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The Effect of a Growth Stimulant Based on Iodine Nanoparticles on *Festuca glauca*

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ABSTRACT

The purpose of the studies was to determine the effects of Stymjod, a growth regulator, on blue fescue (*Festuca glauca*) development and morphological characteristics. The experiment was an attempt to determine the effect of different concentrations of Stymjod on the leaf greenness index, the number and length of leaf blades, the length of roots, and the dry weight of roots and leaves. The plant used in the experiment, *Festuca glauca*, was grown in pots. The following three treatment combinations were used: spraying plants with distilled water only; with Stymjod at a concentration of 2% in the spraying fluid; with Stymjod at a concentration of 4%. Spraying was carried out four times at weekly intervals. It was found that the 2% concentration of Stymjod in the solution significantly improved most morphological. *Festuca glauca* treated with Stymjod was characterized by better efficiency of the photosynthetic apparatus and photosynthetic activity.

Keywords: *Festuca glauca*, plant growth and development regulator, Stymjod, ornamental grass

INTRODUCTION

Plant growth regulators are organic substances which, applied at small doses, are capable of affecting physiological processes determining plant development [Adamczewski and Matysiak, 2009]. Increasing the growth and yield of plants, such substances are the subject of many research papers. Apart from increasing production results by modifying plant growth and development, they can also contribute to the improvement of certain desirable characteristics in crops, vegetables, and ornamental and nursery plants. In industry such characteristics are important in the production of human food and animal feed. In agriculture the efforts to increase the yield of plants create the need to continuously improve growing methods. To this end, scientific research is also needed to study the effects of plant growth and development regulators, playing an increasing role in modern agriculture. Many such products are available on the market, allowing stimulation of various plant characteristics and increasing the weight of roots and leaves. Those products can be used in different concentrations, having different effects on different crop species or ornamental plants. Additionally, they are safe and free of unnecessary chemicals, which is an important feature from an environmental point of view [Gaurilčikienė et al., 2008; Adamczewski and Matysiak, 2009; Piotrowski et al., 2016; Romanowska-Duda et al., 2018, 2019, 2020; Sosnowski et al., 2019, 2020].

In research on growth regulators, the main issue is their effect on biotic and abiotic stress that plants must cope with throughout their life cycle. Stress can trigger a number of processes that can have negative consequences [Burghardt and Riederer, 2006]. Due to their antistress potential, growth and development regulators increase the yield of plants and improve its quality [Adamczewski and Matysiak, 2009].

Iodine is an element naturally occurring in plants. Its adequate content activates the plant's defensive reactions during the occurrence of stress or fungal disease and reduces the drain of nutrients from plants to parasites [Wierzbińska et al., 2011]. The best forms of iodine for the root system to absorb are water-soluble compounds, increasing the uptake of this chemical element up to 2,500 times. The most effective way to apply iodine is to the leaves, where it is absorbed together with water through permeable cuticle [Smoleń, 2009]. Containing iodine nanoparticles, Stymjod is a liquid organic-mineral product in the form of concentrate. It allows reducing the amount of mineral fertilizers, but its application results in other positive effects. It increases the weight of stems, roots, fruits, and leaves, improves resistance of plants to abiotic stress and accelerates regeneration after its occurrence. In result, the yields are of a better quality and structure. Cold plasma technology is applied to produce Stymjod, composed of macronutrients and micronutrients, including amino acids and other organic compounds supporting development of plants [Romanowska-Duda et al., 2018].

Occupying huge areas all over the world, grasses are one of the largest groups of plants. The major grass species are rice, millet, and wheat, and the development of human civilization, including agriculture, to a large extent has been based on them [Xiaohuan et al., 2011]. The variety of colors and forms allows a group of ornamental plants to be distinguished among grass species [Zenkteler and Nitzsche, 1984; Li et al., 2010; Ostapets, 2010]. The advantage of growing ornamental plants is not only their decorativeness, but also their small habitat and care requirements and resistance to diseases and pests [Dernoeden, 2000].

Festuca glauca is an evergreen short-lived ornamental grass. It forms compact clumps, resembling cushions, composed of numerous, very narrow and long silvery blue leaves [Xiaohuan et al., 2011]. Because of their narrowness and length, the leaves are arched. The plant grows up to 15–20 cm, and during flowering forms thick elongated panicles with a height of up to 50 cm. Flowering in the form of such panicles occurs from May to June. When the inflorescences are not cut at the right time, the plant produces seeds and spreads. *Festuca glauca* can also reproduce by cloning. It is a plant with little requirements, preferring light and dry soils. Its growth and development depends on access to light, which is why it requires full sun [Barciak et al., 2007]. Because it has low habitat requirements, it can be used in places unsuitable for many other plant species. Its use is quite extensive, and it is mainly planted in rock gardens, in perennial garden beds exposed to the sun, and just to cover the ground [Ostapets, 2000].

The aim of the experiment was to determine the effect of a plant growth and development regulator with a commercial name of Stymjod on the formation of morphometrics, photosynthetic activity and chlorophyll content of *Festuca glauca*. The studies were meant to determine the effect of different regulator concentrations on the leaf greenness index, the number and length of leaf blades, the length of roots, and the dry weight of roots and leaves, the maximum (F_V/F_m) and actual ($\Delta F/F_{m'}$) photosystem II efficiency, photochemical (qP) and non-photochemical (qN) quenching coefficients, as well as chlorophyll a and chlorophyll b content in leaf blades.

MATERIALS AND METHODS

Conditions of the experiment

The research was conducted in the form of a pot experiment in the breeding room of the University of Natural Sciences and Humanities in Siedlce, Poland, in 2019. Thanks to the use of high-pressure sodium lamps (with 14/10 h day/night), the following conditions were generated in the room: light intensity 200 μmol $m^{-2}s^{-1}$; temperature 24±2/16±2 °C. The species used in the experiment was *Festuca glauca*, grown in 300 mm high pots with 200 mm diameter of the bottom. The experiment was set up in a completely randomized design with a control unit and three replications. Before the experiment, organic carbon content in the soil was 13.5 $g \cdot kg^{-1}$ DM, with total nitrogen content of 1.30 $g \cdot kg^{-1}$ DM. Soil pH of 6.8 was close to neutral, and soil moisture was at 60% of the field capacity. In addition, the soil contained high amounts of available forms of phosphorus and magnesium, but the content of available forms of potassium was moderate. Due to the richness of soil nutrients, mineral fertilizers were not applied.

Plant material

The plants were treated with a growth and development regulator, a product with a commercial name of Stymjod (Table 1). Its different doses constituted different levels of an experimental factor.

Table 1. Composition of Stymjod, an organic-mineral nano-fertilizer

N	P_2O_5	K_2O	MgO	SO_4	B	Cu	Fe	Mn	Mo	Zn
6.3%	4.58%	6.42%	1.69%	1.6%	0.086%	0.17%	0.14%	0.16%	0.028%	0.42%
Iodine (I) – 0.0025%					Humic acids – with about 3.3% C/kg					
Total organic matter – 56.8% (including amino acids and other organic compounds)										

The experiment was conducted with three levels of the experimental factor: F1 – control, sprayed with distilled water; F2 – sprayed with 2% of Stymjod in the spraying liquid; F3 – sprayed with 4% of Stymjod in the spraying liquid. Plants in pots were sprayed until they were completely wet. Treatment was carried out four times at weekly intervals, and the whole experiment lasted 6 weeks.

When plant material was collected, the following characteristics were determined: the SPAD leaf greenness index, the number of leaf blades per pot, the length of leaf blades (cm), the length of roots (cm), dry root weight ($g{\cdot}pot^{-1}$), and dry leaf weight ($g{\cdot}pot^{-1}$). The SPAD leaf greenness index was specified for 10 leaf blades randomly selected from one pot. Additionally, other parameters were determined: the maximum photosystem II efficiency (F_v/F_m), the actual photosystem II efficiency ($\Delta F/F_{m'}$), the photochemical quenching coefficient (qP) and the non-photochemical quenching coefficient (qN). The content of chlorophyll a and b in *Festuca glauca* leaves was also measured. All measurements were carried out when the plants were harvested, on the 15th day after the second Stymjod treatment. The SPAD measurement was conducted with the SPAD-502 portable meter, Minolta, Japan.

Photosynthetic activity of plants was determined by the measurement of chlorophyll fluorescence induction [Sosnowski et al. 2020]. Chlorophyll a and b content was determined with the Arnon et al. [1956] method modified by Lichtenthaler and Wellburn [1983].

Statistical analysis

The results were statistically processed using variance analysis. To determine the significance of the effect of the experimental factor on the values of the characteristics, the F-Fisher-Snedecor test was used, and the $LSD_{0.05}$ value was verified by Tukey's test. The results are presented in the form of graphs using the letter markings of homogeneous groups. Means marked with the same lowercase letters do not differ significantly. The statistical program Statistica 13–2019 was used for the calculations.

RESULTS

During the final analysis of the plant material, the SPAD leaf greenness index was determined using a chlorophyll meter (Table 2). Its highest value was recorded for plants treated with the 2% Stymjod concentration. This value was 31.8, which was 19.55% higher than a control value of 26.6. In contrast, leaves of plants treated with the concentration of 4% reached a value of 28.4. The effect of the 2% Stymjod concentration was significantly higher than 4% treatment and higher than the index for control plants. No significant differences were observed between the control value and that recorded in pots with 4% of the growth regulator.

The effect of growth regulator concentration on the number of leaf blades produced by plants was also determined (Table 2). Plants treated with the 2% concentration of Stymjod developed the highest number of leaf blades per pot, amounting to 262. This value was 20.74% higher than in control with 45 fewer leaves recorded, and the difference was statistically significant. It was also significantly higher, by 34 leaves or 14.91%, than the effect of the 4% Stymjod concentration. The difference between the leaf number in control pots and in those treated with 4% treatment was not statistically significant.

Table 2 presents data on the effect of Stymjod concentrations on the length of the leaf blade. The highest average was recorded for plants treated with 2% Stymjod solution. This value was significantly higher than leaf blade length of control plants (by 4.4 cm, i.e. 17.32%) and of those treated with 4% Stymjod solution (by about 0.9 cm, i.e.3.11%). All the same, leaf blades of plants treated with 4% Stymjod solution were significantly longer (by 3.5 cm, i.e. 13.77%) than those in contro. The length of roots (Table 2) reached its highest value for plants treated with the 4% Stymjod concentration. With 12.6 cm it was significantly higher, by 28.31%, than in control and by 26.76% than roots of plants treated with the 2% concentration. The length of roots for control plants and for plants sprayed with the 2% concentration did not differ significantl. According to the data in Figure 5, the highest dry weight of roots, 4.97 g·pot^{-1}, was recorded for plants treated with the 2% concentration of Stymjod in the solution. This is significantly higher than the values recorded in the control sample and in the 4% sample, by 31.48% and 34.69%, respectively. The dry weight of the roots of plants treated with the 4% concentration was at a similar level as the dry weight of the roots of control plants, with no significant differences. Table 2 presents the effect of different Stymjod concentrations on the dry weight of leaves. The highest dry matter value, 10.1 g·pot^{-1}, was recorded for the 2% sample. However, it did not differ significantly from control units or from plants treated with 4% solution. All the data in Figure 6 were at a similar level, with no statistically significant differences.

The content of chlorophyll a and b in *Festuca glauca* leaves (Table 3) sprayed with Stymjod, a growth stimulant, increased in a statistically significant way. The content of chlorophyll a in the leaves of plants grown on control units was 162 mg 100 g^{-1} freshweight. In those treated with the higher amounts of Stymjod it was 214 mg 100 g^{-1} freshweight for 4% concentration and 188 mg 100 g^{-1} freshweight for 2% concentration. The largest increase of chlorophyll a content compared to control was 24.3%. In the case of chlorophyll b concentration a similar increase of 19.1% was recorded.

Chlorophyll fluorescent measurements determined on *Festuca glauca* leaves showed that the maximum photochemical efficiency of PSII in the dark-adapted state was statistically significantly differentiated by the concentration of the product in the spraying liquid (Table 4). Depending on Stymjod concentration, the treatment increased the Fv/Fm value on average by 26.6%, from 0.549 for control to over 0.6 for plants treated with Stymjod (2% – 0.617, 4% – 0.646). On the other hand, the value of the actual photochemical efficiency, determined under the same conditions, turned out to be on average 28.9% higher (Table 4) for the leaves of plants sprayed with the product – depending on the concentration. The use of Stymjod also contributed to an increase in the value of the non-photochemical quenching coefficient (qN). As indicated by the data from Table 4, this indicator increased by 23.2% compared to control. The present studies did not show a statistically significant effect of the product on the decrease or increase in the photochemical quenching coefficient (qP) of *Festuca glauca* leaves.

DISCUSSION

The positive effect of iodine on ornamental plants as well as on crops has been known for quite a long time [Gaurilčikienė et al., 2008;

Table 2. The effect of Stymjod on *Festuca glauca* morphological characterisitcs

Characteristics	Stymjod concentration		
	Control	2 %	4%
SPAD leaf greenness index	26.6 (±2.24) b	31.8 (±2.86) a	28.4 (±3.01) b
Number of leaf blades	217 (±16.1) b	262 (±10.2) a	228 (±12.2) b
Length (cm) of plant leaf blades	24.5 (±2.02) c	29.8 (±1.12) a	28.8 (±1.42) b
Length (cm) of plant roots	9.82 (±0.82) b	9.94 (±1.10) b	12.6 (±0.74) a
Dry weight (g·pot^{-1}) of plant roots	3.78 (±0.71) b	4.97 (±0.32) a	3.69 (±0.49) b
Dry weight (g·pot^{-1}) of plant leaves	9.18 (±1.02) a	10.1(±1.11) a	8.99 (±0.98) a

* Standard deviation (±SD), means marked with the same letters do not differ significantly

Table 3. The effect of Stymjod on chlorophyll pigment content (mg 100 g^{-1}of fresh weight) leaf greenness index in *Festuca glauca* leaves

Characteristics	Stymjod concentration		
	Control	2%	4%
Chlorofil a	162 (±11.2) c	188 (±18.1) b	214 (±20.1) a
Chlorofil b	89 (±10.4) b	109 (±12.3) a	110 (±11.7) a

* Standard deviation (±SD), means marked with the same letters do not differ significantly.

Table 4. The effect of Stymjod on photosynthetic activity of *Festuca glauca* leaves

Characteristics	Stymjod concentration		
	Control	2 %	4%
(F_v/F_m)	0.548 (±0.05) [b]	0.617 (±0.05) [a]	0.646 (±0.02) [a]
($\Delta F/F_{m'}$)	0.387 (±0.01) [a]	0.501 (±0.07) [a]	0.4988 (±0.03) [a]
(qP)	0.526 (±0.04) [a]	0.532 (±0.03) [a]	0.538 (±0.02) [a]
(qN)	0.112 (±0.03) [c]	0.124 (±0.03) [b]	0.145 (±0.04) [a]

* Standard deviation (±SD), means marked with the same letters do not differ significantly

Piotrowski et al., 2016; Romanowska-Duda et al., 2018, 2019, 2020]. Despite significant needs, however, there have not been many studies on the effect of Stymjod. That is why, the present experiment dealt with the effect of this growth regulator on *Festuca glauca*, while other papers have discussed its impact on other plants as well. By examining the foliar application of Stymjod at different concentrations on *Medicago* x *varia* T. Martyn, Sosnowski et al. [2019] found that only its 1.5% concentration in the solution resulted in an increase in fresh and dry root weight, but a higher concentration did not significantly affect plant characteristics. All Stymjod doses in their research resulted in an increase in the dry weight of stems, but their fresh weight increased only in response to a 3% concentration of the growth regulator. Overall, fresh and dry weight of the whole plant increased for all concentrations. As it turned out, 4.5% concentration of Stymjod significantly affected the growth of hybrid alfalfa leaf blades and stems. Contrary to that, the present studies on *Festuca glauca* indicated that only 2% of Stymjod in solution increased the dry weight of roots, and dry leaf weight did not significantly vary across treatment combinations. In turn, studies on the response of *Dactylis glomerata* to Stymjod [Sosnowski et al., 2020] indicated that this species reacted significantly to spraying with 4.5% Stymjod solution. In the same experiment, an increase in the number of shoots and leaf blades was recorded. The consequence of an increase in these parameters was a higher fresh and dry weight of the plant. However, no increase in fresh and dry root weight was observed. The studies also showed a positive effect of Stymjod on photosynthetic performance for each concentration. Studying the effects of Stymjod on Jerusalem artichoke, Grzesik et al. [2018] reported that the best results in accelerating growth and increasing the yield were achieved by applying a double dose of 15–30 ml of the growth regulator per liter of water, using a three-week interval between sprays. On the other hand, by analyzing the quality and yield of lettuce in response to various methods of applying iodine, Ledwożyw-Smoleń et al. [2011] found that this chemical element could have an adverse effect on the plant. Consequently, a decrease in lettuce growth and the yield was observed. As it turned out, the highest iodine concentration in the substrate resulted in the lowest yield. In addition, lettuce heads from the same sample were of the lowest average weight. The highest yield was observed in a control sample, without iodine. However, by analyzing the research material, the authors also noticed a significant increase in the amount of dry matter with an increase in the amount of iodine added to the substrate. In the present studies, the use of an iodine-containing product did not result in a significant decrease relative to control in the value of the assessed characteristics. In addition, Pekarskas [2005], who conducted research on spring barley with the foliar use of iodine nanoparticles, found that average yields of the cereal treated with Biojodis increased by 3.2% compared to control. An increase in the weight of the above-ground part of plants as a response to rising Stymjod concentrations was noted by Romanowska-Duda et al. [2018]. The authors found that sorghum responded positively to the growing concentrations of Stymjod applied as a spray, and its concentrations of 1.5 and 3% in solution significantly increased plant growth.

According to the literature [Yokoya et al. 2007; Zhao et al. 2016], photosynthetic pigments are responsible for absorbing light and transferring it to photosynthetic reaction centers, and their concentration affects photosynthetic efficiency. In the present experiment, the increase in the content of chlorophyll pigments resulting from the application of Stymjod may be, as otherwise confirmed by Zhao et al. [2016], one of the factors increasing photosynthetic activity. Thus, this product used on the leaves increased photosynthetic efficiency of the plants, resulting from an increase in the content of pigments. Netto and al. [2005] point to a close relationship between the concentration of chlorophyll in the leaves and the level of nitrogen nutrition because most of the nitrogen in the structure of the leaf blade is contained in the chlorophyll molecule. Thus, depending on many factors, the concentration of chlorophyll in the leaf also depends on nitrogen availability for plants.

CONCLUSIONS

The most effective was the 2% concentration of Stymjod in solution. It significantly improved most of the morphological and physiological characteristics of *Festuca glauca*, i.e. the leaf greenness index, the number and length of leaf blades and the dry weight of roots. It turned out that the 4% concentration of Stymjod most favorably influenced the growth of *Festuca glauca* roots. The length of roots treated with this concentration was 28.31% higher than for control plants. Neither of the concentrations significantly increased the dry weight of leaves produced by this grass species. *Festuca glauca* treated with Stymjod, regardless of the concentration, was characterized by better efficiency of the photosynthetic apparatus: (Fv/Fm), ($\Delta F/F_{m'}$) and (qN). All of the Stymjod concentrations increased the photosynthetic activity of plants, resulting from an increase in the content of photosynthetic pigments.

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