and oxidized forms of a compound, such as oxygen [Nowak et al. 2004]. When this homeostasis is disturbed, free radicals appear and the oxidative stress occurs. Plant sensitivity to this stress depends on a quick removal of free radicals and less so on their formation rate. Enzymes such as peroxidases and catalases are involved in the removal of free radicals [Starck and Chołuj 1995]. Preparations tested here increased the activities of catalase and polyphenol oxidase while that of peroxidase decreased. In tall fescue (*Festuca arundinacea*) treated with a preparation containing an extract from the sea weed *Ascophyllum nodosum,* the activity of superthe oxide dismutase increased by 30% (Zhang [1997]. These observations were verified by Fike et al. [2001]. On the other hand, Ayad [1998] reported a reduction in activities of SOD, glutathione reductase and ascorbinian peroxidase in tall fescue treated with the same sea weed extract [Ayad 1998]. According to Zhanng et al. [2006], extracts from algae *Ascophyllum nodosum* increased the SOD activity in *Agrostis stolonifera* infected with a fungus *Sclerotinia homoeocarpa* while reducing the rate of infection. There are no reports on the AAC effects on the oxidoreductase activity. Here it may be concluded that both AlgaminoPlant and Route enhance the responses to the oxidative stress by reducing the levels of hydrogen peroxide. In the first phase of this response the activities of the antioxidative enzymes are enhanced while later that of peroxidase is decreased while catalase and polyphenol oxidase remain highly active.

Ethylene is a gaseous plant hormone. The first reports on its involvement in rhizogenesis date back to 1930’ties but the mechanism of its action is yet to be discovered [Zimmerman and Hitchcock 1933, Wang and Pan 2006]. Published reports document a positive effect of ethylene on root initiation and development [Pan et al. 2002], or a negative one [Nordström and Eliasson 1984] or no effect at all[Harbage and Stimart 1996]. The opinions prevails that the effect may be concentration-dependent [Kawase 1976, Simson 1984], or depend on the physiological state of cuttings, the level of endogenous C2H4 [Liu and Reid 1992, Visser et al. 1996] and its interaction with other hormones, such as a change in tissue sensitivity to auxins stimulated by ethylene [Wang and Pan 2006]. Some woody cuttings, especially conifers, positively respond to treatments with the ethylene-releasing etephone applied prior to the auxin applicatios. Similar effects of stimulation by using Ethrel-A were observed in etiolated cuttings of *Quercus robur* [Istas and Meneve 1981, Simson 1984]. In this study on smoke tree cuttings, the production of endogenous ethylene was reduced after application of AlgaminoPlant and Route.

The results obtained in this work do not provide a complete answer on the mechanisms of action of the biostimulaters in rhizogenesis but they clearly show that several physiological processes are clearly affected, and this may help to make a better use of such preparation in the improvement of ornamental plant production in a changing regulatory environment.

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