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Effect of Bio-Algeen® preparation on growth and mycorrhizal characteristics of Norway spruce seedlings

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ABSTRACT: In this paper, the effect of the Bio-Algeen® product (bio-alginate from the seaweed *Ascophyllum nodosum* (Linnaeus) Le Jolis) was evaluated in relation to basic mycorrhizal and growth characteristics of Norway spruce (*Picea abies* (Linnaeus) H. Karsten) seedlings. The seedlings were planted in a forest nursery in 5 different treatments: 4 treatments with the Bio-Algeen® product in different regimes (soaking, granulate, soaking + granulate, irrigation) and control. Compared to the control, mycorrhizal characteristics of the treated seedlings differed significantly for soaking treatment only, but they were less favourable for all treatments with this product. Among the growth characteristics, the height of aboveground parts was significantly taller compared to the control for soaking and granulate treatments, while the shoot dry weight was significantly higher for granulate treatment only. Both of these characteristics showed higher values across all treatments compared to the control. Although treated seedlings showed worse mycorrhizal characteristics, their growth was not affected by these characteristics. Thus, Bio-Algeen® can be used for an improvement of seedling growth in forest nurseries.

Keywords: *Ascophyllum nodosum*; ectomycorrhiza; nurseries; *Picea abies*; seaweed

Pre-planting application of plant growth regulators and other preparations supporting the growth of plant roots appears to be an affordable way to increase the growth of roots and to improve the tree survival (Scagel, Linderman 2001). Seaweeds and derived products are widely utilized as important sources of organic and inorganic compounds, organic fertilizers, and stimulants to increase plant growth and yield (Khan et al. 2009). *Ascophyllum nodosum* (Linnaeus) Le Jolis is the most frequently used seaweed belonging to the order *Fucales* Kylin. It occurs close to sheltered rocky shores in the North Atlantic (Olsen et al. 2010). From the seaweeds are extracted hydrolysates known as bio-alginates. These are concentrated solutions of selected seaweed gels and natural polysaccharides composed of polyuronic acids. Bio-alginates contain also a wide range of biologically active agents, including amino acids, oligopeptides, organic acids, minerals, trace elements, and phytohormones (auxins). In contact with metals that are present in water, bio-alginates form a gel-flake system that is insoluble in water (Vostroupal 2007).

Growth-promoting effects of bio-alginates on plants have been assessed on important agronomic and horticultural plants, namely flax, potatoes, hops, rapeseed, white mustard, lettuce, bluegrass, maize, barley (Vlk 1990), wheat (Vašáková et al. 1995), clover

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(Sawicki, Szymona 2005), sugar beet (Pospíšil et al. 2006), tomatoes (Dobromilska et al. 2008; Mikiciuk, Dobromilska 2014), carrot (Kwiatkowski et al. 2013), apple trees (von Bennewitz, Hlušek 2006) and orange trees (Spann, Little 2011). There are only a few papers dealing with the effect of bio-alginates on the growth of important tree species in forestry. Such research was carried out on Douglas fir, Engelmann spruce (Scagel, Linderman 2001), lodgepole pine (Scagel, Linderman 2001; MacDonald et al. 2012), white spruce (MacDonald et al. 2013), Norway spruce (Kupka et al. 2015), and European ash (Bulíř 2005).

Ectomycorrhizal symbiosis is a relationship occurring almost solely in forest trees. A part of the root colonized by fungi (mycorrhiza) is frequently turgid, unlike non-colonized roots (Gryndler et al. 2004). Hyphae create a complex, highly branched structure between the root cells, called Hartig net. The tangle of hyphae on the root surface creates a hyphal mantle. From the mantle emanating hyphae grow into the surrounding soil (Peterson et al. 2004). The fungi obtain important organic compounds from the host plant. Plants with well-developed mycorrhizal symbiosis are characterized by increased resistance to low temperature, drought, toxins, changes in pH, root pests, and parasites (Mejstřík 1988; Pešková 2008). Although bio-alginates do not contain any mycorrhizal fungi, they can significantly affect the growth and colonization rate of such fungi, as has been demonstrated for arbuscular mycorrhizal fungi (Kuwada et al. 1999, 2006). Nevertheless, there are no papers available dealing with the effect of bio-alginates on the ectomycorrhizal characteristics of tree species. The aim of the present study was to test the effect of bio-alginate application on growth and mycorrhization in Norway spruce seedlings.

MATERIAL AND METHODS

The effect of Bio-Algeen® was assessed on Norway spruce seedlings in a forest nursery at Obrovice in the Czech Republic (50°16.92’N, 13°12.50’E). The location belongs to Natural Forest Area No. 4 – Doupovské hory Mts. and to the 4th forest vegetation zone – beechwood (Plíva 1987). Mean annual temperature at the location is 7°C, mean annual precipitation is 607 mm. Soil type is Gleyic Cambisols (Němeček et al. 2004). Products of the Bio-Algeen® series were evaluated, namely root concentrate, granulate, and S-90 (all Schulze et Hermsen GmbH, Dahlenburg, Germany). All of these are hydrolysates (Bio-Algeen® granulate is a granulated hydrolysate) extracted from the seaweed *A. nodosum* (Table 1).

The cultivation of Norway spruce seedlings in the forest nursery was as follows: seeds were sown in the first year and cultivated on the seedbeds for 0.5–1 year. Then, the seedlings were transplanted outside in Au-

Table 1. Types of tested Bio-Algeen® products, application methods and rates, costs of the product and costs per seedling (Hanzal et al. 2015)

Bio-Algeen®	Application method and rate	Consumption of the product	Cost	
			product	per seedling (CZK)
Root concentrate (used for root coating before planting)	soaking (root system of plants soaked in a solution of the product root concentrate diluted with water at 1:20 ratio, mechanical planting)	according to the size of the root ball	(1) 200 CZK·l^{-1}	0.01
Granulate (granular form of the *Ascophyllum nodosum* hydrolysate, applied to soils and substrates)	granulate (before planting the granulate at dosage 150 g·m^{-2} applied over the seedbed area, 65 seedlings per 1 m^2)	ca. 2 kg	(2) 150 CZK·kg^{-1}	0.35
Root concentrate + granulate	soaking + granulate (before planting granulate at dosage 150 g·m^{-2} applied over the seedbed area and simultaneously the root system of plants soaked in a solution of the product root concentrate diluted with water at 1:20 ratio, mechanical planting)	ca. 2 kg (according to size of root ball)	(1) + (2)	0.36
S-90 (used for spraying/watering/as solution, containing the concentrate of polyuronic acids, amino acids, phytohormones, and trace elements)	irrigation (after planting, S-90 sprayed over the area in the form of irrigation, diluted with water at 1:200 ratio)	ca. 1 l	220 CZK·l^{-1}	0.22
Control	without any application			0

gust 2011. Five treatments were used with different modes of preparation: soaking, granulate, soaking + granulate, irrigation, and control (Table 1). After transplanting, the seedlings were irrigated. In the following years, the trees received only natural precipitation. Weeds were removed manually or using a mechanical hoe. In spring 2011, additional basic fertilization with Cererit® (a combination of granular fertilizer without chloride, containing N, P, K, B, Mo, Zn, and Cu) was carried out for all treatments. The first assessment phase of the experiment was presented by Kupka et al. (2015).

On 14 March 2014, 10 spruce seedlings growing within a row were removed from each treatment (50 seedlings in total), each time from a row in the centre of the seedbed. Seedlings were carefully cleaned and their growth characteristics were measured: height of aboveground part (measured from the root collar to the terminal bud), length of terminal vegetative shoot, length of the main root (measured from the root collar to the end of the main root), and root collar diameter (mean).

Mycorrhizal characteristics were assessed according to Pešková and Soukup (2006). Mycorrhizae were assessed on roots < 1 mm in diameter, as these roots most sensitively react to changes in environmental conditions. Twenty root sections with the length of the main root 5 cm were randomly selected. The total length of each section was calculated as the length of the main root plus the lengths of lateral roots. Mycorrhizal tips for each root section were then quantified under a stereo microscope with 40 × magnification. These tips were classified into one of the two groups for either active or non-active mycorrhizae. Lighter tips with smooth surface and lacking root hairs, with high turgor and developed hyphal mantle were classified into the group of active mycorrhizae. Darker, wrinkled tips, with a loss of turgor and lacking the hyphal mantle were classified into the group of non-active mycorrhizae (Kocourek 1991). The level of mycorrhization was determined according to Caisová (1994) as density of mycorrhizae and their relative distribution. Density of active and non-active mycorrhizae was calculated as the number of active (non-active) mycorrhizae per 1 cm of root length. Relative distribution of active mycorrhizae was calculated as the number of active mycorrhizae per total number of mycorrhizae (Caisová 1994). After the evaluation of mycorrhizae, the roots were dried in a dryer at 105°C and then weighed to obtain the weight of root dry matter. The weight of aboveground dry matter was obtained in the same manner.

Statistical evaluation was carried out in R version 3.0.2. (R Development Core Team 2013) by Pekár and Brabec (2009) and Zuur et al. (2009). Analysis of variance was used to test the effect of the Bio-Algeen® product on the evaluated mycorrhizal characteristics (density of active mycorrhizae, density of non-active mycorrhizae, relative distribution of active mycorrhizae, weight of root dry matter). Homogeneity of variance was tested using Bartlett's test and correspondence with the Gaussian distribution using the Shapiro-Wilk test applied on the residuals of a relevant linear model (Pekár, Brabec 2009). To assess the effect of the treatment, "treatment contrasts" with the control variant were set up as a reference group and a *t*-test was used. Failing to meet the conditions required for ANOVA, the Kruskal-Wallis (K-W) test and Dunn's test were used (Dunn 1961). A generalized linear model and *t*-test using "treatment contrasts" with the control variant set up as a reference group were used to examine the effects of the mycorrhizal characteristics and Bio-Algeen® product on the growth characteristics of seedlings (height of the aboveground part, length of the main root, root collar diameter, length of the terminal vegetative shoot and weight of aboveground dry matter).

RESULTS

Statistically significant differences in the density of active mycorrhizae between treatments were observed (ANOVA: $N = 50$, $df = 4$, $P < 0.05$; Table 2). All treatments with Bio-Algeen® compared to the control showed lower density of active mycorrhizae (Tables 2 and 3), but these differences were significant for soaked seedlings only (post hoc *t*-test: $P < 0.05$). Close to significant differences in the density of active mycorrhizae compared to the control were seedlings treated with granulate (post hoc *t*-test: $P = 0.06$).

Contrary to density of active mycorrhizae, density of non-active mycorrhizae showed opposite results. It differed significantly between the treatments (K-W test: $N = 50$, $df = 4$, $P < 0.05$; Table 2). All treatments with Bio-Algeen® compared to the control showed higher median values of density of non-active mycorrhizae, but these differences were significant for soaked seedlings only (Dunn's test: $z = 3.153$, $P < 0.01$; Table 2).

Relative distribution of active mycorrhizae showed similar results like density of active mycorrhizae. It differed significantly between the treatments (K-W test: $N = 50$, $df = 4$, $P < 0.05$; Table 2).

Table 2. Statistical tests of the assessed mycorrhizal characteristics

Mycorrhizal parameter		Difference between treatments	Significantly lower than control treatment	Significantly higher than control treatment
Density of	active mycorrhizae	$F = 2.5886$ $P < 0.05$ ANOVA	soaking $P < 0.05$ post hoc t-test	– – –
	non-active mycorrhizae	$\chi^2 = 10.8512$ $P < 0.05$ Kruskal-Wallis test	– – –	soaking $P < 0.01$ Dunn's test
Relative distribution of active mycorrhizae		$\chi^2 = 10.1432$ $P < 0.05$ Kruskal-Wallis test	soaking $P < 0.05$ Dunn's test	– – –
Weight of root dry matter		$F = 1.2554$ $P > 0.05$ ANOVA	– – –	– – –

All treatments with Bio-Algeen® compared to the control showed lower relative distribution of active mycorrhizae, but these differences were significant for soaked seedlings only (Dunn's test: $z = -3.055$, $P < 0.05$; Table 2).

Weight of root dry matter did not differ significantly between the treatments (ANOVA: $N = 50$, $df = 4$, $P < 0.05$; Table 2).

All evaluated growth characteristics were significantly affected by the weight of root dry matter, but only height of the aboveground part and weight of aboveground dry matter were also significantly affected by the mode of treatment (Table 4).

All treatments with Bio-Algeen® showed taller height of the aboveground part compared to the control (Table 3, Fig. 1), but these differences were significant only for granulate (post hoc t-test: $P < 0.001$) and soaking (post hoc t-test: $P < 0.05$) treatments.

A positive effect of Bio-Algeen® was observed also in the weight of aboveground dry matter. All treatments with Bio-Algeen® showed, compared to the control, higher mean values of the growth characteristics (Table 3), but these differences were significant only for granulate treatment (post hoc t-test: $P < 0.01$).

DISCUSSION

In our study, the statistical analysis showed that the weight of aboveground dry matter was significantly higher for granulate treatment only, compared to the control. However, soaking + granulate and irrigation treatments resulted in the higher mean values of the weight of aboveground dry matter than granulate treatment. These contradictory results were caused by the very low weight of root dry matter. The generalized linear model therefore favoured the granulate treatment due to the relatively higher ratio of shoot to root dry weight.

In our study, the irrigation and soaking + granulate treatments showed the highest values for the seedling growth characteristics. Kupka et al. (2015) assessed the effect of the identical prod-

Table 3. Effect of individual treatments on assessed mycorrhizal and growth characteristics

Variable	Soaking	Granulate	Soaking + granulate	Irrigation	Control
Density of active mycorrhizae* (cm^{-1})	3.30 ± 1.10*	3.67 ± 1.45	4.46 ± 1.17	4.59 ± 1.15	4.72 ± 1.24
Density of non-active mycorrhizae* (cm^{-1})	2.56 ± 1.72**	1.64 ± 0.86	1.48 ± 0.81	1.32 ± 0.62	0.85 ± 0.40
Relative distribution of active mycorrhizae*	0.58 ± 0.22*	0.68 ± 0.20	0.76 ± 0.09	0.77 ± 0.09	0.84 ± 0.08
Weight of root dry matter (g)	4.85 ± 3.48	5.09 ± 2.90	6.60 ± 3.47	7.11 ± 4.22	6.16 ± 3.11
Weight of aboveground dry matter (g)***	15.86 ± 11.59	19.29 ± 13.13**	21.01 ± 8.11	22.10 ± 12.48	15.37 ± 6.99
Root collar diameter (cm)	0.67 ± 0.21	0.73 ± 0.18	0.76 ± 0.13	0.79 ± 0.25	0.69 ± 0.14
Height of aboveground parts (cm)***	39.90 ± 11.65*	44.30 ± 7.04***	40.95 ± 7.95	41.60 ± 9.85	32.10 ± 13.86
Length of terminal vegetative shoot (cm)	21.75 ± 6.59	22.55 ± 4.15	23.00 ± 5.39	23.25 ± 5.14	19.60 ± 7.37
Length of main root (cm)	28.8 ± 9.55	28.65 ± 5.83	33.45 ± 10.58	29.75 ± 4.80	29.70 ± 6.80

values: mean ± standard deviation of the assessed characteristics, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 4. Statistical tests of the assessed growth characteristics

Dependent variable	*F*	*df*	*P*-value	Adjusted R^2	Formula
Root collar diameter	85.19	1	$3.27E^{-12}$	0.6321	root collar diameter = weight of root dry matter
Height of above-ground parts	11.83	5	$3.13E^{-7}$	0.5302	log(height of aboveground part) = weight of dry matter + method of treatment
Length of terminal vegetative shoot	17.92	1	$1.032E^{-4}$	0.2567	length of terminal vegetative shoot = weight of root dry matter
Length of main root	7.44	1	$8.884E^{-3}$	0.1162	log(length of main root) = weight of root dry matter
Weight of above-ground dry matter	33.05	5	$7.64E^{-14}$	0.7658	weight of aboveground dry matter = weight of root dry matter + method of treatment

ucts at the same locality in the previous vegetation season. In comparison with the control, Norway spruce seedlings treated with Bio-Algeen® showed in practically all cases higher values for the assessed growth characteristics (weight of aboveground dry matter, weight of root dry matter, root collar diameter, total height of seedlings). However, statistically significant differences for all these characteristics were observed only for the irrigation and soaking + granulate treatments (Kupka et al. 2015). Differences in growth characteristics between treated and control seedlings were less obvious in our study, probably due to environmental factors which had diminished the effect of the product. Statistically higher values for weight of aboveground dry matter, weight of root dry matter, root collar diameter, and total height of Norway spruce seedlings treated with Bio-Algeen® products were recorded also by Hanzal et al. (2015).

It is difficult to compare the results of our study with the other studies where the same product was used due to different evaluated growth characteristics. Bulíř (2005) recorded no significant effect of the Bio-Algeen® product (treatments: soaking, granulate, irrigation) on initial seedling growth for European ash seedlings growing on anthropogenic substrates, but the treated seedlings showed higher survival. Crops treated with Bio-Algeen® S-90 (in our study specified as the irrigation treatment) have been reported to have higher yields in the cases of clover (Sawicki, Szymona 2005), sugar beet (Pospíšil et al. 2006), tomatoes (Dobromilska et al. 2008; Mikiciuk, Dobromilska 2014), and carrot (Kwiatkowski et al. 2013). Bio-Algeen® can therefore influence the growth of various plant species.

Besides the products of the Bio-Algeen® series, other products containing the seaweed *A. nodosum* were tested on forest trees. The product Acadian® (Acadian Seaplants Ltd., Dartmouth, Canada) applied to container lodgepole pine (MacDonald et al. 2012) and white spruce (MacDonald et al. 2013) seedlings had statistically significant effects on the weight of root dry matter, depending on application rate, but those differences were biologically irrelevant

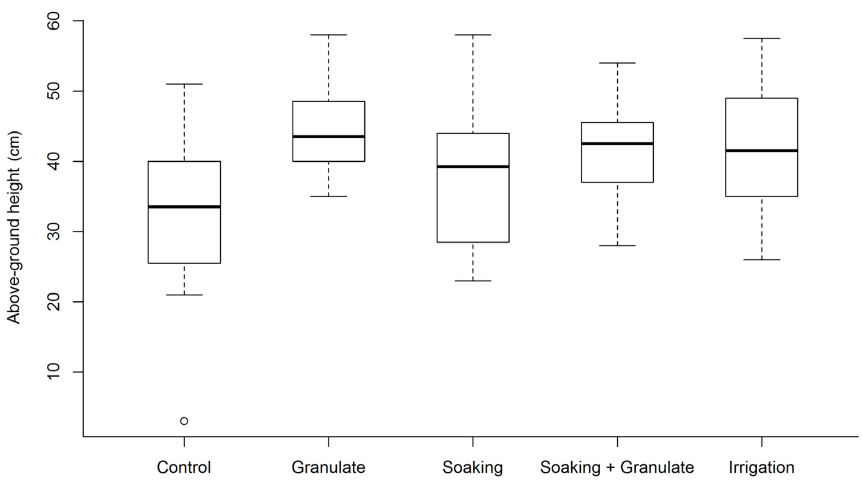

Fig. 1. Height of aboveground parts of seedlings in individual Bio-Algeen® treatments

box – 1st and 3rd quartile, central band – median, whiskers – 1.5 multiple of the interquartile range

(MacDonald et al. 2012, 2013). In our study, the weight of root dry matter did not differ significantly among individual treatments. Kupka et al. (2015) recorded statistically significant increments on English oak, northern red oak, and Norway maple seedlings treated with alginit (an organomineral rock containing seaweeds), compared to control seedlings. For Scotch pine, differences were not statistically significant (Kupka et al. 2015). The results from these papers suggest that bio-alginates and fossils of seaweeds may positively affect the overall growth characteristics of various plant species, but individual characteristics may vary significantly depending upon the treatment and the method of its application.

While the effect of Bio-Algeen® on growth characteristics of Norway spruce seedlings was generally positive in our study, mycorrhizal characteristics were affected negatively (significantly in soaking treatment). Mycorrhizal roots can take up water and nutrients from the soil, accumulate them and pass to the plant better than non-mycorrhizal roots. Therefore, ectomycorrhizal symbiosis is important particularly for plants growing at sites with adverse conditions and during stress (Mejstřík 1988; Gryndler et al. 2004). In such conditions, seedlings treated with products with negative impact on ectomycorrhizae could show worse growth and survival than seedlings with well-developed ectomycorrhizae. Other studies, unlike our study, were focused on plants with arbuscular mycorrhizae (e.g. Hančl 1990; Kudawa et al. 1999, 2006; von Bennewitz, Hlušek 2006).

Financial costs of the Bio-Algeen® product series are bearable. Clearly the lowest costs of seedling treatment were incurred when using Bio-Algeen® root concentrate (soaking treatment) (Hanzal et al. 2015; Table 1).

The results presented in our study illustrate that Norway spruce seedlings treated with products from the Bio-Algeen® series showed higher weight of aboveground dry matter and taller height of aboveground parts. Although treated seedlings showed lower density and lower proportion of active mycorrhizae than untreated seedlings, the growth of seedlings was not significantly affected by these factors. For better knowledge of the effect of bio-alginates on ectomycorrhizae, long-term experiments are needed. There is no certainty that the application of Bio-Algeen® or other bio-alginates will lead to a significant enhancement of the seedling properties. Depending on the tree species, environmental conditions, type of product, technique of its application, and interaction of all the factors, the effect of such a treatment on seedling characteristics can be different. However, in most cases these products affect the growth and development of seedlings positively and, as a rule, not negatively. So, they can be used to enhance the properties of trees in a forest nursery.

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