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Effect of biostimulants on cold resistance and productivity formation in winter rapeseed and winter wheat

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Abstract

The aim of the study was to investigate the effects of biostimulants on the resistance to freezing under laboratory-controlled cold conditions and on the growth, development, overwintering and productivity of winter rapeseed and winter wheat in natural field experiments. The effect of free amino acids, macroelements and microelements that contain biostimulants Ruter AA, Terra Sorb and Razormin was tested on cultivars of rapeseed, 'Hornet H', and winter wheat, 'Skagen' and 'Kovas', applying morphometrical methods. We found that biostimulants applied to rapeseed at BBCH 13–14 stage and to wheat at BBCH 14–15 stage under controlled cold stress conditions increased the freezing tolerance of seedlings. Biostimulants more actively increased the freezing resistance of rapeseed seedlings at –5°C compared to that of wheat seedlings. The temperature of –7°C was mortal to rape seedlings, while the resistance of wheat seedlings increased under the influence of the tested biostimulants compared to that of the control seedlings. In natural field experiments, these biostimulants produced a significant effect on plant growth in autumn, acclimation to the cold, plant overwintering, vegetation renewal and, due to this, formation of productivity elements. The effects of Razormin (200 mL/ha), Terra Sorb (2 L/ha) and Ruter AA (1 L/ha) were significantly higher on growth parameters of winter wheat compared to the productivity of winter rapeseed.

Keywords

Brassica napus L. • *freezing* • *growth regulation* • *resistance* • Triticum aestivum L. • *overwintering*

Introduction

The world's population is steadily increasing, and global food productivity shows a decreasing trend due to the accumulating negative effects of abiotic–biotic stresses. Despite this, agricultural production in the world is rapidly increasing, depending strongly on the use of modern agrochemistry and agrotechniques, as well as suitable growth regulators. Meteorological conditions in European countries, particularly during the periods in which winter crops harden in preparation for wintering, overwintering and vegetation renewal, are unfavourable and change from year to year. Wintering plants such as rape and wheat are stressed in autumn by snow covering unfrozen ground and periodic fluctuations of below-zero and above-zero temperatures during the period of plant acclimation to the cold and later in the season by snowless winters, early springs, etc. (Velička *et al*., 2006; Li *et al*., 2008). In addition, the effect of climate warming on plant growth is problematic too. Thus, the search for tools regulating plant growth, development, acclimation to the cold and productivity formation is important (Zhang *et al*., 2004; Szalai *et al*., 2009). Research on agrotechnical measures applied to winter rape and wheat crops that are important for many countries, including Lithuania, has been intensively carried out, including the selection of suitable soils, fertilisation, sowing time, sowing rate and search for disease-resistant varieties (Brazauskienė and Petraitienė, 2008; Tein *et al*., 2010), as well as investigation on plant autumnal growth, accumulation of cold stress protective compounds and formation of productivity elements (Hayat *et al*., 2012). In the above-mentioned regulation processes, synthetic plant growth regulators of various chemical structures participate: physiological analogues of phytohormones, compounds of contrary action – retardants and herbicides that regulate weed management, amino acids and macroelement and microelement products or biostimulants (Jakienė, 2013; Yang *et al*., 2013; Colebrook *et al*., 2014). Exogenous signals such as synthetic hormones, especially their physiological analogues, play an important role in the regulation of growth response, but the application of hormones in plant cultivation has proved to be ineffective. The effect of this practice is limited for several reasons since the status and proportion of individual endogenous hormones, synthesised at a certain period of ontogenesis, change over time. Therefore, in plant cultivation practice, it is more purposeful to apply synthetic physiologically active compounds – analogues of hormones or retardants (Merkys *et al*., 2007). The latter are the established

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growth regulators that are the so-called special fertilisers or biostimulants that contain free amino acids, macroelements and microelements (Tradecorp catalog, 2010; Abouziena *et al*., 2011; Jakienė, 2013). Free amino acids have lower molecular weight, so they are quickly absorbed by plants, especially through leaves and in limited amounts through roots (Miller *et al*., 2007). Amino acids affect the processes of plant photosynthesis and respiration (Long *et al*., 2006) and increase the content of unsaturated (oleinic) and saturated (linolenic) fatty acids (Jakienė, 2013). It has been suggested that the internal pools of amino acids within plants may indicate the nitrogen status by providing a signal that can regulate nitrate uptake by the plant (Szabados and Savoure, 2010). Several different amino acids – lysine, glutamine and glycine – have been tested for their effects on nitrate and ammonium influx and transcription, with glutamine proving the most effective for these processes (Miller *et al*., 2007). Alongside exogenously applied amino acids, glutamine and especially proline increase free proline content and are involved in the response to cold stress (Dörffling *et al*., 2009; Lehmann *et al*., 2010). Glutamic and aspartic acids are very important for the synthesis of other amino acids. The absence of any amino acid participating in individual protein structure would hinder protein synthesis. Plants synthesise amino acids from inorganic nitrogen (N). During this process, nitrate is assimilated via conversion to nitrite and then via conversion of ammonium ions into amino acids (Miller *et al*., 2007).

Summarising the produced literature data, we have hypothesised that amino acids, especially proline, regulate the acclimation of winter plants to the cold and increase plant freezing tolerance. Thus, scientific investigation and employment of biostimulants – growth regulators with the optimal ratio of amino acids – may be promising. The main task of this research was to investigate the effect of biostimulants that contain free amino acids, macroelements and microelements on freezing resistance in winter rapeseed and winter wheat under controlled cold conditions. We also studied the influence of the tested biostimulants on autumnal growth, plant overwintering, vegetation renewal and productivity formation in these winter crops under natural field conditions.

Materials and methods

Plant material and tested biostimulants

The investigations into the effect of different biostimulants that contain free amino acids, macroelements and microelements on the growth and development of winter rapeseed (*Brassica napus* L.) 'Hornet H' and wheat (*Triticum aestivum* L.) variety 'Skagen' were carried out under controlled cold conditions at the Laboratory of Plant Physiology (Nature Research Centre, Institute of Botany) and under natural field conditions (rapeseed cv. 'Hornet H', wheat 'Kovas') at the Field Experimental Station (Nature Research Centre, Institute of Botany) in 2012–2015. Winter rapeseed cv. 'Hornet H', produced in Germany by the seed growing company Deutsche Saatveredelung AG (Weissenburger Straße 5, Lippstadt), is characterised as tolerant to abiotic stress. Winter wheat cv. 'Skagen', produced in Denmark by the seed growing company Nordic Seed A/S (Kornmarken 1,Galten), is tolerant to the cold. Medium-early, tolerant to wintering winter wheat variety 'Kovas' is produced at the Lithuanian Institute of Agriculture (Instituto al.1, Akademija Kėdainiai distr., Lithuania).

The tested biostimulants differ in the available quantity of free amino acids, macroelements and microelements (Table 1). Ruter AA and Terra Sorb are produced by the Trade Corporation International, S.A. (Spain). Razormin is produced by Atlántica Agrícola (Spain) Terra-Sorb formula contains all biologically active free amino acids: ASP, SER, GLU, GLY, HIS, ARG, THR, PRO, SIS, TYR, VAL, MET, LYS, ILE, LEU, PHE and TRY. This product is suitable for organic agriculture (approved by the Andalusian Committee for Ecological Agriculture). Ruter AA and Razormin are characterised as stimulants for plant rooting and possess anti-stress properties (https://www.bioiberica.com/plant-health/solutions-for-plant-stress/grapevines/terra-sorb-foliar-1/).

Laboratory investigation

The effect of biostimulants on the cold tolerance of winter rape and wheat plants in separate experiments was investigated under controlled laboratory conditions. Rape and wheat seeds were sterilised with 10% sodium hypochlorite for 10 min and washed five times with distilled water before sowing. The

Table 1. The composition of the tested biostimulants (%)

Biostimulants	Free amino acids	N	P_2O_5	K_2O	Fe	Cu	Mn	Mo	Zn	B	Organic matter
Ruter AA	8.4	6.6	6.0	4.2	0.04	-	0.06	0.12	0.08	-	18.0
Terra Sorb	9.3	4.2	-	-	-	-	0.04	-	0.07	0.02	14.8
Razormin	7.0	4.0	4.0	3.0	0.4	0.02	0.1	0.01	0.085	-	25.0

Tradecorp catalog (Nutri-Performance). 2010. http://tradecorp.com.es/.

seeds of each variety were sown in soil (vegetable compost 90%, peat 9%, deciduous ash 1% and fertiliser NPK) in 56 growing dishes each (eight replicates per treatment): 1) control; 2) Ruter AA 0.5 mL/100 mL; 3) Ruter AA 1 mL/100 mL; 4) Terra Sorb 0.5 mL/100 mL; 5) Terra Sorb 1 mL/100 mL; 6) Razormin 0.25 mL/100 mL and 7) Razormin 0.1 mL/100 mL), 16 seeds in a dish. The germination occurred over 24–26 days in a Climacell plant growing chamber (Medcenter Einrichtungen GmbH) at a constant temperature of 18 ± 2°C under illumination of 60 mol·m^2/s and photoperiod 16/8 h (Mackevičius *et al*., 1999). Rape cv. 'Hornet H' seedlings at the BBCH 13–14 (three-leaves) stage and wheat cv. 'Skagen' at BBCH 14–15 (five-leaves) stage (Meier, 2001) were foliar sprayed with water solutions of tested biostimulants (applied in 10 mL solutions for each growing dish of 16 seedlings) using a hand sprayer with dispenser. The seedlings of the control were treated with water. All seedlings were then grown under the same conditions for 24 h. Later, the seedlings (56 growing dishes) were acclimated at 4°C for 96 h in the Friocell chamber. After exposition, all seedlings were subjected to a period of 2 h of gradually lowering temperatures; this was required to avoid cold shock for the plants. Two dishes from each of the above treatments were then placed in separate Climacell growing chambers at -1°C, -3°C, -5°C and -7°C for 24 h. After freezing, in order to determine the survival of the wheat and rape during the freezing, the seedlings were transferred into a plant growing chamber under uniform conditions – a temperature of 18 ± 2°C under illumination of 60 mol/m^2 at a day length of 16/8 for 7 days. After cultivation, the surviving plants were marked and assessed. The effect of the tested biostimulants on the survival of seedlings to freezing was calculated as the percentage ratio of the number of surviving seedlings after freezing to the number of seedlings before freezing. Three biological replicates were carried out for the experiments.

Field experiments

The same regulators with amino acids investigated under controlled laboratory conditions were further studied under natural field conditions at the Field Experimental Station (54º68¢N, 25º26¢E) of the Institute of Botany, Nature Research Centre (Lithuania). The main agrochemical parameters of the arable soil layer were pH 7.0–7.3, P_2O_5 248–250 mg/ha and K_2O 214.0–214.6 mg/kg. Small plot (1 m^2) field trials with winter rapeseed cv. 'Hornet H' and winter wheat cv. 'Kovas' were performed on light loamy Endocalcari-Epihypogleyic Cambisol in sixfold repetition completed in randomised blocks in 2012–2015. Six blocks with seven split plots in each block (control and six variants of biostimulant and concentration) were arranged for each variety/species, i.e. six replications of experiment every year.

Rape and wheat plants were cultivated using standard agrochemical and agrotechnical technologies for these crops (Liakas *et al*., 2000; Tein *et al*., 2010; Balodis and Gaile, 2015). Experiments with winter rapeseed were set in the fourth week of August and those with winter wheat in the second week of September. The fertilisation of rape ($N_{120}P_{90}K_{60}$) and wheat ($N_{100}P_{90}K_{60}$) plants was performed as follows: P, K and N_{30} were used before autumn ploughing of the soil, whereas in spring, N_{90} kg/ha was used before the resumption of the plant vegetation period. The row spacing of rape was 12.5 cm, while that of wheat was 15 cm; rape seeds were placed at 2–3 cm depth and wheat at 4 cm depth. The tested biostimulants were foliar sprayed using water solutions, 100 mL for each plot via a hand sprayer with dispenser at the BBCH 13–14 (fourth–fifth leaves) stage for rape and at BBCH 14–15 leaf development stage for wheat. Plants of the control plots were sprayed with water. In order to assess the success of plant overwintering, the number of plants was calculated in autumn and spring in six plots (1/m^2 each) of the test variants and control. The density of rape in autumn was 80–100 plants/1 m^2 and that of wheat in autumn was 200–220 plants/1 m^2. Plant overwintering was calculated as the percentage ratio of plants in spring to those in autumn. The morphometric parameters of plant growth (stem height, leaf number, rape root column length and diameter, fresh and dry weight) were estimated 20 days after the spraying with tested biostimulants in autumn at BBCH 18–19 stage for rape and at BBCH 22–24 stage for wheat and in spring at the vegetation renewal period at BBCH 33–35 stage with 30–34 plants for each investigated biostimulant and the control sample. From each plot, five plants were randomly rooted out and sampled, with a total of 30 plants per treatment. To assess the effect of regulators on the productivity elements and yield of rapeseeds in the phase of full ripeness –BBCH 89–91 stage – number of seeds per siliqua, 1,000-seed weight and seed weight from each plot (1/m^2 × 6) were evaluated. At the full maturity stage, the structural elements of wheat yield, i.e. the number of productive stems, the number of grain per ear, grain weight per plant, 1,000-grain weight and grain weight from each plot (1/m^2 × 6), were determined. The 1,000-grain weight was determined according to ISO 580-77. The data on 1,000-grain weight and grain weight per plant were adjusted to 10-moisture content.

Statistical analysis

Data were managed using MS Excel (version 7.0). In each year (2012–2015), the effects of biostimulant and its concentration were assessed using multidimensional statistical methods (factorial analysis of variance [ANOVA] or main effects ANOVA) and post hoc comparisons – the Tukey's HSD test (Zar, 1999; Piepho *et al*., 2006; StatSoft, 2010). Before ANOVA, normality of the data was checked using the Kolmogorov–Smirnov *d* test (with confidence level $P < 0.05$ for significant difference from normal distribution). The

Kolmogorov–Smirnov tests were applied to all treatments (control, biostimulants and their concentrations) separately. Most of the data were of normal or near-normal distribution (difference from normal distribution was not significant [NS]); thus, we used linear models. We used numbers instead of percentages when evaluating plant survival and its increase at different temperatures (Onofri *et al.*, 2010), although freezing survival was presented as a percentage of surviving plants. Significance of the difference of winter rapeseed cv. 'Hornet H' and winter wheat cv. 'Kovas' overwintering in control plots and under the influence of biostimulants was tested using Student's *t* test. Calculations were done using Statistica for Windows by former StatSoft, Inc. (currently TIBCO Software Inc.). The minimum confidence level was set to $P < 0.05$. Mean values and standard errors of morphometric measurements presented in Tables 2 and 3 are from three biological and 30 morphometric replicates.

Table 2. Effect of biostimulants on freezing survival of winter rape 'Hornet H' seedlings

Test variant	**Freezing temperature (°C)**			
	-1	**-3**	**-5**	**-7**
	Survived seedlings (%)			
Control	90.4^{c}	61.6^{c}	20.9^{c}	0
Ruter AA (0.5 mL/100 mL)	93.2^{b}	67.5^{b}	22.0^{c}	1.0
Ruter AA (1 mL/100 mL)	95.0^{a}	78.7^{a}	30.1^{a}	1.1
Terra Sorb (0.5 mL/100 mL)	95.0^{a}	63.0^{b}	23.0^{b}	1.1
Terra Sorb (1 mL/100 mL)	95.0^{a}	70.3^{a}	28.7^{a}	1.0
Razormin (0.1 mL/100 mL)	87.9^{c}	64.5^{b}	26.8^{ab}	1.3
Razormin (0.25 mL/100 mL)	91.7^{b}	63.3^{b}	25.8^{b}	1.3

Each data represent three replicates.
Different letters designate statistically significant difference at $P < 0.05$.

Table 3. Effect of biostimulants on freezing survival of winter wheat 'Skagen' seedlings

Test variant	**Freezing temperature (°C)**			
	-1	**-3**	**-5**	**-7**
	Survived seedlings (%)			
Control	95.2^{b}	88.0^{c}	35.7^{c}	4.1^{c}
Ruter AA (0.5 mL/100 mL)	98.0^{a}	95.3^{b}	38.9^{b}	4.5^{c}
Ruter AA (1 mL/100 mL)	100.0^{a}	96.7^{a}	42.8^{a}	5.7^{a}
Terra Sorb (0.5 mL/100 mL)	99.5^{a}	98.5^{a}	40.0^{b}	4.8^{b}
Terra Sorb (1 mL/100 mL)	97.1^{a}	100.0^{a}	47.0^{a}	6.4^{a}
Razormin (0.1 mL/100 mL)	98.1^{a}	97.3^{a}	43.7^{a}	5.7^{a}
Razormin (0.25 mL/100 mL)	100.3^{a}	90.0^{c}	39.7^{b}	4.8^{b}

Explanations under Table 2.

Meteorological data for 2012–2015

The precipitation rate in 2012, especially in October and November; November 2013 and August 2014 was higher than the long-term average, while September 2012, October 2013 and September, October and November 2014 were characterised as relatively dry. In general, the outline amount of precipitation in 2013 was close to the long-term mean and precipitation in March 2013 amounted only to 41% of the average value; however, precipitation in July was about 35% higher than the long-term average (Figure 1). During the investigation years, the air temperature in August, September and October was close to the long-term average and played the main role in the plants' successful germination, growth, development and acclimation to the cold (Figure 1). The temperature in winter and early spring in 2012–2013 was lower than the long-term average: -2.2°C, -0.9°C and -4.6°C in December, January and March, respectively. The year 2013 had a higher air temperature during the flowering and plant productivity formation periods (May and June). The air temperature in May and June of 2014 and 2015 was near to the long-term average, while that in July 2014 was higher than the long-term average. The greatest deviation from the long-term average was recorded in February 2014 and January–February 2015, exceeding the average by 4.3°C and 5°C, respectively (Figure 1). Thus, during the investigation period in 2012–2015, the meteorological conditions were favourable for winter rape and winter wheat growth development and productivity formation.

Results

Experiments under controlled cold conditions

Freezing survival of winter rape 'Hornet H' seedlings depended on temperature (ANOVA, $F = 80{,}770.5$, df = 3, $P < 0.001$), biostimulant ($P < 0.001$), its concentration ($P < 0.001$) and their interaction (factorial ANOVA, biostimulant × concentration $P < 0.001$, temperature × biostimulant × concentration $P < 0.001$). The model was extremely strong ($r^2 = 0.997$, $P < 0.001$).
Evaluation of the effect of tested regulators on the freezing resistance of rape at -1°C revealed that all seedlings treated with biostimulants and control seedlings were resistant to the cold. At lower temperature (-3°C), the effect of tested regulators, depending on their concentrations, was distinctly noticed in the freezing tolerance of rape seedlings. Under the impact of Ruter AA (1 mL/100 mL) and Terra Sorb (1 mL/100 mL), the survival of rape seedlings, compared to the control, increased by 28% and 14%, respectively (Table 2). Difference is significant (Tukey's HSD test, $P < 0.001$).
Freezing survival of winter wheat cv. 'Skagen' seedlings was more dependent on temperature (ANOVA, $F = 76{,}626.3$, P

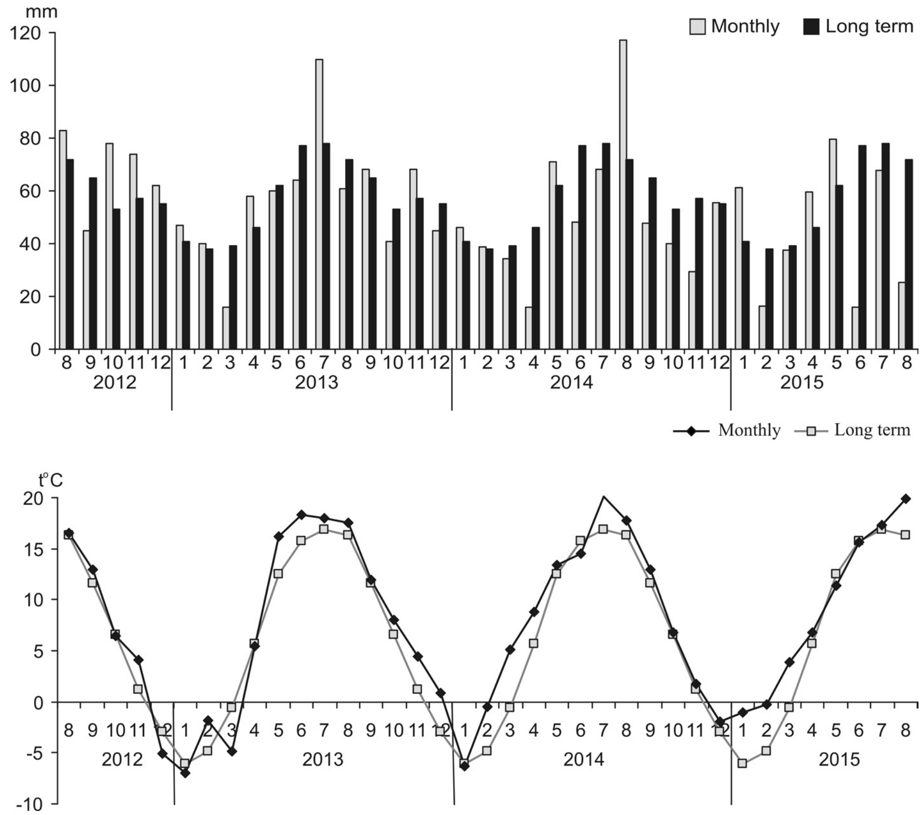

Figure 1. Monthly mean precipitation rates and air temperature in 2012–2015 and long-term averages (Vilnius Hydrometeorological Service under the Ministry of Environment).

< 0.001) and biostimulant ($F = 46.5$, $P < 0.001$) than on the concentration ($P = 0.007$). The model ($r^2 = 0.999$, $P < 0.001$), as well as factor interaction (factorial ANOVA, temperature × biostimulant × concentration $F = 41.9$, biostimulant × concentration $F = 90.6$, temperature × biostimulant $F = 29.7$ and temperature × concentration $F = 17.6$, all cases $P < 0.001$), was strong.

Evaluation of the effect of tested regulators on the freezing resistance of wheat at temperature -3°C increased their survival by 9% and 12% under the impact of Ruter AA (1 mL/100 mL) and Terra Sorb (1 mL/100 mL), respectively, compared to the control. At -5°C, the respective increase in wheat survival was 7.1% and 11.3% (Table 3). Again, differences were significant (Tukey's HSD test, $P < 0.001$).

The most significant differences of the effect of tested biostimulants compared with the control were determined at -5°C. The rape and wheat seedlings under the effect of Ruter AA (1 mL/100 mL), Terra Sorb (1 mL/100 mL) and Razormin (0.1 mL/100 mL) were less damaged by cold compared to the control (Tables 2 and 3). However, the effect of these regulators, especially Ruter AA, on the survival of rape seedlings at the temperature of -5°C is more evident than that of wheat. On the other hand, at this temperature, control rape seedlings were more injured than the control wheat seedlings. The experimental data showed that under controlled cold conditions at the laboratory, the freezing temperature at -7°C is mortal for rape (Table 2). Meanwhile, the wheat seedlings were more tolerant to the cold than the rape seedlings. The tested biostimulants, especially Terra Sorb (1 mL/100 mL), more actively increased the survival of wheat after freezing at -7°C (Table 3) than that of rape seedlings.

Effect of biostimulants on autumnal growth, cold tolerance, wintering, vegetation renewal and formation of productivity elements of winter rape in the field experiment

Analysis of the effect of the tested biostimulants on growth parameters of winter rape 'Hornet H' in autumn revealed that year and biostimulant concentration had significant effects on plant parameters (Wilk's lambda = 0.047, $F = 4.24$, $P < 0.01$ and Wilk's lambda = 0.057, $F = 19.10$, $P < 0.001$, respectively), while the effect did not depend on the biostimulant (Wilk's lambda = 0.17, $F = 1.65$, NS). The strongest effect was determined on the root column diameter ($r^2 = 0.89$, $F_{5,12} = 28.94$, $P < 0.0001$), leaf number ($r^2 = 0.77$, $P < 0.001$), plant height ($r^2 = 0.71$, $P < 0.001$) and fresh weight ($r^2 = 0.65$, $P < 0.005$), with a moderate effect on the root column length ($r^2 = 0.45$, $P < 0.05$). Dry weight did not influence significantly ($r^2 = 0.25$, NS).

Thus, Ruter AA (1 L/ha), Terra Sorb (1 L/ha) and Razormin (200 mL/ha) influenced growth and development of rape: the number of leaves increased by 7%, 10% and 12% in 2012; 15%, 8% and 10% in 2013 and 12%, 7% and 9% in 2014, respectively. Root column diameter increased by 11%, 9% and 14% in 2012; 12%, 8% and 14% in 2013; 20.8%, 14.6% and 12.5% in 2014, respectively, compared to the control sample. The tested regulators, especially Razormin (200 mL/ha) and Terra Sorb (1 L/ha), increased fresh weight and especially dry weight of rape through the stimulation of leaf formation (Table 4). It is known that leaf number and root column development define hardening and overwintering of rapeseeds (Velička *et al*., 2006). The morphometric parameters of plant growth and development of rapeseeds in our field experiment showed that plants were prepared for wintering.

Estimation of the overwintered rape plants showed that the tested biostimulants, especially Ruter AA (1 L/ha) and Terra Sorb (1 L/ha), significantly increased overwintering of rapeseeds compared to that of the control plants (Figure 2). All differences in overwintering of plants between the control and plots under the influence of biostimulants were significant (Student's t test = 3.04–11.31, df = 58, $P < 0.05$–0.0001), with an exception in 2012–2013, when application of Ruter AA (2 L/ha) and Terra Sorb (2 L/ha) had only a tendency of positive effect (Student's t test = 1.71 and 1.94, respectively, df = 58, $P < 0.1$). The strong effect of regulators on overwintering of plants in 2013–2014 was related to the higher temperatures in December and February compared to the temperatures of the same months in 2012–2013 (Figure 1). Terra Sorb (1 L/ha) and Razormin (200 mL/ha) most strongly increased overwintering of the plants in 2014–2015.

Effect of the tested regulators on the plant growth and development in the vegetation renewal period at BBCH 33–35 stage may be defined by the products' influence on rape growth and development in autumn, plant acclimation to the cold, overwintering and favourable meteorological

Table 4. Effect of biostimulants on growth and development of winter rape 'Hornet H' in autumn at BBCH 18–19 stage

Test variant	Plant height (cm)	Leaf number per plant (n)	Roots		Plant weight (g)	
			Length (cm)	Diameter (mm)	Fresh	Dry
2012						
Control	33.8^{b}	8.6^{c}	1.6^{c}	4.4^{c}	9.49^{c}	1.20^{c}
Ruter AA (1 L/ha)	39.1^{a}	9.2^{a}	1.7^{b}	4.9^{a}	10.62^{a}	1.26^{b}
Ruter AA (2 L/ha)	33.4^{b}	8.8^{c}	1.6^{c}	4.5^{c}	9.23^{c}	1.31^{a}
Terra Sorb (1 L/ha)	35.4^{a}	9.5^{a}	1.8^{a}	4.8^{a}	10.10^{a}	1.27^{b}
Terra Sorb (2 L/ha)	31.6^{c}	8.9^{b}	1.6^{c}	4.5^{c}	9.57^{b}	1.21^{c}
Razormin (200 mL/ha)	35.7^{a}	9.6^{a}	1.9^{a}	5.0^{a}	12.02^{a}	1.59^{a}
Razormin (500 mL/ha)	33.8^{b}	8.8^{c}	1.8^{a}	4.5^{c}	11.50^{c}	1.45^{a}
2013						
Control	41.2^{c}	8.9^{c}	1.8^{b}	4.8^{b}	10.39^{b}	1.20^{c}
Ruter AA (1 L/ha)	49.1^{a}	10.2^{a}	1.9^{a}	5.4^{a}	11.42^{a}	1.41^{ab}
Ruter AA (2 L/ha)	42.4^{b}	9.2^{b}	1.7^{b}	4.7^{c}	10.41^{b}	1.12^{c}
Terra Sorb (1 L/ha)	44.4^{a}	9.6^{a}	2.1^{a}	5.2^{a}	11.89^{a}	1.46^{a}
Terra Sorb (2 L/ha)	41.6^{c}	8.9^{c}	1.8^{b}	4.8^{b}	10.27^{c}	1.20^{b}
Razormin (200 mL/ha)	45.8^{a}	9.8^{a}	2.2^{a}	5.5^{a}	14.22^{a}	1.62^{a}
Razormin (500 mL/ha)	42.8^{b}	8.8^{c}	1.9^{a}	4.8^{b}	10.50^{b}	1.35^{b}
2014						
Control	43.1^{c}	9.0^{c}	1.9^{c}	4.8^{c}	11.15^{c}	1.25^{c}
Ruter AA (1 L/ha)	50.3^{a}	10.1^{a}	2.2^{a}	5.8^{a}	12.42^{a}	1.49^{a}
Ruter AA (2 L/ha)	43.5^{c}	9.3^{c}	1.8^{c}	5.0^{b}	11.51^{b}	1.28^{c}
Terra Sorb (1 L/ha)	48.4^{a}	10.5^{a}	2.2^{a}	5.5^{a}	11.89^{a}	1.26^{c}
Terra Sorb (2 L/ha)	44.5^{b}	9.1^{c}	1.9^{c}	4.8^{c}	10.92^{c}	1.52^{a}
Razormin (200 mL/ha)	49.1^{a}	9.8^{a}	2.9^{a}	5.4^{a}	12.51^{a}	1.49^{a}
Razormin (500 mL/ha)	42.5^{c}	9.0^{c}	2.0^{c}	5.0^{b}	11.22^{c}	1.29^{c}

Plant growth and development measurements (the average per plant) were assessed in the BBCH 18–19 stage (20 days after treatment with tested biostimulants).

Different letters designate statistically significant difference at $P < 0.05$.

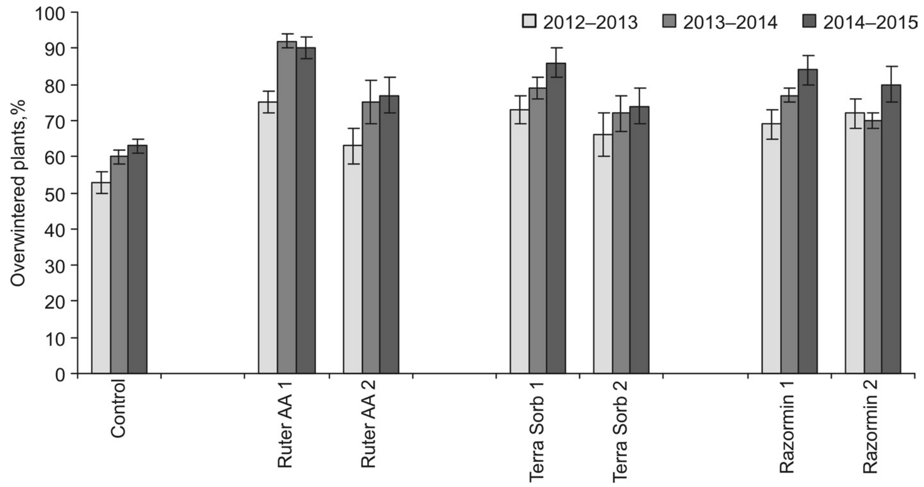

Figure 2. Effect of growth regulators on overwintering of winter rape 'Hornet H' plants, tested in 2012–2013, 2013–2014 and 2014-2015. Ruter AA 1 – 1 L/ha, 2 – 2 L/ha; Terra Sorb 1 – 1 L/ha, 2 – 2 L/ha; Razormin 1 – 200 mL/ha, 2 – 500 mL/ha. Each data represent the mean ± s.e. of 30 replicates. All differences in overwintering of plants between control and biostimulants were significant at $P < 0.05$ or higher (except for 2012–2013 Ruter AA (2 L/ha) and Terra Sorb (2 L/ha), where P was <0.1).

Table 5. Effect of biostimulants on growth and development of winter rape 'Hornet H' in spring at BBCH 33-35 stage

Test variant	Plant height (cm)	Leaf number per plant (n)	Roots		Plant weight (g)	
			Length (cm)	Diameter (mm)	Fresh	dry
2013						
Control	9.0^{c}	9.15^{c}	1.07^{c}	6.0^{c}	9.1^{c}	0.97^{c}
Ruter AA (1 L/ha)	10.6^{a}	11.53^{a}	1.19^{b}	7.6^{a}	10.0^{a}	1.11^{ab}
Ruter AA (2 L/ha)	10.0^{a}	11.22^{a}	1.17^{b}	6.3^{c}	9.3^{c}	0.99^{c}
Terra Sorb (1 L/ha)	9.8^{a}	12.02^{a}	1.39^{a}	6.8^{b}	9.5^{b}	1.12^{a}
Terra Sorb (2 L/ha)	9.6^{b}	11.96^{a}	1.36^{a}	6.2^{c}	9.4^{c}	1.11^{ab}
Razormin (200 mL/ha)	10.3^{a}	11.80^{a}	1.37^{a}	7.1^{a}	10.9^{a}	1.19^{a}
Razormin (500 mL/ha)	9.4^{c}	10.36^{b}	1.26^{b}	6.9^{b}	9.9^{b}	1.09^{b}
2014						
Control	16.2^{c}	8.26^{c}	0.92^{c}	8.0^{c}	12.1^{c}	1.35^{c}
Ruter AA (1 L/ha)	17.5^{a}	10.58^{ab}	1.12^{ab}	9.2^{ab}	13.0^{ab}	1.48^{b}
Ruter AA (2 L/ha)	16.9^{b}	10.47^{b}	1.06^{b}	8.7^{b}	12.8^{b}	1.32^{c}
Terra Sorb (1 L/ha)	18.7^{a}	11.86^{a}	1.24^{a}	9.9^{a}	15.2^{a}	1.81^{a}
Terra Sorb (2 L/ha)	18.2^{ab}	10.73^{ab}	1.00^{b}	9.7^{a}	13.5^{ab}	1.62^{ab}
Razormin (200 mL/ha)	19.3^{a}	12.61^{a}	1.21^{a}	9.2^{ab}	15.9^{a}	1.79^{a}
Razormin (500 mL/ha)	17.2^{b}	12.22^{a}	1.01^{b}	8.7^{b}	13.8^{ab}	1.67^{ab}
2015						
Control	15.8^{c}	10.55^{bc}	1.01^{c}	8.5^{c}	11.5^{b}	1.25^{c}
Ruter AA (1 L/ha)	16.4^{b}	11.42^{a}	1.13^{b}	9.6^{b}	12.8^{a}	1.43^{b}
Ruter AA (2 L/ha)	16.1^{c}	10.80^{b}	1.03^{c}	8.8^{c}	11.0^{c}	1.36^{c}
Terra Sorb (1 L/ha)	17.2^{a}	12.18^{a}	1.28^{a}	9.4^{a}	12.9^{a}	1.71^{a}
Terra Sorb (2 L/ha)	16.5^{b}	9.25^{c}	1.00^{c}	8.3^{c}	10.8^{c}	1.55^{b}
Razormin (200 mL/ha)	18.3^{a}	11.89^{a}	1.19^{a}	9.2^{a}	12.6^{a}	1.63^{a}
Razormin (500 mL/ha)	16.1^{c}	10.25^{c}	1.18^{c}	8.7^{c}	10.8^{c}	1.35^{c}

Plant growth and development measurements (the average per plant) were assessed at BBCH 33–35 stage in the vegetation renewal period.
Different letters designate statistically significant difference at $P < 0.05$.

conditions. Consequently, the tested regulators showed more or less significant stimulating effect on rape morphometrical parameters (Table 5).

The analysed plant parameters were significantly influenced by biostimulant (Wilk's lambda = 0.074, $F_{12,14} = 3.11$, $P < 0.05$) and its concentration (Wilk's lambda = 0.151, $F_{6,7} = 6.57$, $P < 0.05$). The influence varied between years but remained significant (Wilk's lambda = 0.004, $F_{12,14} = 17.93$, $P < 0.0001$). The strongest effect was established on the root column diameter ($r^2 = 0.97$, $F_{5,12} = 102.12$, $P < 0.0001$), dry weight and root column diameter (both $r^2 = 0.90$, $P < 0.0001$) and fresh weight ($r^2 = 0.87$, $P < 0.0001$). A moderate effect was obtained on the root column length ($r^2 = 0.66$, $P < 0.01$), and no effect was obtained on the leaf number ($r^2 = 0.21$, NS).

Effect of the tested products on the productivity formation of winter rape (Table 6) was estimated at the full maturity stage (BBCH 89–91). Seed yield was significantly influenced by year, biostimulant and its concentration ($F_{2,119} = 58.42$, 5.80 and 70.19, respectively, all $P < 0.005$, $r^2 = 0.65$; biostimulant × concentration $F_{2,105} = 4.41$, $P < 0.05$; year × biostimulant × concentration $F_{4,105} = 8.50$, $P < 0.0001$). Seed number per siliqua was influenced by year and biostimulant concentration ($F_{2,119} = 90.82$ and 47.97, respectively, both $P < 0.0001$, $r^2 = 0.29$; biostimulant × concentration $F_{2,608} = 6.69$, $P < 0.005$; year × biostimulant × concentration $F_{4,608} = 2.76$, $P < 0.05$) as well as 1,000-seed weight ($F_{2,119} = 43.89$ and 33.83, respectively, both $P < 0.0001$, $r^2 = 0.49$; biostimulant × concentration $F_{2,105} = 4.37$, $P < 0.02$).

Razormin (200 mL/ha), Terra Sorb (1 L/ha) and Ruter AA (1 L/ha) significantly stimulated the seed number per siliqua (11.2%, 8.4% and 7.7%, respectively), 1,000-seed weight (6.4%, 4.4% and 5.5%, respectively) and seed yield (7.5%, 11.0% and 8.8%, respectively).

Effect of biostimulants on autumnal growth, cold tolerance, wintering, vegetation renewal and formation of productivity elements of winter wheat in the field experiment

Under controlled laboratory conditions, winter wheat proved to be more resistant to cold stress compared to rapeseed. The study of the effect of the tested regulators that contain

Table 6. Effect of biostimulants on productivity formation of winter rape 'Hornet H' at full maturity, BBCH 89–91 stage

Test variant	Seed number per siliqua		1,000-seed weight		Seed yield/m^2	
	n	%	g	%	g	%
2013						
Control	28^{c}	100	6.9^{c}	100	315^{c}	100
Ruter AA (1 L/ha)	30^{a}	107	6.8^{c}	98	331^{b}	105
Ruter AA (2 L/ha)	29^{b}	104	6.8^{c}	98	320^{c}	102
Terra Sorb (1 L/ha)	29^{b}	104	7.0^{b}	101	347^{a}	110
Terra Sorb (2 L/ha)	28^{c}	100	6.9^{c}	100	309^{c}	98
Razormin (200 mL/ha)	30^{a}	107	6.9^{c}	100	351^{a}	111
Razormin (500 mL/ha)	29^{b}	104	7.0^{b}	101	332^{b}	105
2014						
Control	30^{c}	100	7.2^{c}	100	331^{c}	100
Ruter AA (1 L/ha)	33^{b}	110	7.9^{a}	110	368^{a}	111
Ruter AA (2 L/ha)	32^{c}	107	7.0^{c}	97	326^{c}	98
Terra Sorb (1 L/ha)	33^{b}	110	7.8^{b}	108	371^{a}	112
Terra Sorb (2 L/ha)	32^{c}	107	7.7^{b}	107	369^{a}	111
Razormin (200 mL/ha)	35^{a}	117	7.9^{a}	110	348^{b}	105
Razormin (500 mL/ha)	30^{c}	100	7.1^{c}	99	335^{bc}	101
2015						
Control	31^{c}	100	7.4^{c}	100	342^{c}	100
Ruter AA (1 L/ha)	34^{a}	110	7.9^{a}	107	375^{a}	110
Ruter AA (2 L/ha)	30^{c}	97	7.2^{c}	97	342^{c}	100
Terra Sorb (1 L/ha)	35^{a}	113	7.6^{b}	103	378^{a}	110
Terra Sorb (2 L/ha)	33^{b}	106	7.4^{c}	100	352^{c}	103
Razormin (200 mL/ha)	35^{a}	113	8.0^{a}	108	372^{a}	109
Razormin (500 mL/ha)	30^{c}	97	7.3^{c}	99	361^{b}	106

Different letters designate statistically significant difference at $P < 0.05$.

free amino acids on wheat growth and development in autumn 2012, 2013 and 2014 revealed the active products and their most active doses: Ruter AA (2 L/ha), Razormin (200 mL/ha) and Terra Sorb (2 L/ha). The tested regulators stimulated plant growth and induced formation of shoots. It was also noted that Ruter AA (2 L/ha), Razormin (200 mL/ha) and Terra Sorb (2 L/ha), especially in autumn 2013, increased the fresh weight and, of particular importance, dry weight of plants and stimulated plant growth (Table 7).

However, effect of the tested biostimulants (Wilk's lambda = 0.63, $F_{8,18}$ = 0.58) and their concentration (Wilk's lambda = 0.94, $F_{4,9}$ = 0.15) were not statistically significant, as between-year differences were found (Wilk's lambda = 0.003, $F_{4,9}$ = 828.4, $P < 0.0001$).

We found that year, biostimulant and concentration had significant effects on winter wheat fresh plant weight ($F_{5,12}$ = 13.96, $P < 0.001$). The model was weak and NS for winter wheat height, dry plant weight and the number of shoots.

A significantly higher number of overwintered plants were registered under the effect of Ruter AA (2 L/ha), Terra Sorb (2 L/ha) and Razormin (200 mL/ha) compared to other tested concentrations of the products and with the control (difference 10–20%; Figure 3). At that time in winter, especially in December and February 2013–2014 and in December, January and February 2014–2015, the temperature was a few degrees higher than the long-term average (Figure 1). Thus, meteorological conditions were satisfactory for wheat wintering. We did not find a positive significant effect on plant overwintering from applying Terra Sorb (1 L/ha) in any year of the experiment (Student's *t* test < 0.60, all NS), Ruter (1 L/ha) in 2012–2013 (*t* = 0.94) and 2014–2015 (*t* = 0.27) and Razormin (500 mL/ha) in 2013–2014 (*t* = 0.47) and 2014–2015 (*t* =1.17).

Table 7. Effect of biostimulants on growth and development of winter wheat 'Kovas' in autumn at BBCH 22–24 stage

Test variant	**Plant height**		**Number of shoots**		**Plant weight**			
					Fresh		**Dry**	
	cm	**%**	**n**	**%**	**g**	**%**	**g**	**%**
2012								
Control	18.6^{c}	100	4.5^{c}	100	4.0^{c}	100	1.55^{c}	100
Ruter AA (1 L/ha)	17.9^{c}	96	4.7^{c}	104	4.2^{b}	105	1.49^{c}	96
Ruter AA (2 L/ha)	19.5^{b}	105	4.9^{b}	109	4.5^{a}	112	1.61^{bc}	104
Terra Sorb (1 L/ha)	19.2^{c}	103	4.6^{c}	102	4.0^{c}	100	1.58^{c}	102
Terra Sorb (2 L/ha)	20.8^{a}	112	5.1^{a}	113	4.5^{a}	112	1.66^{b}	107
Razormin (200 mL/ha)	21.3^{a}	114	5.2^{a}	115	4.6^{a}	115	1.63^{b}	105
Razormin (500 mL/ha)	18.8^{c}	101	4.6^{c}	102	3.9^{c}	97	1.60^{c}	103
2013								
Control	16.8^{c}	100	4.0^{c}	100	2.3^{c}	100	1.40^{c}	100
Ruter AA (1 L/ha)	17.9^{b}	106	4.4^{bc}	110	2.3^{c}	100	1.40^{c}	100
Ruter AA (2 L/ha)	20.3^{a}	121	5.0^{a}	125	2.8^{ab}	122	1.70^{a}	121
Terra Sorb (1 L/ha)	18.5^{b}	110	4.4^{bc}	110	2.6^{b}	113	1.60^{b}	114
Terra Sorb (2 L/ha)	19.5^{a}	116	4.8^{b}	120	2.7^{ab}	117	1.60^{b}	114
Razormin (200 mL/ha)	19.4^{a}	115	4.9^{a}	122	3.2^{a}	139	1.70^{a}	121
Razormin (500 mL/ha)	17.2^{c}	102	4.3^{c}	107	2.9^{ab}	126	1.40^{c}	100
2014								
Control	17.3^{c}	100	4.8^{c}	100	3.0^{c}	100	1.60^{c}	100
Ruter AA (1 L/ha)	17.7^{c}	102	4.9^{c}	102	3.1^{c}	103	1.55^{c}	97
Ruter AA (2 L/ha)	20.3^{ab}	117	5.3^{b}	110	3.4^{ab}	113	1.70^{b}	106
Terra Sorb (1 L/ha)	20.0^{b}	116	5.0^{c}	104	3.2^{b}	107	1.60^{c}	100
Terra Sorb (2 L/ha)	21.9^{a}	127	5.8^{a}	121	3.6^{a}	120	1.80^{a}	112
Razormin (200 mL/ha)	22.4^{a}	129	5.4^{b}	112	3.6^{a}	120	1.78^{b}	111
Razormin (500 mL/ha)	18.7^{bc}	108	4.8^{c}	100	3.0^{c}	100	1.59^{c}	99

Plant growth and development measurements were assessed at BBCH 22–24 stage (20 days after treatment) with tested biostimulants.

Different letters designate statistically significant difference at $P < 0.05$.

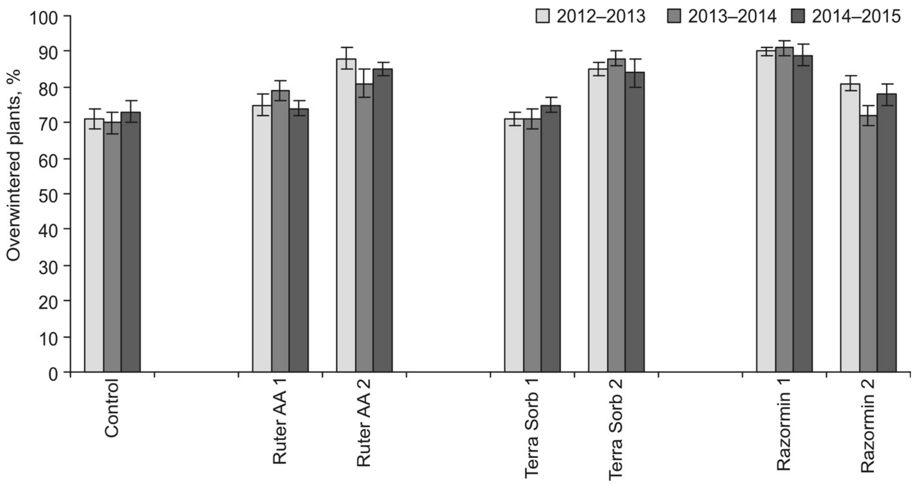

Figure 3. Effect of growth regulators on overwintering of winter wheat ‘Kovas’ plants, tested in 2012–2013, 2013–2014 and 2014–2015. Ruter AA 1 – 1 L/ha, 2 – 2 L/ha; Terra Sorb 1 – 1 L/ha, 2 – 2 L/ha; Razormin 1 – 200 mL/ha, 2 – 500 mL/ha. Each data represent the mean ± s.e. Differences in plant overwintering between control and biostimulants Terra Sorb (1 L/ha) in all years, Ruter (1 L/ha) in 2012–2013 and 2014–2015, and Razormin (500 mL/ha) in 2013–2014 and 2014–2015 are not significant (NS).

We found that at the full maturity stage, the number of productive wheat stems was influenced by year, biostimulant and concentration (r^2 = 0.28, $F_{5,12}$ = 2.32, P = 0.10) and the influence of these parameters on grain number per ear (r^2 = 0.50, F = 4.46, P < 0.02) and grain weight per plant (r^2 = 0.49, F = 4.30, P < 0.02) was significant.

Thus, evaluation of the effect of the tested regulators on the formation of productivity elements of wheat at full maturity stage revealed that Ruter AA (2 L/ha), Terra Sorb (2 L/ha) and Razormin (200 mL/ha) increased the number of productive stems and grain number per ear as well as grain weight per plant. The 1,000-seed weight differed among the test variants and was significantly the highest after treatment with Terra Sorb (2 L/ha). Grain yield also increased after the treatment with Terra Sorb (2 L/ha) (Table 8).

Discussion

Rapeseed and wheat are extremely important crops worldwide. However, cold acclimation and cultivation of winter rapeseed and, to a degree, winter wheat are problematic, especially if genotypes are introduced from countries with a warmer climate to regions of a temperate climate, such as the Baltic countries. High-quality cultivars produced in foreign countries cannot fully realise the genome-encoded productivity potential. Owing to these reasons, cultivars tolerant to stress were chosen for our experiment. Using plant growth regulators, in our case, biostimulants with amino acids, it is possible to regulate winter crop growth processes, freezing tolerance and productivity formation (Hayat *et al*., 2012; Jakienė, 2013).

The plant response to low-temperature stress can be divided into three distinct phases. Cold acclimation (pre-hardening) occurs at low, but above-zero, temperatures. The second stage, during which the full degree of tolerance is achieved, requires exposure to a period of sub-zero temperatures. The final phase is plant recovery after winter (Li *et al*., 2008). It is known that in cold acclimation and freezing tolerance processes of the wintering plants, such as rapeseed and wheat, free proline and glutamine play a significant role (Dörffling *et al*., 2009). For the first time, our experiments showed that biostimulants that contain free amino acids increased freezing tolerance of seedlings in controlled cold stress conditions. Ruter AA (1 mL/100 mL), Terra Sorb (1 mL/100 mL) and Razormin (0.1 mL/ha) significantly increased survival of rapeseeds to cold at -5°C by 44%, 37% and 28%, respectively, compared to the control sample. Resistance of wheat under the same cold conditions increased by 20%, 23% and 39%, accordingly, compared to the control sample.

In addition, winter wheat under controlled stress conditions at -7°C was more resistant to abiotic stress and survived better than winter rapeseed (the seedlings died). Under the identical conditions, the biostimulants Ruter AA (1 mL/100 mL), Razormin (0.1 mL/ha) and especially Terra Sorb (1 mL/100 mL) significantly increased wheat seedlings’ resistance to the cold. Terra Sorb has a bigger amount of free amino acids than Ruter AA and Razormin (Table 1). The literature data indicate that free amino acid proline can serve as a rapidly available source of nitrogen, carbon and reduction equivalents during recovery from cold stress (Hellmann *et al*., 2000). Proline leads to enhanced tolerance to cold stress and to a significant increase in growth and crop yield under environmental stresses (Ashraf and Foolad, 2007).

Table 8. Effect of biostimulants on productivity formation of winter wheat ‘Kovas’ at full maturity, BBCH 96–97 stage

Test variant	Number of productive stems	Number of grains per ear	Grain weight per plant (g)	1,000-grain weight (g)	Grain yield/m^{2} (g)
2013					
Control	4.16^{c}	36.0^{c}	4.32^{c}	58.3^{bc}	510^{c}
Ruter AA (1 L/ha)	4.55^{b}	35.9^{c}	4.57^{b}	59.1^{b}	515^{c}
Ruter AA (2 L/ha)	4.21^{c}	36.7^{c}	4.76^{ab}	60.5^{b}	562^{a}
Terra Sorb (1 L/ha)	4.78^{ab}	36.1^{c}	4.21^{c}	55.7^{c}	520^{c}
Terra Sorb (2 L/ha)	4.87^{a}	37.2^{a}	4.89^{a}	60.6^{b}	577^{a}
Razormin (500 mL/ha)	4.97^{a}	36.8^{b}	4.99^{a}	62.4^{a}	555^{b}
Ruter AA (2 L/ha)	4.24^{c}	36.2^{c}	4.38^{c}	59.7	512^{c}
2014					
Control	4.14^{c}	37.4^{c}	4.92^{c}	57.6^{c}	511^{c}
Ruter AA (1 L/ha)	4.44^{c}	38.3^{c}	4.95^{c}	56.5^{c}	522^{c}
Ruter AA (2 L/ha)	5.17^{a}	40.2^{ab}	4.99^{c}	58.2^{bc}	572^{ab}
Terra Sorb (1 L/ha)	4.55^{c}	35.7^{c}	4.90^{c}	57.2^{c}	538^{bc}
Terra Sorb (2 L/ha)	5.33^{a}	41.0^{a}	5.12^{b}	61.2^{a}	618^{a}
Razormin (200 mL/ha)	5.20^{a}	40.3^{ab}	5.14^{b}	59.3^{b}	605^{a}
Razormin (500 mL/ha)	5.17^{ab}	39.2^{ab}	5.12^{b}	58.2^{bc}	590^{a}
2015					
Control	4.48^{c}	33.4^{c}	4.66^{c}	60.0^{c}	521^{c}
Ruter AA (1 L/ha)	4.01^{c}	33.3^{c}	4.77^{c}	59.9^{c}	530^{c}
Ruter AA (2 L/ha)	4.96^{b}	34.6^{bc}	4.82^{c}	60.3^{c}	578^{b}
Terra Sorb (1 L/ha)	5.20^{ab}	36.4^{ab}	5.22^{b}	61.2^{b}	581^{b}
Terra Sorb (2 L/ha)	5.48^{a}	37.2^{a}	5.45^{a}	64.5^{a}	600^{a}
Razormin (500 mL/ha)	5.37^{a}	37.1^{a}	5.28^{a}	63.8^{a}	628^{a}
Ruter AA (2 L/ha)	4.99^{b}	35.6^{b}	5.08^{b}	60.7^{c}	541^{c}

Different letters designate statistically significant difference at $P < 0.05$.

Overwintering and productivity formation in winter crops, especially rapeseeds, are predetermined by several factors, such as plant acclimation to the cold (preparation for wintering) and meteorological conditions during the vegetation period, especially during autumn (Velička *et al.*, 2006; Balodis and Gaile, 2015).

Meteorological conditions, especially air temperature in autumn 2012–2014, did not differ much from the long-term average, though the precipitation rates in October and November 2012 and 2013 were higher than the long-term average. In 2014, the precipitation rate was low (Figure 1). Thus, meteorological conditions were more or less favourable for rapeseed autumnal growth. The effect of the examined biostimulants, especially Razormin (200 mL/ha) and Ruter AA (1 L/ha), on rape morphometric parameters, i.e. leaf number, root column length and diameter and fresh and dry weights, showed that plants were ready for wintering.

Meteorological conditions of the growing season in Lithuania fully coincide with optimum conditions for winter wheat growth and development, and if plants produce three to four shoots before winter and accumulate a sufficient amount of nutritious material, they survive the winter (Šiuliauskas *et al.*, 2000; Velička *et al.*, 2006). Maybe in this connection, the tested biostimulants with amino acids increased more the productivity of wheat than of winter rapeseed.

Some growth regulators may be able to optimise the physiological and morphological processes of plants and parameters related to winter crop growth and productivity formation (Merkys *et al.*, 2007). We did not find published experimental data about the influence of regulators with amino acids on cold resistance and productivity of wheat, just that Terra Sorb Foliar solutions of 2 L/ha promoted reliably higher spring rapeseed yield (Jakienė, 2013). However, our experiments showed that Terra Sorb of 1 L/ha more intensively activated overwintering and productivity element formation of winter rapeseeds than the 2 L/ha dose. Razormin is considered as a biostimulant based on the investigated higher yield and quality of dill (Radzevičius *et al.*, 2013.). On the other hand, there are many investigations about the effects of microelements and macroelements on plant growth and productivity of wheat and rape (Rengel and Graham, 1995; Mousavi *et al.*, 2012). However, our attention was attracted by free amino acids.

Generalising, our results show positive and significant effects of biostimulants on all analysed parameters of winter rapeseed and winter wheat, despite variation between years and dependence on the applied concentrations. We conclude that biostimulants that contain free amino acids, microelements and macroelements applied to winter rapeseed cv. 'Hornet H' at BBCH 13–14 stage and to wheat cv. 'Skagen' at BBCH 14–15 stage under controlled cold stress conditions significantly increased the freezing tolerance of seedlings. At -5°C, Ruter AA (1 mL/100 mL), Terra Sorb (1 mL/100 mL) and Razormin (0.1 mL/100 mL) had a stronger positive effect on the freezing resistance of rapeseed seedlings compared to that of wheat. At a temperature of -7°C, which is mortal for rape seedlings, resistance of wheat seedlings increased compared to the control sample.

Under natural field conditions, the tested biostimulants produced significant stimulating effects on winter rapeseed cv. 'Hornet H' and wheat cv. 'Kovas' growth in autumn, such as acclimation to the cold, overwintering, vegetation renewal in spring and formation of productivity elements. Winter rapeseed responded better to the effect of the presented doses of Razormin (200 mL/ha), Terra Sorb (1 L/ha) and Ruter AA (1 L/ha) on the above-mentioned parameters than winter wheat. However, Razormin (200 mL/ha), Terra Sorb (2 L/ha) and Ruter AA (2 L/ha) markedly increased (by 11%, 17% and 16%, respectively) winter wheat productivity compared to the control sample and winter rapeseed productivity. Thus, winter wheat is more resistant to the cold than winter rapeseed. This effect was confirmed under controlled cold conditions at the laboratory and in natural field experiments.

In our opinion, the effect of tested regulators on the final result of rapeseed growth and development – plant productivity – may be ascertained as the regulator survival effect on plant growth, development, preparation of acclimation for winter cold, wintering and vegetation renewal in spring. Besides, the obtained data assert that Razormin (200 mL/ha), Terra Sorb (1 L/ha) and Ruter AA (1 L/ha) in the exhibited doses are active regulators for the formation of productivity elements of rapeseeds.

In conclusion, the presented data for the first time showed that regulators that contain free amino acids increased the freezing tolerance of seedlings under cold stress conditions.

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