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ORIGINAL PAPER

The use of biostimulants containing *Ascophyllum nodosum* (L.) Le Jolis algal extract in the cultivation and protection of English oak *Quercus robur* L. seedlings in forest nurseries

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ABSTRACT

Forests in Europe cover an area of 158 million hectares. To preserve them permanently, it is necessary to provide high-quality planting material for renewal and reforestation. With the ever-decreasing number of plant protection products approved in the European Union, searching for alternative methods to improve the condition and development of seedlings in forest nurseries is becoming increasingly popular. One such alternative may be preparations called plant growth and development regulators, or biostimulants. The aim of this study was to evaluate the effect of two biostimulants containing *Ascophyllum nodosum* L. algal extract on the growth and development of pedunculate oak *Quercus robur* L. seedlings. Another goal was to test the preparations in terms of improving resistance to oak powdery mildew *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam infection. These preparations were used in situations where the plants were subjected to severe stress – undercutting of the root systems, a procedure frequently used in forest nurseries. The research was conducted, in parallel, in two forest nurseries in east-central Poland. Both forest nurseries were sprayed twice, fourteen days apart. After two months, 50 seedlings from each variant were collected and the biometric parameters were evaluated: the length of the aerial part, the length of the root systems and the diameter of the root collar. The degree of oak leaf powdery mildew infestation was also compared. The results showed statistically significant differences in the length of the root systems. The seedlings in treatment plots had better developed root system than those in control plots. The other parameters also differed from the control variant, but these differences were not statistically significant. Despite the positive response of the plant to growth, the influence of the biostimulants used on the degree of infection of the oak seedlings by the fungus *E. alphitoides* could not be confirmed.

KEY WORDS

forest nurseries, oak, plant biostimulants, plant stress, seaweed extract

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Introduction

All living organisms, including plants, can be exposed to unfavourable environmental factors. Different intensity and duration of the impact of negative stimuli generate stress. The plant response to a stress factor may be specific for a given stressor or non-specific, *i.e.*, common to several different stress factors. As plants cannot avoid the stressor if it occurs in the area they occupy, they have developed various defence strategies, including (a) the ability to avoid stress (including morphological and biochemical barriers preventing or delaying the activity of the stressor in the cell, *i.e.*, life cycle adaptations) and/or (b) stress tolerance (including alternative pathways that enable the cell to function under stressful conditions, *e.g.*, preventing stress-induced changes, changes in tolerance, or rapid repair mechanisms) (Fraire-Velázquez *et al.*, 2011).

In the face of the difficult task of preventing damage caused by harmful organisms or abiotic stresses in forest nurseries, the production and protection of plants should be based on stimulating their growth and development to ensure high-quality nursery material, whilst reducing threats to humans and the environment. The 'European Green Deal' strategy implemented in the European Union (EU) countries since 2021, distinguishes between two objectives covering plant protection. The first objective assumes a 50% reduction in the use of chemical plant protection products in all EU countries. The second objective includes activities aimed at reducing the risk related to the use of chemical plant protection products (European Commission, 2021). These considerations mainly concern insecticide- and herbicide-building active substances, but do not exclude fungicides. In addition to the 'European Green Deal', an important factor reducing the variety of active substances available in plant protection products on the market, is the suspicion of a negative impact on the hormonal balance of mammals and the harmful effects of some of them on the environment. Some active substances, including fungicides, have been withdrawn due to the lack of interest from the keepers (owners) of the substances in the renewal of their EU registration (Danielewicz *et al.*, 2022). In recent years, in accordance with the European Commission Implementing Regulations (EU) 2018/1500 (2018), 2019/677 (2019), 2020/1498 (2020) and 2020/2087 (2020), many active substances important for fungicide forestry have been withdrawn, including: thiram, chlorothalonil, thiophanate-methyl and mancozeb. The use of chemical plant protection products in forestry is additionally significantly limited by the 'Pesticide Policy' introduced to forests certified in accordance with the Forest Stewardship Council standard (Głowacka and Perlińska, 2015; FSC, 2018, 2019a, b).

In the era of the intense changes described above, the use of biostimulants seems to be the best way to meet the urgent need for alternative organic methods based on new biologically active substances that are environmentally friendly and safe for humans (Posmyk and Szafrańska, 2016). As defined in the European Commission Implementing Regulation (EU) 2019/1009 (2019), biostimulants are products that stimulate plant nutrition processes regardless of the nutrient content of the product, and whose sole purpose is to improve one or more of the following properties of the plant or the plant rhizosphere: (a) nutrient use efficiency, (b) resistance to abiotic stress, (c) quality features, and (d) digestibility of nutrients from forms difficult to access in the soil or the rhizosphere.

There are many preparations on the Polish market that meet the above requirements. One of them are preparations based on the sea algae *Ascophyllum nodosum* (L.) Le Jolis. The extract contains many valuable ingredients that have a positive effect on plant metabolism and the uptake of nutrients. Its main components are plant growth hormones such as cytokinins, gibberellins and auxins, and natural organic molecules, including betaines, mannitol, polysaccharides, and amino acids. These preparations are proven to work, *inter alia*, in agricultural and horticultural crops (Ali *et al.*, 2016). Moreover, they have also proven to be effective in reducing the occurrence of fungal diseases (Jayaraman *et al.,* 2011).

The aim of the research was to evaluate the effect of the Maral (AGRIGES SRL) and Shigeki (FUTURECO BIOSCIENCE, SA) biostimulators containing *A. nodosum* algae extract on 1) the development of two-year-old oak *Quercus robur* L. seedlings with undercut root systems and 2) reducing infection from powdery mildew of oaks *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam.

Materials and method

Maral and Shigeki are one of the many biostimulants available on the Polish market, used in agriculture and horticulture in the event of stress in plants. These preparations contain *A. nodosum* sea algae extract, in addition to many minerals such as boron, copper, iron, manganese and zinc. The content of algae extracts in both preparations was 10.5%. According to the manufacturers' materials, these preparations stimulate plant metabolism which contributes to better plant development and increasing resistance to stress. For the purposes of this experiment, the procedure of undercutting root systems commonly used in forestry, was adopted as the stress factor.

The impact of Maral and Shigeki preparations was assessed in forest nurseries in east-central Poland – Radziwiłłów (geographic coordinates 51°59'13.3" N and 20°20'20.0" E, altitude of 134 m a.s.l.) and Chojnów (geographic coordinates 52°02'49.4" N and 21°04'48.0" E, altitude of 120 m a.s.l.), on two-year-old oak seedlings. For this purpose, plots with an area of 1 m^2 were marked out on the section of the production line that was out of use, and then sprayed in accordance with the manufacturer's instructions contained in the label. For Shigeki, it was a dose of 4 l/ha dissolved in 1000 l/ha of water. For Maral, it was 2 l/ha dissolved in 1000 l/ha of water. Due to the very small volumes of working liquid, spraying was carried out with a hand sprayer. In total, 5 plots were designated for Maral, Shigeki and control plots (where no treatments were performed) in each of the nurseries. Additionally, a 50 cm long buffer zone was used between the plots. The distribution of the plots was random.

The root system undercutting was performed on designated production lines on April 28, 2021 in the Radziwiłłów nursery and on May 14, 2021 in the Chojnów nursery. This treatment was seen as a stress factor for the plants and the use of the preparation was aimed at general improvement of development and resistance after the occurrence of stress. Treatments with the use of biostimulators were carried out twice:

– Radziwiłłów: 14.05.2021 and 25.05.2021.
– Chojnów: 27.05.2021 and 08.06.2021.

Two months after the last treatment, 10 seedlings were randomly collected from each plot (50 seedlings in total, for each variant of the experiment), for which the following were measured: the length of the aboveground part, the length of the roots (measured from the root collar to the end of the longest root), root collar diameter, and the number of leaves infected by the fungus *E. alphitoides* (the cause of powdery mildew of oak). The leaves from the early forcing (present under the treatments at the nurseries) and late forcing (developed after the treatments), were counted separately. For the purposes of this study, the leaves from early forcing were defined as 'old', and from late forcing as 'new'.

All the obtained results were compared with each other using the non-parametric Kruskal-Wallis test at the significance level of α=0.05. This procedure was decided due to the unequal numbers in the groups and the lack of a normal distribution, which was confirmed by the Shapiro-Wilk test. Multiple rank mean comparison test was used to compare the mean pairs between the variants. All calculations were performed with the STATISTICA 13.1 package (TIBCO Software Inc., 2017).

Results

Comparison of biometric parameters. The first part of the experiment included the comparison of biometric parameters (the length of the roots, the length of the aboveground part and the diameter of the root collar) between the tested variants. The conducted analyses showed slight differences between the variants (Table 1). The comparison of the height of the oak seedlings did not show any statistically significant differences between the variants in both forest districts. Despite the undercutting of the root systems in both nurseries, statistically significant differences in the length of the root systems were noted. Interestingly, Maral had a better effect on the growth of roots in the forest nursery in Radziwiłłów, while Shigeki had a better effect in the nursery in the Chojnów. There were also no differences between the widths of the root neck.

Comparison of infested leaves. Symptoms of *E. alphitoides* infection on leaves were observed in all experimental variants in both forest nurseries. The results between the surfaces on which the formulations were tested were very different. In the Chojnów, the highest percentage of seedlings infected with *E. alphitoides* was observed in the variant with the Shigeki preparation, while in Radziwiłłów it was the control variants and the Maral preparation (Table 2). The number of infected leaves ranged from 1 to 78.

Statistical comparison between the variants showed significant differences only when *E. alphitoides* infected new leaves in the Chojnów (Fig. 1). The least number of infected leaves was observed on oaks previously sprayed with Shigeki. No similar tendencies were observed in the forest nursery in the Radziwiłłów.

Discussion

Currently, both in forestry and other branches of the economy related to the cultivation of plants, the aim is to limit the use of chemical plant protection products as much as possible. An alternative to their use may be preparations referred to as plant growth and development regulators or biostimulators (Maciejewska *et al.*, 2007; Gawrońska and Przybysz, 2011; Matyjaszczyk, 2015), plant growth and nutrition stimulators (Joubert and Lefranc, 2008), immune stimulators

Table 1.

Comparison of biometric features of the collected oak seedlings (summary of the median ±quartile and the results of the multiple comparison test of mean ranks, where significant differences are marked with an asterisk)

	Control	Maral	Shigeki	p – value
	Chojnów			
The height of the aboveground part	49 ±14	50 ±11	50 ±16	0.886
Root length	28 ±3	28 ±4	31 ±5*	0.034
Root collar diameter	6.28 ±1.15	6.58 ±1.05	6.38 ±0.85	0.698
	Radziwiłłów			
The height of the aboveground part	37 ±12	38.5 ±13.5	38.5 ±8.5	0.315
Root length	15 ±2	17.5 ±2.5*	15 ±2	0.023
Root collar diameter	11 ±2	11 ±2	10 ±2	0.149

Table 2.
Proportion [%] of seedlings with symptoms of *E. alphitoides* infection

Variant	Leaf category	Seedlings with symptoms of *E. alphitoides* [%]
	Chojnów	
Maral	old	90
	new	76
Shigeki	old	96
	new	78
Control	old	94
	new	7
	Radziwiłłów	
Maral	old	32
	new	66
Shigeki	old	22
	new	52
Control	old	30
	new	68

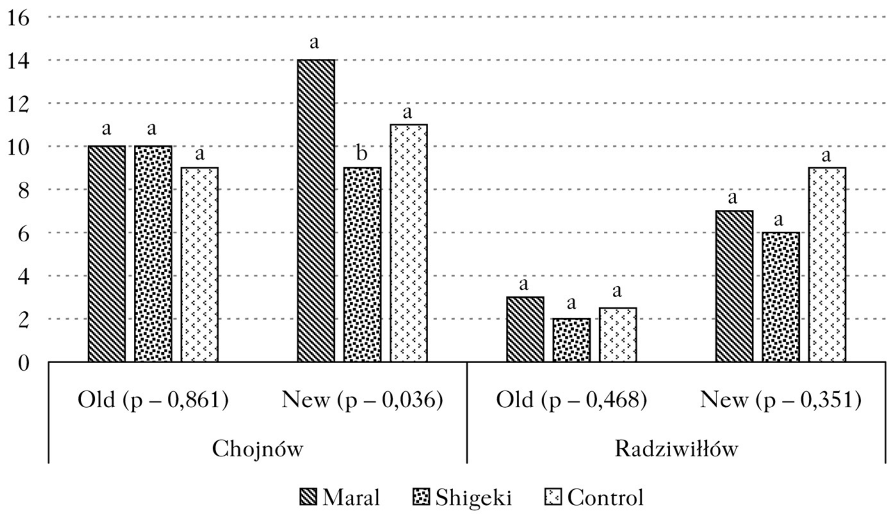

Fig. 1.
Comparison of the number of leaves infested by the fungus *E. alphitoides*

(Koziara *et al.*, 2006; Joubert and Lefranc, 2008), or preparations containing effective microorganisms (Boligłowa and Gleń, 2008; Kaczmarek *et al.*, 2008; Derkowska *et al.*, 2015). These preparations have long been used in agriculture and horticulture, but their use in forest management is a relatively new issue.

The research carried out in the growing season of 2021 showed a positive reaction of plants to the treatments. Significant statistical differences were observed in the development of the root systems. The reconstruction of the root systems is extremely important for the later survival of the plant, especially in unfavourable conditions. Moreover, even in a situation where the above-ground part is small but has a properly developed root system, such trees may develop better at a later stage. Even though these preparations do not act as plant protection products, they may increase resistance to the threat of biotic factors (Jayaraman *et al.*, 2011; Tkaczyk *et al.*, 2016). The conducted comparison of the degree of infection of seedlings by the fungus *E. alphitoides* showed that in the Chojnów forest nursery, seedlings sprayed with the preparation had even more infected leaves than the control seedlings. No statistically significant differences between the variants were observed in the Radziwiłłów nursery, but it was the seedlings sprayed with Maral that had fewer old leaves infected with *E. alphitoides*. Another issue worth noting, is the fact that, in both forest nurseries, the preparation did not affect the degree of infestation of young leaves that appeared during late forcing (after spraying).

Until now, biostimulants containing *A. nodosum* seaweed extract have been tested mainly in agriculture and horticulture. These preparations have been used, inter alia, in tomato *Lycopersicum esculentum* Mill. plants. Nerissa *et al.* (2016) tested the effect of marine algae extract in greenhouse and field conditions. In their experiment, the use of 0.2% concentration of the solution containing *A. nodosum* significantly improved plant height (by 10%) and plant fruit yield (51%) compared to the control conditions. The positive effect of using seaweed extract was also observed on increasing the yield of paprika (Eris *et al.*, 1995). In these experiments, the best effect was obtained using the concentration of 340 g/da. Moreover, the use of *A. nodosum* extract is not limited only to improving plant parameters and improving the quality of crops. Jayaraman *et al.* (2011) tested the effect of algae extract on reducing fungal diseases in greenhouse cucumber. In their experiments, they tested the commercial formulation of Stimplex™ against *Alternaria cucumerina* (Ellis & Everh.) J.A. Elliott, *Didymella applanata* (Niessl) Sacc., *Fusarium oxysporum* Schltdl. and *Botrytis cinerea* Pers. The conducted studies confirmed that the use of this preparation significantly reduced the incidence of all pathogens with which cucumbers were inoculated in greenhouse conditions. With the use of preparations containing seaweed extract, a significant reduction in infection by foliar fungal diseases was observed in carrot crops (Jayaraj *et al.*, 2008). In this experiment, the study confirmed a significant decrease in carrot infestation by *Alternaria radicina* Meier, Drechsler & E.D. Eddy and *B. cinerea*.

In forestry, the use of *A. nodosum* extract has been tested to enhance the spring root growth of dune pine *Pinus contorta* Dougl. var. *latifolia* Engelm. (MacDonald *et al.*, 2012). In these studies, the preparations were applied with the finisher fertilizer in September, the seedling cultivation was continued until harvest in December, and then the seedlings were stored in a freezer. In the spring of the following year, only partial growth of seedlings in some variants was recorded. The results are partially in line with the results carried out in our experiment. In the experiments described above, a statistically significant increase in the length of the root systems was also observed, however, different preparations worked in different nurseries (Shigeki in a forest nursery in Chojnów and Maral in a forest nursery in Radziwiłłów). However, it is important to note that the effect of the preparations on the oaks was observed two months after the treatments. In the case of *P. contorta*, a partially positive effect was seen after a much longer time (MacDonald *et al.*, 2012). Moreover, the research described in this paper shows that even if no statistically significant differences were observed between the treatments (in terms of growth and thickness), the biometric parameters were higher among trees sprayed with biostimulators than in control seedlings. Perhaps a positive effect in the form of statistically significant differences in both the height of the seedlings and the thickness of the root collar would be observed after a longer period of time, which may be indicated by the presented results.

The biggest novelty of the research carried out is the testing of preparations containing the extract of *A. nodosum* to reduce the occurrence of powdery mildew caused by the fungus *E. alphitoides*. The use of various preparations referred to as biostimulants against oak mildew have been reported with different effects. In a study published by Buraczyk *et al.* (2020), the use of preparations based on humic acids and chitosan polymers reduced powdery mildew infestation of oak but did not affect the growth and development of the seedling itself. In another study, Tkaczyk *et al.* (2014) confirmed that the use of phosphite-based preparations also reduced the infestation of oak seedlings by *E. alphitoides*, although it was a side effect of the conducted research. The Maral and Shigeki biostimulators tested in this study, containing *A. nodosum* seaweed extract, may possibly have a positive effect on the development of seedlings after the occurrence of stress, but their beneficial effect on increasing natural immunity and reducing infection from *E. alphitoides* has not been reported.

Authors' contributions

All authors substantially conceived the ideas, contributed to conceptualization, resources, writing the original draft, and reviewing and editing the text.

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Conflicts of interest

Authors declare no personal circumstances or interests that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

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STRESZCZENIE

Zastosowanie biostymulatorów zawierających ekstrakt z alg *Ascophyllum nodosum* (L.) Le Jolis w uprawie i ochronie sadzonek dębu szypułkowego *Quercus robur* L. w szkółkach leśnych

Lasy w Europie zajmują powierzchnię 158 milionów hektarów. Aby je zachować, konieczne jest zapewnienie wysokiej jakości materiału sadzeniowego do odnowienia i ponownego zalesienia. Przy stale malejącej liczbie dopuszczonych w Unii Europejskiej środków ochrony roślin coraz popularniejsze staje się poszukiwanie alternatywnych metod poprawy kondycji i rozwoju sadzonek w szkółkach leśnych. Jedną z takich alternatyw mogą być preparaty zwane regulatorami wzrostu i rozwoju roślin lub biostymulatorami. Celem pracy była ocena wpływu dwóch biostymulatorów zawierających ekstrakt z alg *Ascophyllum nodosum* (L.) na wzrost i rozwój siewek dębu szypułkowego *Quercus robur* L. Kolejnym celem było przetestowanie preparatów pod kątem poprawy odporności na infekcje mączniakiem dębu *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. Preparaty te stosowano w sytuacjach, gdy rośliny poddawane były silnemu stresowi – podcinaniu systemów korzeniowych, co jest zabiegiem często stosowanym w szkółkach leśnych. Badania prowadzono równolegle w dwóch szkółkach leśnych w środkowo-wschodniej Polsce. Obie szkółki leśne opryskiwano dwukrotnie w odstępie 14 dni. Po dwóch miesiącach zebrano po 50 sadzonek z każdego wariantu i oceniono parametry biometryczne: długość części nadziemnej, długość systemów korzeniowych oraz średnicę szyjki korzeniowej (tab. 1). Porównano również stopień porażenia liści mączniakiem dębu. Wyniki wykazały istotne statystycznie różnice w długości systemów korzeniowych – siewki na poletkach zaprawianych miały lepiej rozwinięty system korzeniowy niż na poletkach kontrolnych. Pozostałe parametry także wykazały różnice w porównaniu do wariantu kontrolnego, ale nie były one istotne statystycznie. U dębów potraktowanych algami nie stwierdzono zmniejszenia stopnia porażenia mączniakiem *E. alphitoides*, a jedynie zwiększony wzrost korzeni (tab. 2, ryc. 1).