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[1] Faculty of Medical and Health Sciences, University of Siedlce, B. Prusa 14, 08-110 Siedlce, Poland
[2] Faculty of Agricultural Sciences, University of Siedlce, B. Prusa 14, 08-110 Siedlce, Poland
* e-mail: godlewskaa@uws.edu.pl

AGNIESZKA GODLEWSKA[1]*, GRAŻYNA ANNA CIEPIELA[1], MARCIN BECHER[2], DAWID JAREMKO[2], ADAM MATYSZCZAK[2]

The influence of biostimulants used in the cultivation of Italian ryegrass (*Lolium multiflorum* Lam.) on nitrogen uptake and the efficiency of nitrogen fertilization

Wpływ biostymulatorów stosowanych w uprawie Italian ryegrass (*Lolium multiflorum* Lam.) na pobranie azotu i efektywność nawożenia azotem

Abstract. The aim of the study was to determine the effect of different biostimulants applied in the cultivation of Italian ryegrass cv. Dukat on nitrogen uptake and effectiveness of nitrogen fertilisation. A two-year field experiment was arranged as a randomised subblock design (split-plot) with three replicates. The following factors were examined: a) type of biostimulant: Algex, Tytanit, Asahi SL and a control (no biostimulant addition); b) nitrogen application rate: 0 (control), 120 and 180 kg ha^{-1}. The total nitrogen content in the plant material was determined and nitrogen uptake with yield, agricultural and physiological efficiency were calculated. The application of biostimulants increased the nitrogen content in the Italian ryegrass biomass compared to the control. The differences between the tested biostimulants were insignificant. The amount of nitrogen in the biomass was significantly influenced by the rate of nitrogen. The effect of the year of the study was insignificant. The biostimulants used had a significant impact on the total nitrogen uptake during the vegetation year by Italian ryegrass. The values of this parameter depended on the type of biostimulant. Similarly, the value of this parameter was significantly affected by the year of the study, i.e. the total nitrogen uptake was significantly lower in the second year of the study. In the case of the agricultural efficiency fertilisation of Italian ryegrass, no significant differences were found for the influence of the tested experimental factors. The obtained values of the physiological efficiency fertilisation of Italian ryegrass fertilization did not differ significantly in the years of the study.

Keywords: nitrogen uptake, content of nitrogen, Algex, Tytanit, Asahi SL, biostimulants

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INTRODUCTION

In recent years, there has been observed a considerable increase in the number of biostimulant-based products. Biostimulants are classified as fertilising products [Du Jardin 2015, Kocira et al. 2018] because their registration is governed by legal regulations pertaining to fertilisers and plant protection products.

Biostimulants have different origins. They are made from natural substances such as seaweeds (trade names: Algex, Kelpak SL), humic and fulvic acids, synthetic compounds, e.g. phenolic compounds (the trade names: Asahi SL, Atonik) which in nature occur in plants, and titanite (the trade name: Tytanit) [Godlewska and Ciepiela 2018, Kocira et al. 2020, Canellas et al. 2023]. One of natural biostimulants deserving attention is an extract of the sea algae *Ascophyllum nodosum* (L.) Le Jolis which is applied to crops worldwide. In general, there are positive reports on the effect of this product [Murawska et al. 2017, Stamatiadis et al. 2021]. As far as synthetic products are concerned, Asahi SL and Tytanit are products which deserve attention as both of them have been quite frequently applied to agricultural and horticultural crops for many years (their composition is given in table 2). The effect of Asahi SL is diverse and dependent upon the crop plant [Przybysz et al. 2014, Szparaga et al. 2018]. The role of Tytanit is to improve crop performance through a stimulatory effect of enzymes; however, its mode of action remains unknown [Lyu et al. 2017, Malinowska et al. 2020]. An introduction of biostimulants into agricultural production makes it possible to reduce cultivation-related costs [Thomas and Singh 2019] by limiting an application of mineral fertilisers [Kamilova et al. 2014] and, simultaneously, enhancing yield quality. Increasing interest in such products [Hassan et al. 2021] is also associated with their positive impact on plant growth and resistance to biotic and abiotic stress [Renuka et al. 2018] and, as a result, higher yields of improved quality and nutritional value, which is expected by increasingly fastidious consumers. It should be stressed, however, that biostimulants are not a direct (major) source of nutrients, but substances which support the plant protection agents applied and mineral fertilisation [Bashir et al. 2021]. By Regulation (EU) 2019/1009 specifies the function of biostimulant is to stimulate plant nutrition processes. Their influence is due to stimulation of the process of plant nutrition [Caradonia et al. 2019] by e.g. enhancing the development of the rooting system, the ability to hold water, and the process of photosynthesis. The positive effect of biostimulants cannot be extended to all crop plants [Battacharyya et al. 2015, Basile et al. 2020)], thus, it seems necessary to determine application methods, timing and rates for each species, taking into account various habitats.

A variety of research works can be found in the source literature reporting results of an application of biostimulants to crop plants [Soppelsa et al. 2019, Pačuta et al. 2021]. However, there has been a paucity of reports on the use of biostimulants on grasslands.

Nitrogen fertilisation is one of key factors affecting the amount and quality of yields obtained from fodder grasses. Nitrogen plays an important part in enhancing plant production, its shortage being the most frequent factor hampering plant growth [Zotarelli et al. 2008]. The effectiveness of nitrogen fertilisation is reflected in quantitative and qualitative yield characteristics, as well as physiological effectiveness (plant ability to transform the nitrogen taken up from fertiliser into usable yield) and agricultural effectiveness (yield increase per one unit of N provided with fertiliser) [Małecka and Blecharczyk 2005, Ciepiela et al. 2012]. Unfortunately, plants do not fully utilise the nutrients, including nitrogen, provided with fertiliser which are then readily leached into deeper soil strata and contribute to environment degradation [Guo et al. 2010]. The society's increasing awareness of the negative effects of an excessive application of artificial fertilisers has made growers search for novel solutions which would make it possible to reduce amounts of mineral fertilisers applied. Organic manures, which have been applied for years, cannot sufficiently meet the nutrient-related needs of crop plants [Wuang et al. 2016].

Scientific reports by other authors [Kováčik et al. 2018, Świerczyński et al. 2021] have confirmed that an application of biostimulants combined with the rate of mineral fertilisers reduced by half results in much better plant growth parameters compared with the full rate of mineral fertilisation. Przybysz et al. [2010] report that increased plant biomass obtained following an application of biostimulant results from improved efficiency of the photosynthetic apparatus, which translates into a higher leaf area. Battacharyya et al. [2015] claim that seaweed extracts enhance nutrient uptake, photosynthesis and crop yield. Earlier research demonstrated a significant effect of an application of various biostimulants, that is Algex, Tytanit and Asahi SL, on the dry matter yield of Italian ryegrass [Ciepiela and Godlewska 2019]. In the research, the dry matter in units fertilised with 120 kg N per 1 ha and treated with a biostimulant was similar to the yield obtained in plots fertilised with 180 kg N per 1 ha without biostimulant. In this context, superior effects were recorded for Algex. The study [Ciepiela and Godlewska 2019] suggests that biostimulants combined with nitrogen regime will allow the grower to reduce nitrogen fertiliser by around 30%.

These facts led the authors of the present work to determine the effect of different biostimulants applied in Italian ryegrass cultivation on nitrogen uptake and effectiveness of nitrogen fertilisation. In this study, we address the research issue of agricultural and physiological effectiveness of Italian ryegrass fertilisation with nitrogen combined with an application of three different biostimulants. Hence, in the work, we attempt to check the following hypotheses: an application of natural and synthetic biostimulants enhances the agricultural and physiological effectiveness of nitrogen fertilisation; an effectiveness of nitrogen utilisation by Italian ryegrass depends on nitrogen fertiliser rate and biostimulant type.

MATERIALS AND METHODS

Study location and field experiment design

A field experiment was conducted on the ploughed fields of Siedlce University of Natural Sciences and Humanities Experimental Unit (Poland, DMS: 52°10'1.733"N 22°17'14.747"E), on soil formed from outwash sand. According to the Polish Soil Classification [Kabała et al. 2019], the soil is classified as follows: order – anthroogenic soils, type – culturozems, subtype – hortisols or anthrosols (the English terms follow Świtoniak et al. [2016]) whereas in the IUSS Working Group WRB [2015], the soil is a hortic anthrosol. Before the experiment was set up, soil samples were taken from the humus layer to determine basic physical and chemical properties using methods which are customarily utilised in agrochemical laboratories (tab. 1) [Kabała et al. 2019].

The field experiment was set up in mid-August 2013. Plots (2.5 × 4.0 m) were arranged following the split plot design in three replicates, which gives a total of 36 plots. In order to study the effect of biostimulants and the nitrogen fertilisation during a two-year field experiment (2014 and 2015), the following were used: 3 types of biostimulants (brand names): Algex, Tytanit and Asahi SL (tab. 2); 2 rates nitrogen fertiliser rates of ammonium nitrate (in kg N ha^{-1}): 120 and 180. The control treatment included neither biostimulants nor nitrogen fertilisation.

Table 1. Selected properties of the humus layer of the experimental site soil

Soil property	Unit	Value
Sand fraction (2.0–0.05 mm)	%	72
Silt fraction(0.05–0.002 mm)	%	23
Clay fraction (below 0.002 mm)	%	3
Granulometric group	–	loamy sand
pH (in 1 M KCl)	–	6.80
SOM (soil organic matter)	g kg$^{–1}$	37.8
Nog	g kg$^{–1}$	1.30
Available P	mg P kg$^{–1}$	74.8
Available K	mg K kg$^{–1}$	114
Available Mg	mg Mg kg$^{–1}$	84.0

N_{og} – total nitrogen

Table 2. Description of biostimulants used in the experiment

Biostimulant type	Selected information
Algex	An extract of the sea algae *Ascophyllum nodosum* which contains phytohormones (auxins, cytokinins, gibberellins), vitamins, amino acids, polysaccharides and betaine, and is also enriched with macroelements (in % m/v): N − 8%, P − 3.6% (P_2O_5), K − 7% (K_2O), and microelements: B − 0.036%, Zn − 0.025%, Cu − 0.009%, Fe − 0.016%, Mn − 0.036% and Mo − 0.0036% [https://agrosimex.pl/nawoz-algex-rosier]
Tytanit	A mineral biostimulant which contains 8.5 g Ti dm^{-3} (0.8%) in the form of Ti-ascorbate [https://intermag.pl/produkt/tytanit/]
Asahi SL	It contains the following phenolic derivatives (m/v): sodium p-nitrophenolate − 0.3%, sodium o-nitrophenolate − 0.2% and sodium 5-nitroguaiacolate − 0.1% [https://pl.uplonline.com/download_links/L3cKweW8VdgMkfcmBuEMxrJP6YoHOeLUnbX6ovjJ.pdf]

The test plant was Italian ryegrass (*Lolium multiflorum* Lam. cv. Dukat). The sowing rate in individual experimental plots corresponded to 31 kg ha$^{–1}$ (thousand-grain weight – 2.8 g). In October 2013, when the seeds were sown, only one cut was performed at the plant height of 6 cm. Over the study period (the years 2014 and 2015), the cutting regime consisted of three harvests of green mass per year, each time after about 30 days of growing.

In 2013, when seeds were sown, neither biostimulants nor nitrogen fertiliser was applied. The experimental factors were applied throughout the growing season of 2014 and 2015. Three applications of biostimulants were performed by spraying an aqueous solution at a rate recommended by the producer, the timing being as follows: three weeks before the first cutting, two weeks after the first harvest and three weeks after the second harvest. The total nitrogen amount was split into three equal rates which were applied to each regrowth (in spring after plants resumed their growth, and five days after the first and second cutting was harvested). Phosphorus and potassium fertilisers were applied to all the plots following the regime:

– P was applied once in spring (triple superphosphate at the rate of 40 kg ha^{-1} P_2O_5),
– K (160 kg ha^{-1} K_2O) was split into three equal rates and applied to each regrowth (in spring after plants resumed their growth, five days after the first and second cutting was harvested).

Climate and weather conditions

According to the climate classification by Köppen-Geiger [Kottek et al. 2006], the experimental site was located in the fully humid warm temperate climate zone with warm summers. The weather conditions during the study period are given in table 3. Parameters of weather conditions were recorded at the Meteorological Station in Siedlce which is situated 3 km from the experimental site. Meteorological conditions during the study period differed from mean values for the years 2006–2015. The precipitation pattern was irregular. Non-standard water shortages occurred in September 2014, and August 2015. The average monthly air temperature in the 2015 growing season was by 2 degrees lower than the monthly mean across 2006–2015.

Table 3. Values of selected weather-related parameters at the experimental site

Years	Months						
	Apr.	May	June	July	Aug.	Sept.	Apr.–Sept.
Mean monthly air temperature (°C)							
2014	9.70	13.7	15.1	20.4	17.8	13.7	15.1
2015	8.10	12.3	16,5	14.3	21.1	8.80	13.5
2006–2015 (mean)	9.60	14.0	17.2	19.9	18.4	13.6	15.5
Precipitation sum (mm)							
2014	39.5	79.3	50.3	62.5	66.3	26.7	324.6
2015	29.7	100.6	41.1	68.3	12.0	77.5	329.2
2006–2015 (mean)	26.9	68.9	64.6	55.8	65.3	44.3	325.8

Laboratory analyses of plant material

Representative plant material samples (around 500 g) were collected from each plot during harvest. The plant material was cut and dry matter was determined (by the weighing method, following drying at 105°C). The remaining part of the sample was left for drying at room temperature in a ventilated room and then it was shredded and ground in a mill to obtain particles with the diameter of 0.25 mm. The obtained samples were used to determine N-total by the Kjeldahl method [Kalembasa 1995].

Laboratory analyses were performed in 3 replicates. Their results were related to absolute dry matter of the plant material (in air-dry samples dry matter content was determined after drying at 105°C).

Calculations of nitrogen fertilisation effectiveness

An agricultural effectiveness of nitrogen fertilisation is a ratio of the difference between the yield in plots where N was applied and control yield (no N) to the N rate applied. The value of agricultural effectiveness of nitrogen fertilisation was expressed as kg d.m. kg^{-1} N.

The physiological effectiveness of nitrogen fertilisation is a ratio of the difference between the yield in plots where N was applied and control yield (no N) to the difference between the N uptake with plant yield in a given unit and N uptake with plant yield in control (no N).

The value of physiological effectiveness of nitrogen fertilisation was expressed as kg d.m. kg^{-1} N uptake by plants [Małecka and Blecharczyk 2005].

All the indicators were calculated based on the total yield of three cuts, means across three cuts, total nitrogen content and total nitrogen rate (applied per year).

The yield level values used in the above calculations have been taken from previous work [Ciepiela and Godlewska 2019].

Statistical analysis

Statistical analysis was performed using the Statistica 13 PL statistical program (TIBCO Software Inc., Palo Alto, California, USA). The average values of the examined factors were compared with the 3-way analysis of variance and Tukey's post-hoc test, where the experimental factor was the year of research, the level of nitrogen fertilization and the type of biostimulant. Means were grouped into homogeneous groups at a significance level <0.05.

RESULTS AND DISCUSSION

Tables 4 and 5 present only the data which was used to calculate the effectiveness of Italian ryegrass fertilisation with nitrogen. Total nitrogen content in Italian ryegrass varied, and depended on nitrogen rate and type of applied biostimulant (tab. 4). The levels of this element in the biomass of grasses ranged from 14 g kg^{-1} d.m. for the control in 2014 to 27.9 g kg^{-1} d.m. in Asahi-treated plots fertilised with the highest nitrogen rate in 2015. Increasing nitrogen fertiliser rates contributed to a significant increase in the biomass content of this nutrient in the test grass, regardless of the study years or biostimulants. The highest nitrogen increase in the biomass of Italian ryegrass (31.9%) was associated with the nitrogen rate of 180 kg ha^{-1}.

The highest increase in the plant content of nitrogen due to an application of the biostimulants was recorded in plots without nitrogen fertilisation although their effect was not uniform. The highest increase in the nitrogen content in Italian ryegrass biomass, as much as 51.7%, was found in units without nitrogen fertiliser but treated with Algex. In their experiment, also Godlewska and Ciepiela [2016] observed increased nitrogen contents in grasses treated with sea algae extract. Similarly, Murawska et al. [2017] reported a significant increase in protein content in wheat treated with the biostimulants Asahi SL and Tytanit. According to Szabo et al. [2011], natural biostimulants contain substances which positively affect protein synthesis, which translates into an increased nitrogen content in plants. Joubert and Lefranc [2008] claim that active substances in sea algae extracts act as phytoactivators, which may explain an altered chemical composition in plants treated with these extracts. Results of the study reported here may point to a possibility of reducing nitrogen inputs, which is desirable because it generates lower production costs and contributes to environment protection as reported by Stamatiadis et al. [2021]. According to these workers, an application of *Ascophyllum nodosum* (L.) Le Jolis extract increased nitrogen accumulation in winter wheat grain so it is possible to reduce nitrogen rates and enjoy economic benefits without a negative impact on yield quantity and quality.

Table 4. The content of total nitrogen (g kg^{-1} d.m.) in Italian ryegrass (mean from three cuts)

Year (C)	N rate kg ha^{-1} (B)	Biostimulant (A)				Mean for N rate	Mean for year
		without biostimulant (control)	Algex	Tytanit	Asahi SL		
2014	0	14.0	21.7	18.5	18.2	18.1	21.3[a]
	120	19.2	21.8	25.0	20.6	21.7	
	180	22.5	23.1	24.0	26.8	24.1	
2015	0	15.7	23.5	20.3	18.3	19.5	22.5[a]
	120	20.3	21.5	25.9	22.6	22.6	
	180	23.6	25.0	25.6	27.9	25.5	
Mean for N rate and biostimulant	0	14.9	22.6	19.4	18.2	18.8[a]	–
	120	19.8	21.7	25.5	21.6	21.9[b]	–
	180	23.1	24.1	24.8	27.4	24.8[c]	–
Mean for biostimulant	–	19.2[a]	22.8[b]	23.2[b]	22.4[b]	–	–
$LSD_{0,05}$	B ×– 0.19; B × C – 0.27; A × B – 0.43; B × A – 0.39; A × B × C – 0.60; B × A × C – 0.55						

a, b, c – homogenous groups of means at 0.05 significance level

Nitrogen uptake with Italian ryegrass yield varied and was related to nitrogen fertiliser rate, biostimulant type and study years (tab. 5). Increasing nitrogen fertiliser rates significantly increased nitrogen uptake by plants. At the nitrogen rate of 180 kg ha^{-1}, the uptake of this nutrient by grasses was almost twice as high as the uptake by the control plants. In earlier research by Godlewska and Ciepiela [2017], nitrogen uptake with the yield of grasses was significantly affected by nitrogen rates.

Table 5. Annual total nitrogen uptake (kg ha^{-1}) with the yield of Italian ryegrass

Year (C)	N rate kg ha^{-1} (B)	Biostimulant (A)				Mean for N rate	Mean for year
		without biostimulant (control)	Algex	Tytanit	Asahi SL		
2014	0	176	384	292	264	279	407[b]
	120	334	488	505	385	428	
	180	428	538	533	555	513	
2015	0	88	254	197	167	176	291[a]
	120	201	340	373	310	306	
	180	293	472	394	407	392	
Mean for N rate and biostimulant	0	132	319	245	215	228[a]	–
	120	268	392	439	347	367[b]	–
	180	360	505	464	481	453[c]	–
Mean for biostimulant	–	253[a]	413[c]	382[bc]	348[b]	–	–
$LSD_{0,05}$	B – 8.01; B × C – 11.33; A × B – 17.59; B × A – 16.02; A × B × C – 24.88; B × A × C – 22.65						

Explanations as in table 4.

The effect of biostimulants mainly consists in increasing the amount of chlorophyll and root mass, which enhances nutrient uptake by limiting their loss, which is particularly true for nitrogen [Pacholczak et al. 2013, Jankowski et al. 2014]. In light of earlier studies and agricultural practice, an application of enhancing products has been shown to allow reducing intensive mineral fertilisation, nitrogen fertiliser in particular.

It should be pointed out that the highest increase in nitrogen uptake was recorded in plots where biostimulants were applied but no nitrogen applications were made. Compared with control, the greatest increase in nitrogen uptake (as much as 142%) was found in units treated with Algex. The obtained results confirmed earlier findings by Godlewska and Ciepiela [2017] who applied the biostimulant Kelpak SL to *Dactylis glomerata* (L.) and Festulolium brauni (K. Richt) at various nitrogen fertiliser levels, and reported similar relationships.

A supreme yield performance of grasses was recorded in the first study year when nitrogen uptake was the greatest in this year, this being helped by favourable meteorological conditions (tab. 3).

The agricultural effectiveness of nitrogen fertilisation, defined as a yield increase per 1 kg N applied as a fertiliser, is a measure of an efficacy of plant fertilisation with nitrogen [Ciepiela et al. 2012]. Values of this indicator fell within a narrow range of 31.1 to 45.0 kg d.m. kg^{-1} N (tab. 6). Nitrogen fertilisation with 180 kg ha^{-1} significantly reduced the agricultural effectiveness of nitrogen fertilisation (by 8%). Also Marino et al. [2004] reported a decline in the value of the above parameter at higher nitrogen rates applied to annual ryegrass. The effectiveness decreased following an increase in N rate because yield does not tend to increase linearly due to increased N input [Albrizio et al. 2010].

Table 6. Agricultural efficiency of fertilisation of Italian ryegrass with nitrogen (kg d.m. kg^{-1} N) as affected by biostimulant type and study year

Year (C)	N rate kg ha^{-1} (B)	Biostimulant (A)				Mean for N rate	Mean for year
		without biostimulant (control)	Algex	Tytanit	Asahi SL		
2014	120	40.0	39.2	36.7	35.0	37.7	35.9[a]
	180	35.6	31.1	35.6	34.4	34.2	
2015	120	35.8	41.7	39,2	38.3	38.8	37,5[a]
	180	37.8	45.0	31.7	30.6	36.3	
Mean for N rate and biostimulant	120	37.9	40.4	37.9	36.7	38.2[a]	–
	180	36.7	38.1	33.6	32.5	35.2[a]	–
Mean for biostimulant	–	37.3[a]	39.2[a]	35.8[a]	34.6[a]	–	–
$LSD_{0,05}$	B – 1.67; B × C – 2.26; A × B – 4.37; B × A – 3.34; A × B × C – 6.19; B × A × C – 4.72						

Explanations as in table 4.

Foliar applications of biostimulants may increase an effectiveness of nitrogen fertilisation [Carillo and Rouphael 2022]. An application of Algex contributed to an increase in the value of the discussed indicator, by 5.20%, on average, the differences being statistically insignificant. In the previous research by Godlewska and Ciepiela [2017], the authors demonstrated that an application of sea algae extract in the cultivation of *Dactylis glomerata* and *Festulolium braunii* increased the value of the indicator by 29.7%. According to Stamatiadis et al. [2021], *Ascophyllum nodosum* extract applied to winter wheat increased the nitrogen use efficiency by 11%. Goñi et al. [2021] recorded an increase in nitrogen fertilisation efficiency of barley treated with an extract of *Ascophyllum nodosum*; however, it was much lower than in the study reported here. Cozzolino et al. [2021] demonstrated that an application of sea algae extract improved nitrogen use, which makes it possible to reduce the rate of this nutrient by about 35%. Regardless of the study year and nitrogen rate, there was noted a varied effect of an application of Tytanit and Asahi SL on the agricultural effectiveness of nitrogen fertilisation. In the majority of fertilised units, Tytanit and Asahi SL contributed to a decline in the value of this parameter in both the study years. However, in the units fertilised with 180 kg N ha^{-1} in the first study year, and 120 kg N ha^{-1} in the second study year, an application of Tytanit was followed by enhanced agricultural effectiveness. An increase in the value of this parameter, compared with units untreated with biostimulants, was 2.57% and 9.60%, respectively. Asahi SL applied in the second study year and accompanied by nitrogen fertiliser at the rate of 120 kg ha^{-1} contributed to a 7.90% increase in the value of the parameter discussed. The above results indicate that the effect of biostimulants is to a large degree dependent upon the type of biostimulant, which has been confirmed in other works [Szczepanek et al. 2018, Di Mola et al. 2020]. However, their modes of action differ substantially and have not been fully understood yet.

Physiological effectiveness is a measure of a plant's ability to transform the nitrogen it has taken up into usable yield, which also bespeaks the efficiency of nitrogen management processes in the plant [Kruczek 2000]. The value of this parameter (tab. 7) was slightly lower than the value of agricultural effectiveness, the likely reason behind this discrepancy being too large an amount of this nutrient being taken up which the plants were unable to transform.

Table 7. Physiological efficiency fertilisation of Italian ryegrass with nitrogen (kg d.m. kg^{-1} N uptake by plants) depended on biostimulant type and study year

Year (C)	N rate kg ha^{-1} (B)	Biostimulant (A)				Mean for N rate	Mean for year
		without biostimulant (control)	Algex	Tytanit	Asahi SL		
2014	120	30.4	45.1	20.7	34.6	32.7	30.1[a]
	180	25.5	36.3	26.6	21.3	27.4	
2015	120	38.0	58.2	26.7	32.1	38.8	34,6[a]
	180	33.2	37.0	28.9	22.8	30.5	
Mean for N rate and biostimulant	120	34.2	51.6	23.7	33.4	35.7[b]	–
	180	29.4	36.7	27.7	22.1	29.0[a]	–
Mean for biostimulant	–	31.8[a]	44.2[b]	25.7[a]	27.7[a]	–	–
$LSD_{0,05}$	B – 1.14; B × C – 1.62; A × B – 3.00; B × A – 2.29; A × B × C – 4.24; B × A × C – 3.23						

Explanations as in table 4.

Regardless of the study years and biostimulant type, the nitrogen rate of 180 kg ha^{-1} contributed to a significant decline in physiological effectiveness which amounted to 18.8%. Also Godlewska and Ciepiela [2017] reported that, in their study, physiological effectiveness decreased as nitrogen rates increased. However, the differences were statistically insignificant.

There were observed varied effects of the biostimulants applied in the study reported here. Also Murawska et al. [2017] observed that the cultivation effects observed depend on the type of applied biostimulant and nitrogen rate. An application of *Ascophyllum nodosum* extract (Algex) significantly increased physiological effectiveness, that is by 50.9% and 24.8% at the nitrogen rate of 120 and 180 kg ha^{-1}, respectively. However, previous research by Godlewska and Ciepiela [2017] demonstrated a decline in physiological effectiveness following an application of the biostimulant Kelpak SL (extract of the seaweed *Eclonia maxima* (Osbeck) Papenfuss) in two species of grasses. Therefore, the effect of seaweed-based biostimulants may also be associated with crop plant species [Battacharyya et al. 2015], which confirms the need to continue this line of research. An application of Tytanit and Asahi SL increased physiological activity only in the first study year. In plots amended with 180 kg N ha^{-1} + Tytanit, and units fertilised with 120 kg N ha^{-1} + Asahi SL, physiological effectiveness increased by 4.31% and 13.8%, respectively.

CONCLUSIONS

1. The hypothesis which assumed that both natural and synthetic biostimulants increase the agricultural and physiological effectiveness of nitrogen fertilisation was partially confirmed. Algex applied in both the study years positively affected values of the two parameters which increased by 5.67% and 64.2%, respectively. The effect of the tested biostimulants varied and depended on study years and nitrogen fertiliser rate.

2. Of the biostimulants tested in the study, Algex was associated with the highest yields, increased nitrogen content and uptake by Italian ryegrass, as well as improved agricultural and physiological effectiveness.

3. Nitrogen fertilisation increased yields as well as nitrogen content and uptake of the test grasses. However, the highest nitrogen rate, that is 180 kg N·ha^{-1}, contributed to significant decline in both agricultural and physiological effectiveness.

4. The biostimulants can be alternative for conventional mineral fertilisers. However, such a statement requires further research including other plant species, rates and timing of biostimulant application.

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