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The Effect of Applied Biostimulants on the Yielding of Three Non-Genetically Modified Soybean Cultivars

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Abstract: Background: Soybean is one of major crop plants cultivated in numerous parts of the world, which is due to an increasing demand for plant protein. Both in Europe and Poland, much attention is paid to enhancing the production of their own fodder protein, as to reduce the import of soybean meal produced from genetically modified plants. Climate warming and breeding progress have made it possible to grow soybeans in central Europe. The yield potential of plants, including soybeans, can be enhanced by an application of biostimulants, which alleviate negative effects of stresses disturbing the life processes of plants. The objective of the present work was to evaluate, under the climatic conditions of central-eastern Poland, the yielding of three non-modified soybean cultivars treated with biostimulants. Methods: A field experiment was conducted in the years 2017–2019 in eastern Poland (central Europe). The soil of the experimental field belonged to the Haplic Luvisol group. The experimental factors included three non-GMO soybean cultivars (Abelina, Merlin, and SG Anser) and two biostimulants (Asahi SL and Improver). Results: Soybean seed yields were affected by the climatic conditions during the growing season, cultivars, and biostimulant applications. Regardless of cultivars and biostimulants, the highest yields were produced by plants grown in 2017 (on average, 3.41 Mg·ha^{-1}), them being slightly lower in 2019 (on average, 3.0 Mg·ha^{-1}) and the lowest in the dry 2018 (on average, 2.48 Mg·ha^{-1}). Significant differences were recorded between cv. SG Anser (the average yield 2.73 Mg·ha^{-1}) and Merlin (the average yield 3.31 Mg·ha^{-1}). An application of biostimulants resulted in a significant increase in soybean seed yield compared with the control. Biostimulants contributed to a significant increase in the values of the remaining characteristics, i.e., 1000-seed weight, seed number per pod, and average number of seeds per pod.

Keywords: biostimulants; *Glycine max* (L.) *Merr*.; soybean seed yield; yield-forming characteristics

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1. Introduction

Soybean is believed to be one of the most valuable leguminous plants [1–3]. It is due to the crop being widely used for consumption (mainly oil), feed (soybean meal), processing (e.g., cosmetics, plastic materials, paints), and pharmaceutical purposes [4,5]). Moreover, soybean exerts a positive impact on the physical and chemical properties of soil as it improves soil structure and water–air balance [6,7]. Its developed root system allows the plant to take up nutrients from deeper soil strata. The ability to fix atmospheric nitrogen through symbiosis with nodulating bacteria allows soybean plants to introduce a substantial amount of nitrogen into the soil, which contributes to an increase in the yield of the following plants while reducing fertiliser expenses [8,9]. Furthermore, nitrogen fixed by bacteria is much less slowly leached from the soil than mineral nitrogen, which results in a more stable soil pool of this element.

Similarly to other EU countries, attempts are made in Poland to increase feed protein production, as to limit the import of soybean meal obtained from genetically modified plants. Soybean is the plant that seems to respond to this strategy. Thus far, soybean cultivation has been popular in neither the EU nor Poland. It has been due to the crop's substantial thermal and soil requirements and response to the length of day as the agro-climatic conditions in Europe are not ideal for widespread soybean cultivation [10]. At present, biological progress and climate change make it possible to grow this crop plant under European conditions; however, to obtain high stable yields under these conditions, it is necessary to choose drought-resistant varieties, which require a short growing season and have a high yielding potential [11].

In addition to cultivar and meteorological conditions, appropriate agrotechnology seems to be the major factor conditioning soybean yielding [12–16]. However, conventional cultivation practices are often not enough to achieve high good-quality yields due to numerous biotic and abiotic stresses which pose a risk to plants. Hence, to increase the yielding potential of crop plants grown in adverse climatic or otherwise unfavourable conditions, modern farming tends to rely on an application of biostimulants [17–19], which are defined as products supporting plant physiological processes while promoting their growth and development under optimal or suboptimal conditions [20]. An application of biostimulants may affect plant metabolism and enhance biochemical, morphological, and physiological processes taking place in plants [21,22]. Biostimulants improve plant growth and development (by stimulating root, leaf, and flower development), as well as nutrient uptake and assimilation, increase tolerance to environmental conditions (e.g., drought, low or high temperature, salinity), and reduce heavy metal toxicity. Additionally, they induce natural immunity mechanisms in the plant and thus directly limit pest development, e.g., they strengthen plant cell wall by creating a natural barrier against pests and pathogens. Van Oosten et al. [23] claimed that biostimulants increased yields by soil conditioning and improving their ability to retain water.

Biostimulants can contain organic and inorganic compounds; they can be synthetic or natural [18]. Most frequently, natural biostimulants contain humic substances or amino acids. Synthetic biostimulants contain growth regulators, phenolic compounds, inorganic salts, and nutrients (e.g., aluminium, cobalt, sodium, selenium, sulphur) [24–26].

An application of biostimulants is desirable for crops that are extra-sensitive to adverse climatic conditions. This group includes leguminous plants such as soybean, which is sensitive to both low and high temperatures as well as heavy rainfall [27]. Low temperatures at germination hinder this process and contribute to plants being attacked by soilborne pathogens, which results in the development of weak shoots or even lack of shoots [28]. In turn, high temperature events, even of short durations, negatively affect plant yielding [29]. At flowering, they may unfavourably influence seed number per plant [30]. Moreover, very high temperatures prevailing from flowering and pod formation to the grain fill stage result in a decline in seed weight, which leads to yield drop [31].

As biostimulants have become part of new cultivation technologies of various plant species, it was attempted to investigate an application of biostimulants in the cultivation of three soybean cultivars. Such research is fully justified in the face of increasing popularity of this crop plant and changing climate, which is conducive to an occurrence of plant stressors.

The objective of the study reported here was to determine the yield response of three soybean cultivars grown under the central European conditions as affected by the applied biostimulants.

The research hypothesis assumed that the biostimulants would significantly enhance the yield-forming potential of cultivars by increasing the total yield and yield-related characteristics.

2. Materials and Methods

2.1. Description of the Experiment

A field trial was carried out at Łączka, Eastern Poland (52°15′ N, 21°95′ E) from 2017 to 2019. Soybean cultivars (Abelina, SG Anser, Merlin) were the first experimental factor, and types of biostimulants (no biostimulant, Asahi, Improver) were the second factor. Asahi contains sodium p-nitrophenolate, sodium o-nitrophenolate, and sodium 5-nitroguaiacolate, whereas Improver contains potassium p-nitrophenolate, potassium o-nitrophenolate, and potassium 5-nitroguaiacolate. Both the biostimulants contain components that activate metabolic processes, support natural life processes of plants, and enhance plant resistances to stressors. The soybean sowing material was purchased from Saatbau, the company that is the distributor of soybean seeds for Poland and a holder of a certificate confirming that the sowing material does not contain GMO impurities. The seeds were ready for sowing because they had been coated with the nodulating bacteria (*Bradyrhizobium japonicum*) and a binder, which was also a conserving and protecting agent, during the technological process. The experiment was set up on soil which, according to the World Reference Base for Soil Resources [32], was classified as a representative of the Haplic Luvisol group. The soil had average organic carbon, total nitrogen, and phosphorus contents, a high potassium content, and a low content of plant-available magnesium forms (Table 1).

Table 1. Selected soil properties in the layer 0–0.25 m prior to the commencement of the experiment in 2017–2019.

Soil Properties	Year		
	2017	2018	2019
pH (in KCl)	6.9	7.1	7.2
C_{org} (gkg^{-1})	9.0	8.9	9.3
N_t (gkg^{-1})	0.75	0.77	0.81
Fe_t (gkg^{-1})	995	990	997
B_t (gkg^{-1})	0.70	0.68	0.74
P_{av} ($mgkg^{-1}$)	55.8	57.1	56.2
K_{av} ($mgkg^{-1}$)	132.8	130.3	131.6
Mg_{av} ($mgkg^{-1}$)	26.5	25.9	26.4

Maize was the crop preceding soybean. In each year, the following fertilisers were applied, taking into account the soil availability of each nutrient: nitrogen at a rate that corresponded to 30 kg N introduced into the soil, 30 kg P and 90 kg K per 1 ha.

Seeds were planted in plots whose area was 9 m^2, at a row spacing of 22 cm and a depth of around 4 cm, the number of seeds being 70 per 1 m^2. The sowing dates were 4 May 2017, 5 May 2018, and 1 May 2019. Plots were maintained to be weed-free using the soil herbicide Stomp Aqua 455 CS, which was applied up to 5 days post-sowing at a rate of 1.5 l per 1 ha, and Focus Ultra 100 EC, applied during vegetation at a rate of 2 l per 1 ha. The biostimulants were sprayed following the methodology regime after the development of the trifoliolate leaf on the third node at the BBCH 13–15 stage and at the beginning of flowering (BBCH 61) at the rate 0.6 dm^3ha^{-1} (Asahi SL) and 1.0 dm^3ha^{-1} (Improver).

Plant number per 1 m^2 was determined at full emergence (BBCH 10), and (BBCH 97) 20 plants were selected from each plot prior to harvest to obtain biometric parameters and determine yield structure elements at maturity. The following morphological characteristics were measured: plant height, first pod height, pod length, pod number per plant, seed number per pod, and 1000-seed weight. Whole plants were harvested at the stage of full maturity (BBCH 99).

2.2. Weather Conditions

Growing season precipitation and thermal conditions during the study period were changeable (Table 2).

The warmest and the coldest growing seasons were in, respectively, 2018 and 2017, the respective average temperatures across April–September being 17.0 and 14.7 °C. The highest precipitation sum was recorded for the growing season in 2017 (425 mm), it being the lowest in 2019 (306 mm). In 2017 and 2018, August was the warmest month of the soybean growing season, June being such a month in 2019. The highest precipitation sum was recorded for September in 2017, July in 2018, and May in 2019. Analysis of insolation, i.e., the time during which direct radiation reached the earth surface, demonstrated that 2017 received the lowest insolation sum (1343 h) during the growing season compared either with 2018 (1728 h) or 2019 (1495 h). The highest monthly values of this parameter were recorded in May, June, and August (respectively, 347, 303, and 306 h), it being the lowest in September 2017 (119 h).

Table 2. Air temperature atmospheric precipitation and insolation values in 2017–2019.

Month	Precipitation			Temperature			Insolation		
	2017	2018	2019	2017	2018	2019	2017	2018	2019
April	82	52	9	7.1	12.5	9.4	174	284	235
May	46	26	114	13.1	16.4	13.0	266	347	295
June	56	75	29	17.6	18.3	21.5	277	303	286
July	76	96	40	17.6	19.7	18.0	246	254	251
August	53	29	72	19.0	19.9	19.3	259	306	268
September	112	42	42	13.9	15.2	14.0	119	235	160
Sum/Mean (April–Sept.)	425	320	306	14.7	17.0	15.9	1343	1728	1495

2.3. Research Material and Statistical Analyses

In the present work, it was attempted to analyse yielding as well as selected characteristics associated with yield, i.e., 1000-seed weight (TSW), pod number per plant, seed number per pod, seed number per plant, pod length, pod height, plant number per 1 m^2, and plant height of three soybean cultivars as affected by the test biostimulants. Prior to harvest, random samples were collected from each plot (20 plants per each plot) to determine pod number per plant, seed number per pod, and seed number per plant. Pod height was measured from ground level to the point where the first pod was attached at the lowest node. After the harvest, the yield obtained from each experimental plot (9 m^2) was converted into Mg per ha. A 1000-seed weight was determined at the seed moisture of 15%.

It was a two-factor split-plot arrangement with three replicates. Soybean cultivars were the first experimental factor (factor A): A1—Abelina; A2—SG Answer; and A3—Merlin. Types of biostimulants were the second factor (factor B): B1—Control (no biostimulant); B2—Asahi SL; and B3—Improver.

The results were analysed statistically using a two-way variance analysis following the split-plot model:

$$y_{ijl} = m + a_i + g_j + e_{ij}^1 + b_l + ab_{il} + e_{ijl}^2,$$

where:

y_{ijl}—value of the variable,
m—population mean,
a_i—effect of the i-th level of factor A i = 1,2, ... , a = 3,
g_j—effect of the j-th replicate, j = 1,2, ... ,n; n = 3,
e_{ij}^1—error 1 (due to an interaction between factor A and replicates),
b_l—effect of the l-th level of factor B l = 1,2, ... , b; b = 3,
e_{ijl}^2—random effect.

Means were compared by means of the Tukey's test at the significance level of $\alpha = 0.05$. All the calculations were performed using the software Statistica 13.3 (Analytic tools–Experiment Design).

3. Results

Soybean yield was affected by conditions throughout the growing season, biostimulants, and cultivars. Moreover, the following interactions were found to be significant: years × cultivars, years × biostimulants, cultivars × biostimulants, and years × cultivars × biostimulants. Regardless of cultivar and biostimulant, average soybean yields were higher in 2017 (3.41 $Mgha^{-1}$) than in the remaining years, which meant the growing conditions in this year were the most conducive to the growth and development of this legume. Regardless of the remaining experimental factors, higher yields were produced by cv. Merlin rather than Abelina or SG Anser. Such a pattern was predominantly the result of the growing season in 2019 when cv. Merlin produced superior yields (3.88 Mgha^{-1}) and the growing seasons of 2017 and 2018 when the cultivar's yields were also high, although they were not significantly different from the yields of the remaining cultivars (Table 3).

Table 3. Soybean yield performance according to years, cultivars, and biostimulants (Mgha^{-1}).

	Year			Mean
Cultivar	2017	2018	2019	
Abelina	3.45 A ± 0.23	2.37 A ± 0.09	2.78 AB ± 0.18	2.87 B ± 0.14
SG Anser	3.36 A ± 0.17	2.40 A ± 0.11	2.42 B ± 0.19	2.73 B ± 0.18
Merlin	3.40 A ± 0.18	2.65 A ± 0.15	3.88 A ± 0.23	3.31 A ± 0.14
Mean	3.41 a ± 0.22	2.48 b ± 0.15	3.0 a ± 0.21	
Biostimulant	2017	2018	2019	
Control	2.71 C ± 0.13	2.12 C ± 0.24	2.68 B ± 0.20	2.50 C ± 0.11
Asahi SL	3.61 B ± 0.18	2.46 B ± 0.21	3.45 A ± 0.23	3.17 B ± 0.15
Improver	3.90 A ± 0.18	2.84 A ± 0.22	32.94 A ± 0.16	3.23 A ± 0.18
		Biostimulant		
Cultivar	Control	Asahi SL	Improver	
Abelina	2.36 B ± 0.13	3.08 AB ± 0.21	3.16 B ± 0.18	
SG Anser	2.43 B ± 0.21	2.94 B ± 0.22	2.81 C ± 0.20	
Merlin	2.72 A ± 0.20	3.50 A ± 0.19	3.71 A ± 0.16	

Means in rows followed by different letters in lowercase: a and b (for years) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A, B, and C (for cultivars, biostimulants, and the interaction: years × cultivars, years × biostimulants, and cultivars × biostimulants) differ significantly at the $p \leq 0.05$; ±Se (standard error) value.

An application of the biostimulant Improver was followed by a significant increase in soybean yield of over 29% (regardless of cultivar) compared with the control, whereas Asahi SL contributed to a 0.7 Mgha^{-1} yield increase. Moreover, the yield of soybean treated with Improver rose by over 2% compared with Asahi SL (Table 3). The effect of biostimulants on soybean yield performance was affected by growing seasons. Considerable differences between the biostimulants were observed in the first and second study year, when an increase in the yield was recorded following an application of Improver compared with Asahi SL. In 2019, the differences between the test biostimulants were not confirmed (Table 3). An analysis of the three-year results demonstrated a significant interaction between the cultivars and biostimulants, which meant that the test cultivars responded in a different manner to the applied biostimulants. Cv. Abelina and SG Anser produced higher yields compared with control plants, although no differences were confirmed between these cultivars following treatment with Asahi SL or Improver. Unlike these two cultivars, cv. Merlin responded to an application of Asahi SL and Improver, the latter product contributing to a 6% yield increase compared with crops harvested from plots treated with Asahi SL (Table 3). However, the effect of biostimulants on the yield performance of cultivars was much more complicated when their response was considered in the light of growing conditions, as indicated by the interaction years × cultivars × biostimulants (Figure 1). In the wet year 2017, SG Anser was the only cultivar that remained unresponsive to an application of biostimulants. Cv. Abelina and Merlin produced better yields when treated with biostimulants compared with control yields, the yield-forming effect of the test biostimulants being similar. A similar response was observed for cv. Merlin in the dry 2017 and for cv. Abelina in 2019. Under the growing conditions of 2018 and 2019, there were no significant yield changes due to spraying with Asahi SL for cv. Abelina and SG Anser compared with the control, whereas this was true for the year 2019 for only cv. Merlin.

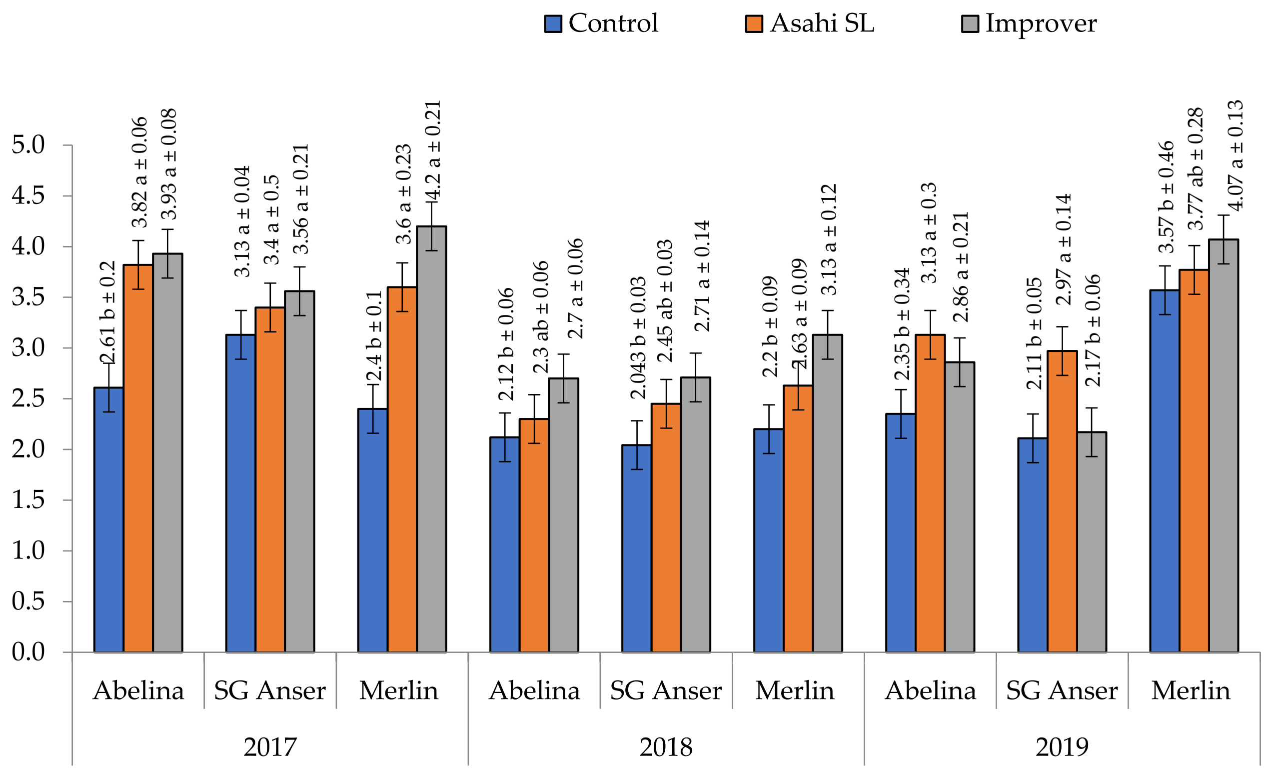

Figure 1. Soybean yield performance according to cultivars and biostimulants in 2017–2019. Means followed by different letters in lowercase: a and b differ significantly at the $p \leq 0.05$; ±Se (standard error) value.

The factors that significantly influenced the 1000-seed weight included study years, cultivars, and biostimulants. Additionally, the following interactions were significant: years × cultivars, years × biostimulants, and cultivars × biostimulants. Soybean seeds harvested in 2017 had a higher TSW (172.78 gha^{-1}) compared with the remaining study years. Regardless of the remaining experimental factors, a higher TSW was determined for cv. Merlin compared with cv. Abelina or SG Anser (respectively, 169.85, 148.85, and 155.07 gha^{-1}). The TSW of the test cultivars was affected by the meteorological conditions in the study years, as indicated by a significant interaction between these factors. In two growing seasons (2017 and 2019), a lower 1000-seed weight was recorded for cv. Abelina compared with the remaining two test cultivars. By contrast, no such differences between the test cultivars were found in 2018.

The best effect of biostimulants on 1000-seed weight was confirmed for the Improver, as the TSW values for soybean treated with this product were much higher compared with Asahi SL and by over 20 g higher compared with control. Merlin was the only cultivar which produced seeds with a higher 1000-seed weight following an application of either Asahi SL or Improver. The 1000-seed weights of cv. SG Anser and Abelina were similar (Table 4).

An interaction of cultivars and biostimulants in terms of 1000-seed weight was much more complex when viewed through the prism of growing seasons as confirmed by the significance of the interaction years × cultivars × biostimulants (Figure 2). The impact of biostimulants on TSW was determined in 2017 only. The seeds of cv. Abelina had a lower 1000-seed weight in the control unit compared with Asahi- and Improver-treated plots, there being no significant differences confirmed for cv. Merlin. Improver contributed to an increase in TSW compared with the control or Asahi SL. In the remaining study years, the 1000-seed weight of the test cultivars was similar, regardless of the biostimulant applied.

Table 4. 1000-seed weight according to years, cultivars and biostimulants (gha^{-1}).

Cultivar	Year 2017	2018	2019	Mean
Abelina	152.89 B ± 6.45	140.89 A ± 1.86	152.78 B ± 3.91	148.85 B ± 3.34
SG Anser	181.11 A ± 6.29	136.56 A ± 6.45	147.56 A ± 5.28	155.07 B ± 7.40
Merlin	184.33 A ± 3.89	147.00 A ± 4.57	178.22 A ± 6.32	169.85 A ± 5.6
Mean	172.78 a ± 6.82	141.48 c ± 9.51	159.52 b ± 8.07	
Biostimulant	**2017**	**2018**	**2019**	
Control	148.22 C ± 4.16	133.11 B ± 3.2	153.89 A ± 4.56	145.07 C
Asahi SL	176.11 B ± 5.23	139.78 AB ± 8.01	162.33 A ± 6.8	159.41 B
Improver	194.00 A ± 8.61	151.56 A ± 6.56	162.33 A ± 4.12	169.30 A
		Biostimulant		
Cultivar	**Control**	**Asahi SL**	**Improver**	
Abelina	137.89 A ± 5.7	152.44 B ± 4.51	156.22 B ± 4.56	
SG Anser	146.00 A ± 4.12	158.78 AB ± 6.28	160.44 B ± 6.21	
Merlin	151.33 A ± 7.21	167.00 A ± 8.21	191.22 A ± 4.28	

Means in rows followed by different letters in lowercase: a, b and c (for years) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A, B, and C (for cultivars, biostimulants, and the interaction: years × cultivars, years × biostimulants, and cultivars × biostimulants) differ significantly $p \leq 0.05$; ±Se (standard error) value.

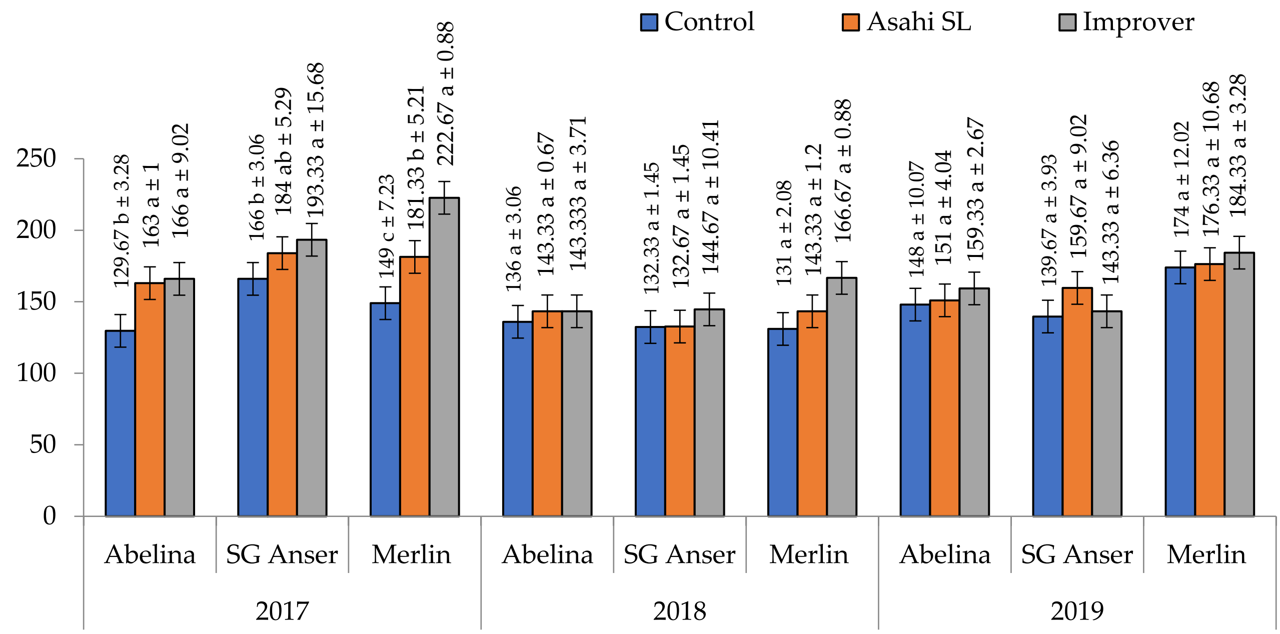

Figure 2. 1000-seed yield according to cultivars and biostimulants in 2017–2019. Means followed by different letters in lowercase: a, b, and c differ significantly at $p \leq 0.05$; ±Se (standard error) value.

Seed number per pod was affected by cultivar-related characteristics, weather conditions, and test biostimulants. Particularly low numbers of seeds per pod were formed by soybean plants in the warm and dry 2018 (1.86 seeds). Superior seed numbers were determined in 2019 (2.07 seeds). The weather conditions that were the most favourable for seed formation prevailed in 2017 (2.17 seeds). In the experiment reported here, the cultivar's ability to form seeds was genetically conditioned, cv. Merlin being much better in this respect than cv. Abelina. However, this genetic potential of the test cultivars was modified by the conditions of the growing season, as indicated by the significance of the interaction years × cultivars. During the growing season of 2017 and 2018, cv. Merlin formed more seeds, on average, compared with Abelina or SG Anser, the number of seeds developed by Merlin in 2019 being similar to the remaining test cultivars (Table 5).

Table 5. Seed number per pod according to years, cultivars, and biostimulants.

Cultivar	Year			Mean
	2017	2018	2019	
Abelina	2.10 B ± 0.096	1.76 B ± 0.056	2.11 A ± 0.038	1.99 B ± 0.060
SG Anser	2.17 AB ± 0.064	1.82 B ± 0.059	2.07 A ± 0.076	2.02 AB ± 0.052
Merlin	2.24 A ± 0.038	1.99 A ± 0.041	2.02 A ± 0.032	2.08 A ± 0.040
Mean	2.17 a ± 0.069	1.86 c ± 0.075	2.07 b ± 0.038	
Biostimulant				
Control	1.98 B ± 0.059	1.77 B ± 0.053	2.03 A ± 0.037	1.93 C ± 0.044
Asahi SL	2.17 AB ± 0.040	1.87 B ± 0.075	2.04 A ± 0.031	2.03 B ± 0.040
Improver	2.36 A ± 0.065	1.93 A ± 0.082	2.12 A ± 0.040	2.14 A ± 0.042

Means in rows followed by different letters in lowercase: a, b and c (for years) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A, B, and C (for cultivars, biostimulants, and the interaction: years × cultivars, years × biostimulants) differ significantly $p \leq 0.05$; ±Se (standard error) value.

Regardless of the remaining experimental factors, the greatest average seed number per pod was recorded for the treatment with Improver, it being significantly lower for Asahi SL and the lowest for control. The influence of the biostimulants on the seed number per pod changed in the growing seasons, as indicated by the significant interaction years × biostimulants. In the wet 2017 and dry 2018, the Improver-treated soybean formed more seeds per pod, which indicated that the biostimulant alleviated the negative environment-related influences. The conditions in 2019 did not affect seed number per pod.

Unlike the interaction between cultivars and biostimulants, which was insignificant (Table 5), the interaction of cultivars × biostimulants × years was significant for seed number per pod. The number increased for soybean cv. Abelina and SG Anser treated with Improver in 2017 and for soybean cv. SG Anser and Merlin in 2018. An application of the biostimulant Asahi SL positively affected seed number per pod formed by cv. Abelina in 2017 and by cv. SG Anser and Merlin in 2018. There were no significant differences between seed numbers per pod between the biostimulant-treated soybean plants and the control (Figure 3).

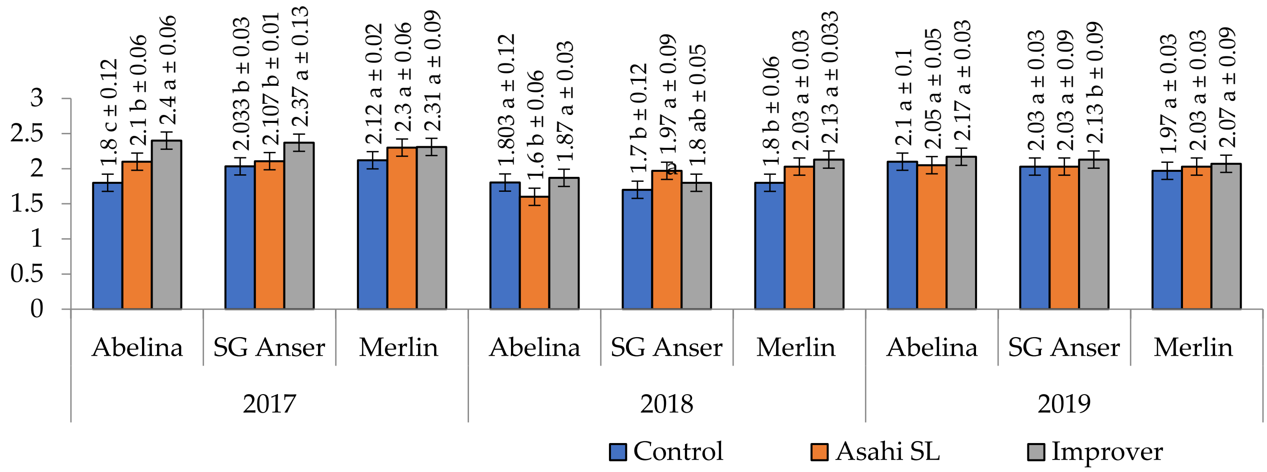

Figure 3. Seed number per pod according to biostimulants and cultivars in 2017–2019. Means followed by different letters in lowercase: a, b, and c differ significantly at $p \leq 0.05$; ±Se (standard error) value.

The factors that significantly affected pod number per plant included cultivars, biostimulants, and years. Additionally, significant interactions were confirmed, namely: years × cultivars, cultivars × biostimulants, and years × cultivars × biostimulants. Regardless of cultivar, soybean plants produced fewer pods in 2018 and 2019 (respectively, 20.96 and 23.21 pods) than in 2017 (25.39 pods). The cultivar factor significantly affected the number of pods formed by a soybean plant. In the experiment reported here, regardless of the remaining factors, the average pod number per plant was higher for cv. Merlin versus cv. Abelina or SG Anser.

Meteorological conditions influenced pod formation by plants, as indicated by the significant interaction between years and cultivars. Only in 2017 were there differences between the pod number per plant for the test cultivars. On average, cv. Merlin formed more pods compared with the remaining cultivars investigated in the present work (Table 6).

Table 6. Pod number per plant according to years, cultivars, and biostimulants.

Cultivar	Year			Mean
	2017	2018	2019	
Abelina	23.17 B ± 0.98	20.07 A ± 0.96	21.29 A ± 0.71	21.51 B ± 0.87
SG Anser	21.57 B ± 1.21	20.02 A ± 1.11	25.61 A ± 0.88	22.40 B ± 1.12
Merlin	31.43 A ± 0.70	22.78 A ± 1.32	22.73 A ± 1.12	25.64 A ± 1.21
Mean	25.39 a ± 2.32	20.96 b ± 0.98	23.21 ab ± 1.06	
Cultivar	Biostimulant			
	Control	Asahi SL	Improver	
Abelina	19.79 A ± 1.13	20.97 B ± 0.94	23.77 B ± 1.2	
SG Anser	20.76 A ± 1.26	24.41 AB ± 1.41	22.03 B ± 1.45	
Merlin	19.42 A ± 1.28	28.19 A ± 1.26	29.31 A ± 1.11	
Mean	19.99 b ± 1.08	24.52 a ± 1.39	25.04 a ± 1.32	

Means in rows followed by different letters in lowercase: a and b (for years and biostimulants) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A and B (for cultivars, biostimulants, and the interaction: years × cultivars, years × biostimulants) differ significantly at $p \leq 0.05$; ±Se (standard error) value.

Regardless of the remaining factors, the test biostimulants contributed to an increase in the pod number developed by one plant, although no significant differences were confirmed between the Asahi SL and Improver. A significant interaction between the cultivars and biostimulants indicated that the cultivars responded in a different manner to the biostimulants applied in the study. In the control unit, there were insignificant differences between pod numbers developed by the test cultivars. An application of Asahi SL was followed by an increase in the pod number per plant for cv. Merlin compared with Abelina. A similar response was observed for the Improver, which contributed to a higher number of pods per plant in cv. Merlin compared with the remaining two cultivars (Table 6). The interaction between cultivars and biostimulants was more complex, as the pod number per plant was also affected by weather conditions. Throughout the whole study period, cv. Abelina formed a similar number of pods per plant regardless of the biostimulant applied. Cv. SG Anser responded positively to Asahi SL in 2017 and Improver in 2018, when it produced more pods per plant compared with the control plants, the same response being confirmed for cv. Merlin treated with either Asahi SL or Improver applied in the first and final study years (Figure 4).

Pod length was influenced by conditions throughout the growing season and cultivar. Moreover, it was found that if the characteristic was dependent upon cultivar, and it was also affected by weather conditions in the study years. Regardless of the remaining factors, pods produced in 2017 were, on average, shorter (4.15 cm) compared with the remaining study years, and cv. Merlin formed shorter pods (3.68 cm) in comparison with the remaining cultivars (Table 7).

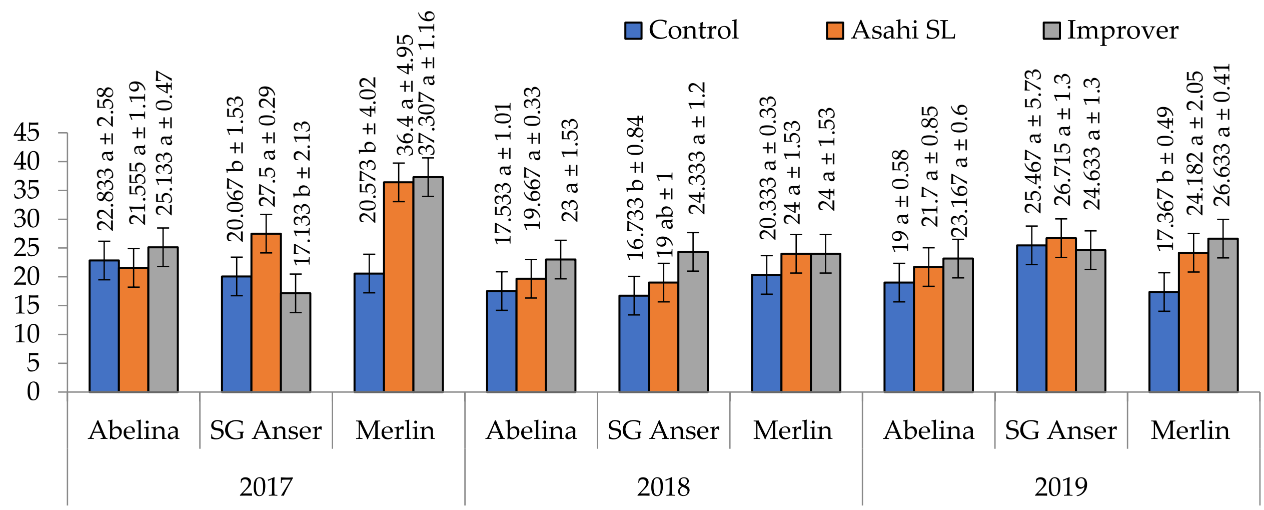

Figure 4. Pod number per plant according to biostimulants and cultivars in 2017–2019. Means followed by different letters in lowercase: a and b differ significantly at $p \leq 0.05$; ±Se (standard error) value.

Table 7. Pod length according to years and cultivars (cm).

Cultivar	Year			Mean
	2017	2018	2019	
Abelina	4.34 B ± 0.09	3.68 AB ± 0.15	4.45 A ± 0.07	4.16 A ± 0.10
SG Anser	4.56 A ± 0.10	3.97 A ± 0.11	4.15 AB ± 0.09	4.23 A ± 0.09
Merlin	3.55 C ± 0.16	3.61 B ± 0.12	3.89 B ± 0.15	3.68 B ± 0.10
Mean	4.15 a ± 0.16	3.75 b ± 0.13	4.17 a ± 0.14	

Means in rows followed by different letters in lowercase: a and b (for years) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A, B, and C (for cultivars and the interaction: years × cultivars) differ significantly at $p \leq 0.05$; ±Se (standard error) value.

The first pod height, measured from ground level to the point where the first pod was attached at the lowest node, was affected by weather conditions, cultivars, and biostimulants. In 2017 and 2019, the height was similar and equated to around 13.8 cm, on average. Of the test cultivars, the first pod was attached lowest and highest at the first node for cv. Merlin and AG Anser, respectively. As confirmed by the significant interaction years × cultivars, weather conditions during the growing season affected pod height. In 2017 and 2019, the greatest first pod height was determined for cv. SG Anser, the opposite being true for cv. Merlin. No significant differences were confirmed between SG Anser and Merlin in 2018. As far as biostimulants are concerned, Improver was the biostimulant that contributed to an increase in the first pod height of soybean plants, regardless of the remaining factors (Table 8).

The number of plants per 1 m^2 depended on the weather conditions during the growing season as well as the cultivars. It was also confirmed that this characteristic had different mean values for the test cultivars grown in the study years. Regardless of the remaining factors, the lowest average number of plants per 1 m^2 was recorded in 2018, it being higher in 2019 and the highest in 2017 (respectively, 21.41, 42.37, and 52.70 plants/m^2). Of the cultivars, cv. Abelina established the greatest number of plants per 1 m^2, it being much lower for Merlin and the lowest for SG Anser. The number of plants each cultivar established per 1 m^2 was affected by the meteorological conditions of the growing season. In 2017, the number of plants per 1 m^2 was similar for each test cultivar, whereas cv. Abelina established more plants per 1 m^2 compared with the remaining cultivars in 2018 and 2019, them having similar mean values of this characteristic in 2019 (Table 9).

Table 8. First pod height according to years, cultivars, and biostimulants (cm).

Cultivar	Year 2017	Year 2018	Year 2019	Mean
Abelina	13.42 B ± 0.56	10.11 A ± 0.85	14.43 B ± 0.62	12.66 B ± 0.64
SG Anser	16.74 A ± 0.48	7.53 B ± 0.67	15.61 A ± 0.31	13.30 A ± 1.01
Merlin	11.27 C ± 0.62	6.78 B ± 0.69	11.55 C ± 0.54	9.87 C ± 0.59
Mean	13.81 a ± 0.84	8.14 b ± 0.79	13.86 a ± 0.75	
	Biostimulant			
Cultivar	Control	Asahi SL	Improver	
Abelina	12.27 ± 0.81	12.32 ± 0.80	13.37 ± 0.91	
SG Anser	13.08 ± 0.90	13.28 ± 0.65	13.52 ± = 0.93	
Merlin	9.03 ± 0.85	9.88 ± 0.83	10.69 ± 0.72	
Mean	11.46 b ± 0.87	11.83 b ± = 0.95	12.53 a ± = 0.61	

Means in rows followed by different letters in lowercase: a and b (for years and biostimulants) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A, B, and C (for cultivars and the interaction: years × cultivars) differ significantly at $p \leq 0.05$; ±Se (standard error) value.

Table 9. Number of plants per 1 m^2 according to years and cultivars (plants/m^2).

Cultivar	Year 2017	Year 2018	Year 2019	Mean
Abelina	53.00 A ± 2.16	24.11 A ± 2.15	45.00 A ± 2.40	40.70 A ± 3.52
SG Anser	52.67 A ± 1.55	19.22 C ± 2.01	40.44 B ± 1.41	37.44 C ± 3.15
Merlin	52.44 A ± 2.41	20.89 B ± = 1.92	41.67 B ± 1.52	38.33 B ± 3.42
Mean	52.70 a ± 1.92	21.41 c ± 2.19	42.37 b ± = 2.21	

Means in rows followed by different letters in lowercase: a, b and c (for years) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A, B, and C (for cultivars and the interaction: years × cultivars) differ significantly at $p \leq 0.05$; ±Se (standard error) value.

Soybean plant height was predominantly affected by the main factors examined in the study, i.e., years, cultivars, and biostimulants. The highest plants were observed in 2017, followed by slightly lower ones in 2019 and the lowest in 2018 (respectively, 83.77, 75.02 and 46.20 cm). Cv. Merlin had the lowest plants, whereas cv. SG Anser and Abelina did not differ significantly in terms of this characteristic. The biostimulant Improver was associated with the highest plants, regardless of the remaining factors. No significant differences were found between the height of biostimulant-treated plants and the control plants (Table 10).

Table 10. Plant height according to years, cultivars, and biostimulants (cm).

Cultivar	Year 2017	Year 2018	Year 2019	Mean
Abelina	89.93 ± 5.31	47.77 ± 2.85	78.54 ± 2.52	72.08 A ± 4.98
SG Anser	91.52 ± 3.89	47.70 ± 2.76	83.40 ± 3.18	74.21 A ± 4.92
Merlin	69.84 ± 2.56	43.14 ± 2.64	63.12 ± 4.21	58.70 B ± 3.53
Mean	83.77 a ± 5.33	46.20 c ± 3.96	75.02 b ± 4.23	
	Biostimulant			
Cultivar	**Control**	**Asahi SL**	**Improver**	
Abelina	66.94 ± 4.30	70.38 ± 6.54	78.92 ± 6.10	
SG Anser	69.37 ± 4.98	75.16 ± 5.89	78.10 ± 6.20	
Merlin	55.80 ± 5.17	57.03 ± 6.21	63.28 ± 5.71	
Mean	64.04 b ± 4.30	67.52 b ± 4.96	73.43 a ± 5.68	

Means in rows followed by different letters in lowercase: a, b and c (for years and biostimulants) differ significantly at $p \leq 0.05$. Means in columns followed by different letters in uppercase: A and B (for cultivars) differ significantly at $p \leq 0.05$; ±Se (standard error) value.

4. Discussion

As soybean is a short-day plant with substantial temperature-related demands [33–35], it is rather difficult to obtain high yields in temperate climate conditions. Major restrictive factors for the cultivation of this species include temperature and precipitation during germination and flowering [2]. These conditions, when extreme, become stressors, which disturb plant growth and development, which, as a consequence, may contribute to reduced yields (by as much as 70%) of a poorer quality [36–38].

Under the conditions of a temperate climate, the stressors include high temperature, photoperiod (the length of day and night), and soil water shortages (periodical droughts) [36]. Many studies have demonstrated that negative plant response to stress can be reduced by an application of biostimulants that increase plant yields [3,24,39]. The aforementioned relationships have been confirmed in the experiment reported here. Both yield and yield-forming characteristics were considerably affected by the conditions during the growing season, with temperature and precipitation being the most important of these. In 2017, when precipitation and temperature were regularly distributed, soybean plants produced superior yields. Particularly beneficial hydrothermal conditions in the spring contributed to good plant germination and emergence, which resulted in the highest number of plants per 1 m^2. The beneficial conditions in 2017 allowed plants to form the greatest number of seeds per pod as well as the highest 1000-seed weight. Soybean plants produced the poorest yields in 2018 because at the beginning of the growing season, i.e., in May, there occurred an intense drought event. The monthly atmospheric precipitation sum for this month was barely 26 mm, and it was accompanied by quite high temperatures (the monthly average was 16.4 °C). Drought, when combined with high air temperature, leads to an increase in water shortages [40], resulting in yield decreases, which may be as high as a half of the usual yield [41]. Dornbos and Mullen [42] confirmed that there was a linear decrease in the weight and number of seeds produced by soybean plants due to progressing water and temperature stress. High temperatures contributed to a decline in 1000-seed weight and seed number. According to Ergo et al. [43], a combination of drought- and temperature-related stresses during seed fill may disturb photosynthesis and, in this way, hinder metabolism, which results in a decline in seed weight and number causing poorer soybean yields.

Soybean response to environmental conditions was affected by cultivars. The highest yield potential was displayed by cv. Merlin, which produced the highest yields and the greatest 1000-seed weight in 2019 when the early growing season saw very good water supply (precipitation sum in May was 114 mm). In 2017 and 2019, lower 1000-seed weight values were recorded for cv. Abelina, which might point to its high demand for thermal resources, as the average monthly air temperature in May was 13 °C in both the study years. The differences between genotypes in resistance to temperature-related stresses have been reported by, e.g., Gass et al. [35], Kurosaki et al. [44], Hume and Jackson [45] and Karges et al. [46]. Soybean is temperature-sensitive throughout the whole growing season, its biological minimum range being 17–18 °C. Prolonged periods, when the average daily temperature drops are below 15 °C, slow down plant growth and hinder leaf and shoot formation, whereas temperatures below 10 °C disturb the flowering process [47,48]. Janas et al. [49] demonstrated that stress due to cold temperatures at early development stages brought about the inhibition of soybean shoot and root growth.

An occurrence of stressors during the growing season causes physiological changes in plants that close their stomata to prevent water loss and delay photosynthesis processes, which is followed by the inhibition of metabolic processes [50]. In the experiment reported here, the adverse effects of stressors were considerably alleviated by the applied biostimulants, which led to increased yield, seed weight, and seed number per pod in soybean plants. A positive impact of biostimulants under stress conditions consisted of enhanced tolerances to stress and facilitated repairs of damage caused by unfavourable conditions [51]. Treatment with biostimulants that provides plants with an additional source of amino acids makes it easier for the plants to open their stomata, whose functioning has been disturbed by adverse weather conditions. An application of biostimulants in plant cultivation facilitates their water retention and, as a result, stimulates photosynthesis and the rate and direction of metabolic processes [52].

In 2017 and 2018, plants produced better yields due to a higher 1000-seed weight as a result of an application of the biostimulant Improver compared with Asahi SL. In 2019, there was no significant effect confirmed of the biostimulants on seed numbers. A positive influence of Asahi SL on soybean yielding under Polish conditions was reported by Kozak et al. [1]. Kapela et al. [53] found that Asahi SL, Improver, and Zeal enhanced the yield, 1000-seed weight, and seed number per ear of maize. A similar response due to various biostimulants was reported by Boghdady et al. [54], who investigated chickpea, mustard, and pea [55]. In turn, Kocira et al. [56] claimed that one application of Asahi SL beneficially influenced bean yielding by increasing seed number and weight as well as pod number.

5. Conclusions

The research reported in the present work has confirmed the hypothesis assuming that an application of biostimulants in soybean cultivation under the conditions of temperate climate, which is very variable, enhances the yield-forming potential of cultivars. In the field experiment, soybean yield and yield-forming characteristics were affected by the hydrothermal conditions during the growing season. The highest yield, TSW, and seed number were recorded in 2017, which had the highest precipitation sum (425 mm) and regular precipitation distribution, while having the coldest growing season (its average temperature was 14.7 °C). Both yield and its characteristics were cultivar-related, superior yields being produced by cv. Merlin, which also had the highest TSW, pod number, and seed number per pod. At the same time, the cultivar had the lowest average plant height and the lowest pod height. Biostimulants contributed to enhanced soybean yields by alleviating negative environmental influences, in particular, in 2017 and 2018. Plants sprayed with either Improver or Asahi SL produced yields which were, respectively, 29 and 27% higher compared with the control, the increase being mainly due to a positive effect of the products on TSW and seed number per pod. The response of plants to biostimulants was cultivar-related, the highest yield increases being determined for biostimulant-treated plants of cv. Merlin and Abelina, regardless of weather conditions. The response of cv. SG Anser to treatment with biostimulants was also positive, although the differences were not always statistically significant. A similar response of cultivars was found for the remaining characteristics. Under the growing conditions, the test cultivars treated with biostimulants increased seed numbers per pod, TSW, and pod number, although, in some cases, the increase was statistically insignificant.

An application of biostimulants may be a suitable strategy for increasing soybean yields under changeable climatic conditions. Further research into the impact of biostimulants on soybean yields should encompass an application of various rates of biostimulants in order to determine the optimal input.

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