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Biostimulants as a Response to the Negative Impact of Agricultural Chemicals on Vegetation Indices and Yield of Common Buckwheat (*Fagopyrum esculentum* Moench)

Mateusz Krupa [1,*] and Robert Witkowicz [2]

[1] Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, Al. 29 Listopada 46, 31-425 Krakow, Poland
[2] Department of Agroecology and Crop Production, University of Agriculture in Krakow, Al. Mickiewicza 21, 31-120 Krakow, Poland
* Correspondence: mateusz.krupa@urk.edu.pl

Abstract: Weed control during common buckwheat cultivation is hindered by the crop's high sensitivity to agrochemicals. This study evaluates whether biostimulants (Asahi SL, Kelpak SL, B-Nine) could reduce the adverse effect of abiotic stress caused by these substances on buckwheat's vegetation indices and yield. To this end, a four-factor field experiment was performed according to the 3^{4-1} Box–Behnken design on chernozem soil with silt texture at the Experimental Station of the Agricultural University of Krakow (Poland, 50°07′ N, 20°04′ E). The results showed that calcium cyanamide fertilization was effective in reducing the abundance of dicotyledonous weeds by 39% and the dry weight of weeds per unit area by 20% relative to ammonium nitrate-fertilized sites. However, the most effective method of weed control was the application of metazachlor together with clomazone. The mixture of these active substances reduced the abundance of monocotyledonous weeds, dicotyledonous weeds, and dry weight of weeds by 83%, 40.5%, and 36.4%, respectively. The use of herbicides adversely affected the leaf area index (LAI). Nitrophenol treatment of buckwheat grown on soil fertilized with calcium cyanamide resulted in increased achene yield and number of seeds per plant compared to ammonium nitrate fertilization. The application of daminozide on chemically protected plants resulted in improved vegetation indices such as normalized difference vegetation index (NDVI) and soil plant analysis development (SPAD) compared to sites not exposed to herbicides.

Keywords: crop protection; crop nutrition; plant biostimulants; LAI; SPAD; NDVI; segetal flora

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1. Introduction

In the early stages of growth, common buckwheat (*Fagopyrum esculentum* Moench) can succumb to heavy weed infestation, resulting in compromised achene yield [1]. However, weed control is problematic for buckwheat due to its high sensitivity to the chemicals contained in herbicides and also to the low availability of legally permitted compounds in buckwheat protection [2–5]. Studies presenting the use of new active substances, such as linuron, metazachlor, and clomazone, confirm their negative effects on the growth and development of buckwheat, although the research is essentially limited to assessing their phytotoxicity [6].

One of the new methods for weed control in buckwheat cultivation is the use of fertilizers containing calcium cyanamide [7–10]. Dobrzanski [11] noticed that calcium cyanamide effectively controls weeds in the cultivation of *Rheum rhaponticum*, a species from the same family as buckwheat (*Polygonaceae*). Fertilizers containing calcium cyanamide have a low risk of nitrogen leaching and weaken the pressure of phytopathogens and pests. In a study by Dixon [12], the systematic application of calcium cyanamide at 1000 kg·ha^{-1} on plantations with plants from the *Brassicaceae* family effectively reduced the occurrence of *Plasmodiphora brassicae* in the soil, and, when applied at 80 g·m^{-2} and 200 g·m^{-2} in the greenhouse cultivation of cucumber, reduced the pressure from *Fusarium oxysporum* that causes Panama disease affecting roots and shoots [13]. Calcium cyanamide also regulates pest populations, including *Schistosoma japonicum* larvae [14,15]. On the other hand, calcium cyanamide decomposition products are phytotoxic for crop plants, such as free cyanamide or dicyandiamide, the latter being found in soils with a low concentration of organic matter [16–18]. Dicyandiamide is a nitrification inhibitor and affects nitrifying bacteria such as *Nitrosommonas*, *Nitrobacter*, and *Nitrococcus*. As a result, the activity of the bacteria is reduced, leading to a slowdown in the nitrification process [19].

Reducing the adverse impact of agrochemicals on the vegetation indices and yield of buckwheat is a key challenge in buckwheat cultivation. One of the increasingly popular solutions to mitigate the effects of abiotic and biotic stresses on buckwheat is the use of biostimulants that contain chemicals of natural or synthetic origin and selected microorganisms [20–22]. The active compounds contained in biostimulants include humic substances, complex organic substances, beneficial chemical elements, inorganic substances, seaweed extracts, chitin and chitosan derivatives, free amino acids, and substances containing nitrogen [23]. Some studies indicate that an adequate composition of biostimulants could result in a synergic effect [24]. Available scientific studies do not present research on the use of biostimulants to reduce abiotic stress caused by the influence of active substances contained in agrochemicals on vegetation indices and the yield of buckwheat. Due to the extremely low availability of registered active substances that can be used for the protection of buckwheat, we have undertaken this research into new agrochemicals and biostimulants designed to reduce their phytotoxicity.

2. Materials and Research Methods

2.1. Plant Material and Field Experiment

A four-factor field experiment was set up according to the Box–Behnken plan [25–27] in two replicates and in three blocks at the Experimental Station of the Agricultural University of Krakow, in 2016–2018 (Poland, 50°07′ N, 20°04′ E). Each block contained eighteen 12 m^2 plots (Table 1, Figures A1–A3).

Table 1. Experimental factors and their levels.

Factor		Treatment Level of Experimental Factor		
I	Cultivar	Kora	Panda	MHR Smuga
II	Fertilizer	0 kg $CaCN_2$·ha^{-1} (50 kg N·ha^{-1}–NH_4NO_3, 0 kg N·ha^{-1}–$CaCN_2$, 126.5 kg CaO·ha^{-1}–calcium oxide, 0 kg CaO·ha^{-1}–Perlka) BBCH 00 F0	78.5 kg $CaCN_2$·ha^{-1} (25 kg N·ha^{-1}–$CaCN_2$, 25 kg N·ha^{-1}–NH_4NO_3, 63.5 kg CaO·ha^{-1}–calcium oxide, 63.5 kg CaO·ha^{-1}–Perlka) BBCH 00 F1	157 kg $CaCN_2$·ha^{-1} (50 kg N·ha^{-1}–$CaCN_2$, 0 kg N·ha^{-1}–NH_4NO_3, 0 kg CaO·ha^{-1}–calcium oxide, 126.5 kg CaO·ha^{-1}–Perlka) BBCH 00 F2
III	Herbicide	No herbicide	Linuron (Linurex 500 SC) 400 g·ha^{-1} BBCH 02	Metazachlor (Metazanex 550 SC) 750 g·ha^{-1} and clomazone (Command 480 EC) 96 g·ha^{-1} BBCH 02
IV	Biostimulants	Daminozide (Bi-Nine 85 SG) BBCH 14	Nitrophenols (Asahi SL) BBCH 14	Seaweed *Ecklonia maxima* extract (Kelpak SL) BBCH 14

The first experimental factor was buckwheat cultivar (Kora, Panda, and MHR Smuga). Elite buckwheat seed was obtained annually from the Palikije Cultivar Breeding Station (Malopolska Plant Breeding, Krakow, Poland). The germination capacity of the tested buckwheat cultivars in the years studied ranged from 90% to 93%. Buckwheat was sown from May 21 to June 6.

The amount of calcium cyanamide in nitrogen fertilization was the second research factor (0 kg $CaCN_2 \cdot ha^{-1}$—F0; 78.5 kg $CaCN_2 \cdot ha^{-1}$—F1; 157 kg $CaCN_2 \cdot ha^{-1}$—F2). The applied Perlka fertilizer contained 62% $CaCN_2$. Total nitrogen doses for all levels of this factor were balanced (Table 1). Perlka fertilizer was applied before sowing buckwheat.

Herbicide was the third research factor (1. No herbicide, 2. Linuron, 3. Metazachlor + clomazone) (Table 1). Linuron, the active ingredient of Linurex 500 SC, was applied at 400 $g \cdot ha^{-1}$, and metazachlor, the active ingredient of Metazanex 500 SC herbicide, was applied at 750 $g \cdot ha^{-1}$. Command 480 EC contains clomazone, the dose of which was 96 $g \cdot ha^{-1}$. Metazachlor and clomazone are chemical compounds recommended and registered for weed control in crops from the *Brassicaceae* genus [28,29]. Herbicides were applied at the BBCH 02 stage of buckwheat growth (up to 3 days after sowing) (Table 1). The doses were in line with the manufacturers' recommendations, except for Linuron, where it was 20% lower than recommended due to the previously determined phytotoxicity.

The final experimental factor was the use of three biostimulants (1. B-Nine 85 SG, 2. Asahi SL, 3. Kelpak SL). The content of daminozide in B-Nine 85 SG (Arysta Life Science Ltd., Praha, Czech Republic) was 850 $g \cdot kg^{-1}$. The B-Nine formula was applied at a rate of 1.5 $kg \cdot ha^{-1}$, in accordance with the manufacturer's recommendations. Biostimulant Asahi SL (Arysta Life Science Ltd., Praha, Czech Republic) is a commercial product containing plant-derived nitrophenols: sodium para-nitrophenolate ($NaC_6H_4NO_3$, 0.3%), sodium ortho-nitrophenolate ($NaC_6H_4NO_3$, 0.2%), and sodium 5-nitroguaiacolate ($NaC_7H_6NO_4$, 0.1%) [30]. Asahi biostimulant was applied at 0.6 $l \cdot ha^{-1}$. Biostimulant Kelpak SL (Kelp Products International (Pty) Ltd., Cape Town, South Africa) is an extract of algae *Ecklonia maxima*, containing polysaccharides such as laminarin, alginates, and carrageenins, as well as micro- and macronutrients, sterols, N-containing compounds such as betaines, and phytohormones [31]. Biostimulant Kelpak SL was applied at 3 $l \cdot ha^{-1}$. The biostimulants were applied on the leaves at the BBCH 14 stage of buckwheat growth (Table 1).

Two weeks before the scheduled harvest date, buckwheat was desiccated at the BBCH 80–89 stage with ammonium glufosinate at a dose of 450 $g \cdot ha^{-1}$ (Basta 150 SL). Buckwheat achenes were harvested with a plot harvester (Wintersteiger Seedmaster, Austria). Achenes yield was given after conversion based on 14% moisture content. In 2016–2018, harvesting dates were between 6 August and 22 August.

Post-emergence plant density of buckwheat plants was determined on each experimental plot using the frame method (0.5 m^2) (three replicates). The post-harvest density of buckwheat plants was evaluated twice on each experimental plot using the frame method (1 m^2). The number of achenes per buckwheat plant was determined on a sample of 15 randomly taken plants from each experimental plot. The weight of one thousand seeds is the average of four determinations made for each experimental plot [32].

The abundance and dry weight of the segetal flora community in the buckwheat experimental field were measured at the BBCH 20–29 stage (three weeks after herbicide application) using the frame-weight method (1 m^2) on each experimental plot (three replicates).

2.2. Vegetation Indices

The leaf area index (LAI) was measured using the Sunscan System (Delta T, Cambrige, UK) at three developmental stages of buckwheat: 1. BBCH 20–29 (lateral shoot development), 2. BBCH 50–59 (inflorescence development) and 3. BBCH 60–69 (flowering). In each experimental plot, the measurement was carried out three times. Chlorophyll content in soil plant analysis development (SPAD) units was measured using a portable Chlorophyll Meter Spad 502 DL spectrophotometer (Konica Minolta Sensing, Inc., Sakai, Osaka, Japan) on the 10 youngest fully developed leaves in each experimental plot at the developmental stages BBCH 20–29 and BBCH 50–59. The normalized difference vegetation index (NDVI) was estimated as (ρ_NIR–ρ_red)/(ρ_NIR+ρ_red), where ρ_NIR denotes reflectance in the near-infrared spectral range and ρ_red denotes reflectance in the red spectral range [33].

The NDVI was measured at three developmental stages of buckwheat (1. BBCH 20–29, 2. BBCH 50–59, and 3. BBCH 60–69) using a GreenSeeker Handheld Optical Sensor Unit (NTech Industries, Inc., Dalton, GA, USA) in each experimental plot.

2.3. Soil Conditions

According to the international classification of the World Reference Base for Soil Resources (WRB), the soil of the experimental field was classified as chernozem with silt texture [34]. Analysis of the soil's granulometric composition was carried out using an ANALYSETTE 22 MicroTec plus laser analyzer (FRITSCH GmbH, Idar-Oberstein, Germany). The pH (KCl) was determined in accordance with ISO 10390 [35] as 5.5 in the arable level of the soil. The macronutrient content of the soil of the experimental field was as follows: N—2.0 g·kg^{-1}; P—91.0 mg·kg^{-1}; K—185.1 mg·kg^{-1}; and Mg—86.0 mg·kg^{-1}. Soil phosphorus and potassium were determined using the Egner–Riehm method, and magnesium was determined using atomic absorption spectrometry (ASA). The concentration of total N in the soil was determined using the Kjeldahl method according to ISO 11261 [36]. The concentration of organic C was determined using the Thiurin method [37]. The average C content in the soil arable level was 11.4 g·kg^{-1}.

2.4. Weather Conditions

Hydrothermal conditions for the months covering the buckwheat growing season were characterized by the Selyaninov hydrothermal coefficient, estimated based on the equation $K = P/0.1\sum t$, where P is the sum of monthly precipitation and $\sum t$ is the monthly sum of average air temperatures above 0 °C [38]. The equation for estimating GDD was adopted from Liu et al. [39]. The base temperature was taken as 10 °C [40]. The presented hydrothermal conditions in 2016–2018 indicate a shortage of water against the background of multi-year precipitation in the years 1988–2018 (Table 2). The year 2016 may be classified as a dry growing season, and in the other years of the field experiment, the average temperatures and precipitation were generally higher than in 2016 (Table 2).

Table 2. Rainfall, air temperature, hydrothermal coefficient of Selyaninov and growing degree days.

Month	Sum of Temperature [°C]	Rainfall [mm]	Difference between Rainfall and Rainfall over Many Year [mm] [#]	Hydrothermal Coefficient of Selyaninov *	Characteristic of the Month	Growing Degree Days [°D]
			2016			
May	448.4	41.4	−35.3	0.92	Dry	0–712
June	564.9	59.8	−22.2	1.06	Dry enough	
July	566.5	92.8	−3.4	1.64	Moderately wet	
August	573.0	62.0	−9.8	1.08	Quite dry	
			2017			
May	433.2	83.8	7.2	1.93	Moderate wet	0–718
June	563.0	45.2	−36.8	0.80	Dry	
July	593.9	84.4	−11.8	1.42	Optimal	
August	627.8	83.8	12.0	1.33	Optimal	
			2018			
May	541.2	62.4	−14.3	1.15	Dry enough	0–710
June	554.8	85.6	3.6	1.54	Optimal	
July	615.5	119.8	23.6	1.95	Moderately wet	
August	646.0	56.2	−15.6	0.87	Dry	

[#]—Rainfall from 1988 to 2018 base on the data resources of the meteorological Station in Krakow–Balice of the Institute Meteorology and Water Management. *—$K \leq 0.4$, Extremely dry; $0.4 < K < 0.7$—Very dry; $0.7 < K \leq 1.0$—Dry; $1.0 < K \leq 1.3$—Dry enough; $1.3 < K \leq 1.6$—Optimal; $1.6 < K \leq 2.0$—Moderately wet; $2.0 < K \leq 2.5$—Wet; $2.5 < K \leq 3.0$—Very wet; $K > 3.0$—Extremely wet [38].

2.5. Statistical Analyses

The results obtained from the four-factor field experiment were subjected to analysis of variance according to the experimental design (Box–Behnken 3^{4-1} design) using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA) [41]. The Box–Behnken 3^{4-1} design is a type of response surface design that is used to optimize the response of a process or system. The design involves four factors, each with three levels, and one center point. The design is useful for minimizing the number of repetitions required while maximizing the information gained from the experiment [25–27]. The field experiment design diagram is attached (Figure A1).

3. Results

3.1. Yield and Yield Components

Buckwheat achene yield and yield components developed differently in the individual years of the study. The year 2016 saw the highest achene yield (3177 kg·ha^{-1}) and the highest number of seeds per plant (76.9 pcs). In 2017, we found the lowest achene yield and lower values of yield components than in 2016. On the other hand, in 2018, plant density after emergence (218 pcs·m^{-2}) was significantly higher than in 2016 (190 pcs·m^{-2}), similar to plant density before harvest (186 pcs·m^{-2} in 2018 and 161 pcs·m^{-2} in 2016) and thousand-seed weight (25.1 g in 2018 and 24.4 g in 2016) (Table 3). The buckwheat cultivars differed in achene yield, plant density after emergence, and thousand-seed weight (Table 4). The highest achene yield was observed for the Smuga cultivar (2672 kg·ha^{-1}); moreover, the plants of the Smuga cultivar developed achenes with a higher thousand-seed weight by 5.6% compared to the Kora cultivar. A smaller post-emergence plant density compared to the other cultivars (quadratic effect) was found in the Panda cultivar (163 pcs·m^{-2}).

Table 3. Yield and yield components of the buckwheat in 2016–2018.

Parameter	Year *		
	2016	2017	2018
Yield of the achenes [kg·ha^{-1}]	3177 $^{b\,**}{}_{A\,***}$	1917 $_{B}$	2113 $^{a}{}_{A}$
Plant density after emergence [pcs·m^{-2}]	190 $^{a}{}_{A}$	201 $_{A}$	218 $^{b}{}_{A}$
Plant density before harvesting [pcs·m^{-2}]	161 $^{a}{}_{A}$	155 $_{B}$	186 $^{b}{}_{A}$
The number of seeds per plant [pcs]	76.9 $^{b}{}_{A}$	58.7 $_{B}$	51.2 $^{a}{}_{A}$
Thousand-seed weight [g]	24.4 $^{a}{}_{A}$	23.3 $_{B}$	25.1 $^{b}{}_{A}$

*—means were compared separately for parameters. **—the linear effect is described in superscript (lowercase—a, b), which, for the factor with three levels, indicates a comparison of values obtained at extreme levels (2016 and 2018) of the factor. Only the means denoted by lowercase letters are compared, and means with different lowercase letters were significantly different at $p \leq 0.05$. ***—square effect is described in subscript (capital letters—A, B), which, for the factor with three levels, means a comparison of the value obtained for the central factor level with the average of the values obtained for the extreme levels (the lowest and the highest) of the factor. The means were significantly different at $p \leq 0.05$.

Table 4. Yield and yield components of common buckwheat under the influence of cultivar, calcium cyanamide fertilization, herbicide use, and PGP application.

Parameter	Cultivar *			Fertilization *			Herbicide *			PGP *		
	Kora	**Panda**	**Smuga**	**F0 #**	**F1**	**F2**	**No herbicide**	**Linuron**	**Metazachlor and Clomazone**	**Daminozide**	**Nitrophenols**	***Ecklonia maxima* Extract**
Yield of the achenes [$kg \cdot ha^{-1}$]	2465 $^{a\,**}_{A\,***}$	2269 $_{B}$	2672 $^{b}_{A}$	2436 $^{a}_{A}$	2418 $_{A}$	2330 $^{a}_{A}$	2403 $^{a}_{A}$	2403 $_{A}$	2401 $^{a}_{A}$	2353 $^{a}_{A}$	2313 $_{A}$	2458 $^{a}_{A}$
Plant density after emergence [$pcs \cdot m^{-2}$]	206 $^{a}_{A}$	197 $_{B}$	213 $^{a}_{A}$	207 $^{a}_{A}$	203 $_{A}$	199 $^{a}_{A}$	209 $^{a}_{A}$	199 $_{B}$	205 $^{a}_{A}$	199 $^{a}_{A}$	204 $_{A}$	200 $^{a}_{A}$
Plant density before harvesting [$pcs \cdot m^{-2}$]	175 $^{a}_{A}$	163 $_{A}$	170 $^{a}_{A}$	163 $^{a}_{A}$	169 $_{A}$	167 $^{a}_{A}$	172 $^{a}_{A}$	167 $_{A}$	162 $^{a}_{A}$	163 $^{a}_{A}$	169 $_{A}$	168 $^{a}_{A}$
The number of seeds per plant [pcs]	62.9 $^{a}_{A}$	60.6 $_{A}$	65.9 $^{a}_{A}$	64.6 $^{a}_{A}$	62.3 $_{A}$	60.0 $^{a}_{A}$	59.9 $^{a}_{A}$	62.3 $_{A}$	64.5 $^{a}_{A}$	62.5 $^{a}_{A}$	59.0 $_{A}$	63.5 $^{a}_{A}$
Thousand-seed weight [g]	23.6 $^{a}_{A}$	24.2 $_{A}$	25.0 $^{b}_{A}$	24.2 $^{a}_{A}$	24.2 $_{A}$	24.4 $^{a}_{A}$	24.2 $^{a}_{A}$	24.4 $_{A}$	24.0 $^{a}_{A}$	24.8 $^{b}_{A}$	24.2 $_{B}$	24.1 $^{a}_{A}$

*—the means were compared separately for the factors (Cultivar, Fertilization, Herbicide, PGP). **—the linear effect is described in superscript (lowercase—a, b), which, for the factor with three levels, indicates a comparison of values obtained at extreme levels (the lowest and the highest) of the factor. Only the means denoted with lowercase letters are compared, and means with different lowercase letters were significantly different at $p \leq 0.05$. ***—square effect is described in subscript (capital letters—A, B), which, for the factor with three levels, means a comparison of the value obtained for the central factor level with the average of the values obtained for the extreme levels (the lowest and the highest) of the factor. The means were significantly different at $p \leq 0.05$. #—F0: 0 kg $CaCN_2 \cdot ha^{-1}$; F1: 78.5 kg $CaCN_2 \cdot ha^{-1}$; F2: 157 kg $CaCN_2 \cdot ha^{-1}$.

Fertilization with different forms of nitrogen fertilization did not statistically significantly influence achene yield and other yield elements, but the application of the active substances contained in herbicides reduced post-emergence plant density (Table 4). Non-toxic effects of herbicides were found on the yield and yield elements of buckwheat. The plants protected by linuron saw a decrease in post-emergence plant density by 6 pcs·m^{-2} compared to the use of metazachlor and clomazone and by 10 pcs·m^{-2} compared to the chemically unprotected sites (control). The thousand-seed weight was affected in various ways by using biostimulants (Table 4). The highest increase was observed after using daminozide (by 24.8 g). However, biostimulants did not affect achene yield or other yield components.

The achene yield varied depending on the combinations of different types of nitrogen fertilization and biostimulants (fertilization × biostimulant interaction). The fertilization of buckwheat plants with ammonium nitrate and the application of *Ecklonia maxima* algae extract resulted in lower achene yields compared to the application of daminozide or nitrophenols. The fertilization of buckwheat plants with calcium cyanamide followed by the application of nitrophenols resulted in a slightly higher yield of achenes compared to plants where the other biostimulants were applied (Figure 1A). The effect of fertilization with different forms of nitrogen and the application of the tested biostimulants (fertilization × biostimulant interaction) on the number of seeds per plant was close to statistical significance (α = 0.051). Buckwheat grown on soil fertilized with ammonium nitrate developed a higher number of seeds after the application of daminozide or nitrophenols (statistical trend). On sites fertilized with calcium cyanamide, an increase in the number of seeds was observed after the application of nitrophenols (Figure 1B).

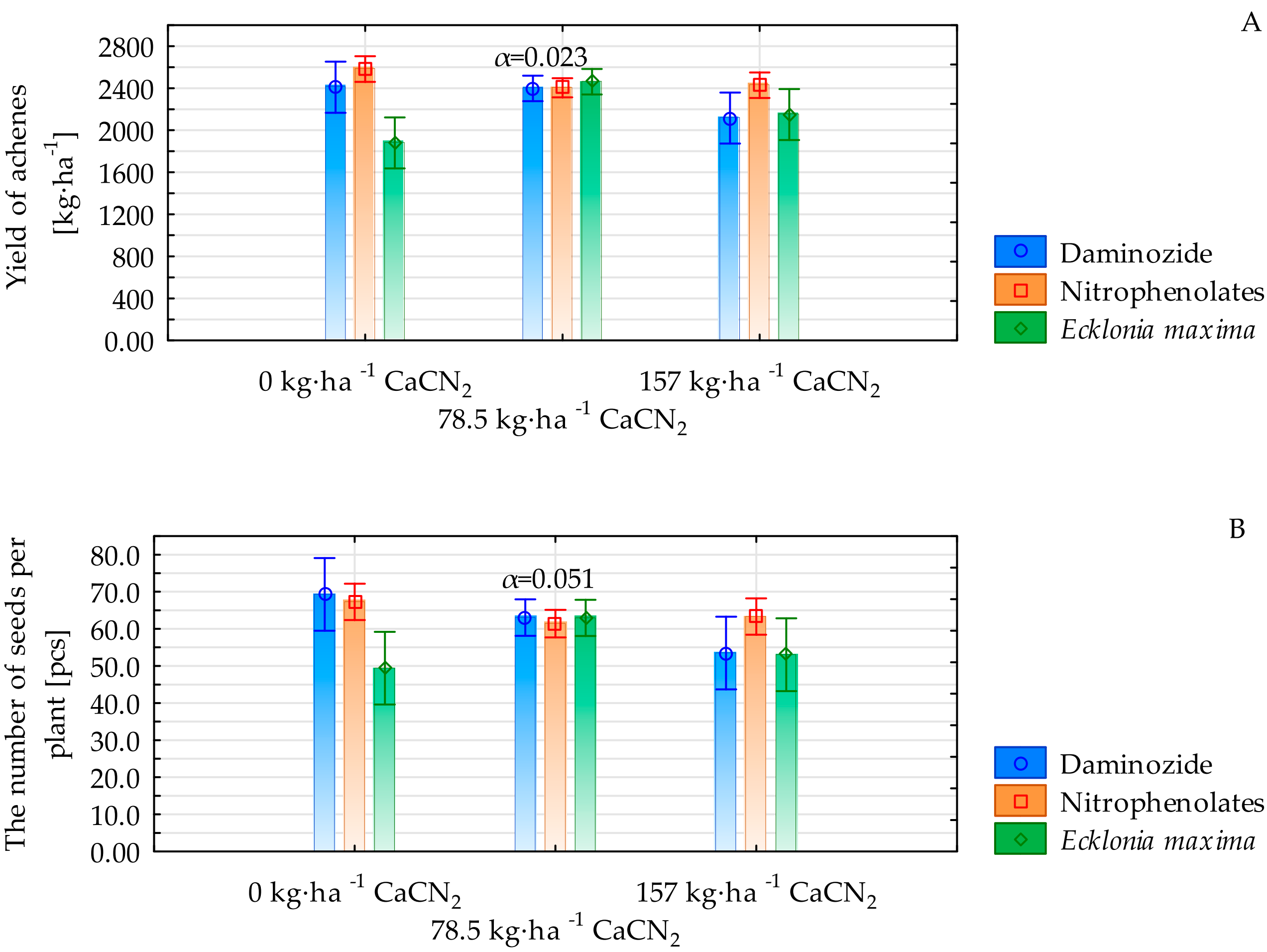

Figure 1. Achenes yield (**A**) and number of seeds per plant (**B**).

3.2. Vegetation Indices

Buckwheat canopy vegetation indices showed statistically significant differences between the years of the study and between developmental stages (Table 5). In 2017, we observed the highest leaf area index (LAI) (5.87 $m^2 \cdot m^{-2}$). The LAI increased linearly with buckwheat growth and development, reaching a maximum at the BBCH 60–69 stage (7.55 $m^2 \cdot m^{-2}$). In addition to the variation in LAI across the years of the study, there was also a variation in SPAD. In 2016, buckwheat plants had the highest SPAD, at 39.47 (Table 5). Statistically significant differences in SPAD were also observed between developmental stages. Younger plants at the BBCH 20–29 stage were characterized by higher SPAD values (40.45) than plants at BBCH 60–69 (34.80). The decrease in chlorophyll concentration during plant growth and development is the result of dilution caused by an intensive increase in assimilation surface area.

Table 5. Parameters of common buckwheat by year and growth stage.

Parameter	Year *			Plant Growth Stage *		
	2016	2017	2018	BBCH 20-29	BBCH 50-59	BBCH 60-69
LAI ($m^2 \cdot m^{-2}$)	4.49 $^{b\,**}{}_{A\,***}$	5.87 $_B$	5.69 $^a{}_A$	2.44 $^a{}_A$	6.05 $_B$	7.55 $^b{}_A$
SPAD	39.47 $^b{}_A$	35.94 $_B$	37.48 $^a{}_A$	40.45 ^{b}	-	34.80 ^{a}
NDVI	0.559 $^a{}_A$	0.646 $_B$	0.651 $^a{}_A$	0.605 $^a{}_A$	0.661 $_B$	0.589 $^b{}_A$

*—the means were compared separately for the year and plant growth stage. **—the linear effect is described in superscript (lowercase—a, b), which, for the factor with three levels, indicates a comparison of values obtained at extreme levels (the lowest and the highest) of the factor. Only the means denoted with lowercase letters are compared, and means with different lowercase letters were significantly different at $p \leq 0.05$. ***—square effect is described in subscript (capital letters—A, B), which, for the factor with three levels, means a comparison of the value obtained for the central factor level with the average of the values obtained for the extreme levels (the lowest and the highest) of the factor. The means were significantly different at $p \leq 0.05$.

There was also a statistical variation in the NDVI of buckwheat between different years of the study (Table 5). In 2018, the highest NDVI (0.651) was observed. The NDVI of buckwheat also statistically significantly different between the analyzed developmental stages, with the highest NDVI observed at BBCH 50–59 (0.661).

The vegetation indices also differed between the tested buckwheat cultivars (Table 6). The Kora (5.45 $m^2 \cdot m^{-2}$) and Smuga (5.41 $m^2 \cdot m^{-2}$) cultivars had higher LAIs compared to Panda (5.28 $m^2 \cdot m^{-2}$). The SPAD also differed depending on the cultivar. The highest SPAD and NDVI was found for the Kora cultivar canopy at 38.68 and 0.633, respectively. The application of different forms of nitrogen fertilizers resulted in different vegetation indices (Table 6). The buckwheat grown on soil fertilized with calcium cyanamide had the lowest LAI (5.04 $m^2 \cdot m^{-2}$). The same fertilization resulted in a SPAD decrease of 1.19 units compared to ammonium nitrate fertilization. The use of ammonium nitrate had the highest NDVI (0.627), while calcium cyanamide resulted in the lowest NDVI (0.605).

Table 6. LAI, SPAD, and NDVI of common buckwheat under the influence of cultivar, calcium cyanamide fertilization, herbicide use, and PGP application.

Parameters	Cultivar *			Fertilization *			Herbicide *			PGP *		
	Kora	Panda	Smuga	F0 #	F1	F2	No herbicide	Linuron	Metazachlor and Clomazone	Daminozide	Nitrophenols	*Ecklonia maxima* Extract
LAI ($m^2 \cdot m^{-2}$)	5.45 $^{a\,**}{}_{A\,***}$	5.28 $_{B}$	5.41 $^{a}{}_{A}$	5.53 $^{b}{}_{A}$	5.40 $_{B}$	5.04 $^{a}{}_{A}$	5.43 $^{b}{}_{A}$	5.36 $_{A}$	5.23 $^{a}{}_{A}$	5.32 $^{a}{}_{A}$	5.36 $_{A}$	5.34 $^{a}{}_{A}$
SPAD	38.68 $^{b}{}_{A}$	37.48 $_{A}$	36.94 $^{a}{}_{A}$	38.19 $^{b}{}_{A}$	37.65 $^{a}{}_{A}$	37.00 $^{a}{}_{A}$	37.56 $^{a}{}_{A}$	37.62 $_{A}$	37.70 $^{a}{}_{A}$	37.72 $^{a}{}_{A}$	37.69 $^{a}{}_{A}$	37.36 $^{a}{}_{A}$
NDVI	0.633 $^{b}{}_{A}$	0.618 $_{A}$	0.606 $^{a}{}_{A}$	0.627 $^{b}{}_{A}$	0.620 $_{A}$	0.605 $^{a}{}_{A}$	0.615 $^{a}{}_{A}$	0.618 $_{A}$	0.623 $^{a}{}_{A}$	0.613 $^{a}{}_{A}$	0.617 $_{A}$	0.627 $^{a}{}_{A}$

*—the means were compared separately for the factors (Cultivar, Fertilization, Herbicide, PGP). **—the linear effect is described in superscript (lowercase—a, b), which, for the factor with three levels, indicates a comparison of values obtained at extreme levels (the lowest and the highest) of the factor. Only the means denoted with lowercase letters are compared, and means with different lowercase letters were significantly different at $p \leq 0.05$. ***—square effect is described in subscript (capital letters—A, B), which, for the factor with three levels, means a comparison of the value obtained for the central factor level with the average of the values obtained for the extreme levels (the lowest and the highest) of the factor. The means were significantly different at $p \leq 0.05$. #—F0: 0 kg $CaCN_2 \cdot ha^{-1}$; F1: 78.5 kg $CaCN_2 \cdot ha^{-1}$; F2: 157 kg $CaCN_2 \cdot ha^{-1}$.

The applied herbicides only influenced the LAI of the buckwheat. The application of metazachlor together with clomazone reduced the LAI compared to the LAI of the chemically unprotected crops. No differences were observed in the SPAD and NDVI after the application of herbicides.

The applied biostimulants did not alter buckwheat vegetation indices. It should be noted, however, that treating the buckwheat canopy with *Ecklonia maxima* algae extract showed a tendency to increase the NDVI and decrease the SPAD compared to the use of daminozide and nitrophenols.

The LAI for buckwheat grown on soil fertilized with different forms of nitrogen developed differently when treated with biostimulants (fertilization × biostimulant interaction). Full substitution of ammonium nitrate with calcium cyanamide resulted in reduced LAI following the application of daminozide and nitrophenols. The LAI of buckwheat treated with *Ecklonia maxima* algae extract in combination with the full substitution of ammonium nitrate with calcium cyanamide did not differ from the LAI following the use of ammonium nitrate and calcium cyanamide or ammonium nitrate alone. A significantly higher value of this parameter was found in plants fertilized with ammonium nitrate and treated with daminozide (Figure 2A).

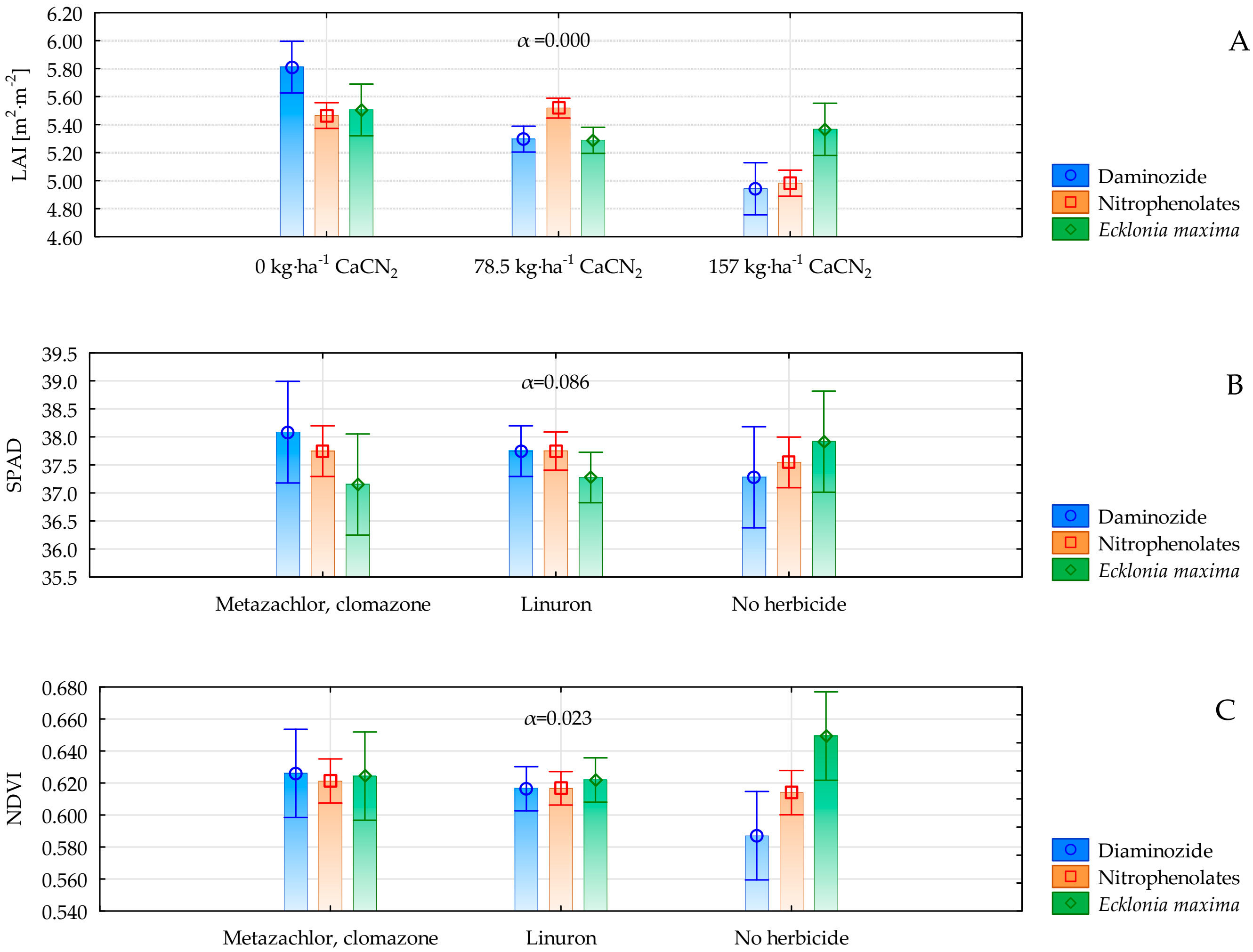

Figure 2. LAI (**A**), SPAD (**B**), and NDVI (**C**) of buckwheat.

Following the applied methods of chemical weed control and the application of biostimulants (herbicide x biostimulant interaction), the SPAD showed no statistically significant differences (statistical trend α = 0.086). The application of *Ecklonia maxima* algae extract to buckwheat plants protected from weeds by linuron, as well as metazachlor and clomazone, resulted in reduced SPAD. On the chemically unprotected plot, a slight increase in the SPAD was observed after the application of *Ecklonia maxima* algae extract. The application of daminozide, as well as nitrophenols, to herbicide-protected plants tended to increase the SPAD, especially in chemically unprotected sites (Figure 2B).

The simultaneous use of herbicides and biostimulants (herbicide x biostimulant interaction), resulted in a statistical variation in the NDVI. On the non-chemically protected plants, the application of *Ecklonia maxima* algae extract resulted in a clear increase in the NDVI, while the application of daminozide resulted in its considerable decrease. The application of daminozide on chemically protected sites resulted in a similar NDVI to other combinations of chemical protection (Figure 2C).

3.3. The Segetal Flora

The abundance of monocotyledonous and dicotyledonous weeds and the dry weight of weeds differed between the individual years of the study (Table 7). By far the highest density of monocotyledonous and dicotyledonous weeds was observed in 2016 (11.86 pcs$\cdot$m^{-2} and 3.35 pcs$\cdot$m^{-2}, respectively). The tested buckwheat cultivars differed in the abundance and dry weight of monocotyledonous weeds (Table 7). For the Panda cultivar, the number of monocotyledonous weeds (6.93 pcs$\cdot$m^{-2}) and their dry weight (1.72 g) were higher than in other cultivars due to the dominance of *Echinochloa crus-galli*. Fertilization with different forms of fertilizer nitrogen resulted in significant differences in the abundance of dicotyledonous weeds and dry weight of weeds per unit area (Table 7). Calcium cyanamide fertilization reduced the abundance of dicotyledonous weeds by nearly 39% compared to the effect of ammonium nitrate. In addition, the dry weight of weeds from the calcium cyanamide fertilized site was nearly 20% less than that following the use of ammonium nitrate. Herbicide protection significantly reduced the abundance as well as the dry weight of weeds (Table 7). The greatest reduction in the abundance of monocotyledonous and dicotyledonous weeds was observed after the application of metazachlor in combination with clomazone (by 83.0% and 40.5%, respectively). Their application also reduced the dry weight of weeds per square meter by 36.4% compared to the chemically unprotected site.

Table 7. The abundance and dry weight of the segetal flora.

Parameters	Year			Cultivar *			Fertilization *			Herbicide *		
	2016	**2017**	**2018**	**Kora**	**Panda**	**Smuga**	**F0 #**	**F1**	**F2**	**Control (No Herbicide)**	**Linuron**	**Metazachlor and Clomazone**
Number of monocotyledonous weeds [pcs·m^{-2}]	$11.86^{b\,**}_{A\,***}$	3.87_{B}	2.75^{a}_{A}	5.67^{a}_{A}	6.93_{B}	4.93^{a}_{A}	6.54^{a}_{A}	6.19_{A}	5.71^{a}_{A}	8.43^{b}_{A}	7.27_{B}	1.43^{a}_{A}
Number of dicotyledonous weeds [pcs·m^{-2}]	3.34^{b}_{A}	2.67_{A}	1.26^{a}_{A}	2.38^{a}_{A}	2.51_{A}	2.25^{a}_{A}	2.97_{A}	2.44_{A}	1.82^{a}_{A}	2.15^{b}_{A}	3.06_{B}	1.28^{a}_{A}
Dry weight of weeds [g·m^{-2}]	-	2.03^{b}	0.97^{a}	1.34^{a}_{A}	1.72_{B}	1.18_{A}	1.74^{b}_{A}	1.44_{A}	1.39^{a}_{A}	1.73^{b}_{A}	1.58_{A}	1.10^{a}_{A}

*—the means were compared separately for the factors (Cultivar, Fertilization, Herbicide, PGP) and year. **—the linear effect is described in superscript (lowercase—a, b), which, for the factor with three levels, indicates a comparison of values obtained at extreme levels (the lowest and the highest) of the factor. Only the means denoted with lowercase letters are compared, and means with different lowercase letters were significantly different at $p \leq 0.05$. ***—square effect is described in subscript (capital letters —A, B), which, for the factor with three levels, means a comparison of the value obtained for the central factor level with the average of the values obtained for the extreme levels (the lowest and the highest) of the factor. The means were significantly different at $p \leq 0.05$. #—F0: 0 kg $CaCN_2 \cdot ha^{-1}$; F1:78.5 kg $CaCN_2 \cdot ha^{-1}$; F2:157 kg $CaCN_2 \cdot ha^{-1}$.

4. Discussion

4.1. Yield and Yield Components of Common Buckwheat

Common buckwheat is a relatively unpopular crop that is often underestimated in terms of its nutritional value [42]. Its yield depends on many factors, including variability in pluviothermic conditions during the growing season, sensitivity to agricultural chemicals, and lack of an effective method of weed control [43–46].

Pluviothermic conditions during the years of the study influenced the levels of buckwheat yields. The high yield of achenes in 2016 followed the favorable precipitation in that year. That year, the hydrological deficiency, characteristic of Poland, decreased over the growing season and remained relatively small at the stage of flowering and seed maturation.

The dominant cultivar in terms of achene yield was Smuga (2672 kg·ha^{-1}). Fang et al. [47], who also studied three buckwheat cultivars, reported higher achene yield in the cv. Youqiao no. 2 buckwheat compared to cv. Ukraine daliqiao by nearly 27%. Amelin et al. [48], on the other hand, showed higher yields in cultivars with determinate growth compared to cultivars with indeterminate growth by nearly 25%. Bavec et al. [49] also reported 34% higher achene yields for the Darja cultivar relative to locally occurring buckwheat genotypes. The Smuga cultivar was also characterized by higher plant density at emergence, number of seeds per plant, and thousand-seed weight compared to the other cultivars tested.

Calcium cyanamide is used as a source of nitrogen for plants [50]. However, nitrogen fertilizers containing calcium cyanamide in their composition are not without disadvantages because, during the decomposition of calcium cyanamide in the soil, chemical compounds such as dicyandiamide or free cyanamide are formed that negatively affect the growth and development of crops [7,12]. In the presented research, we found no negative effect of calcium cyanamide on the yield of buckwheat. The scientific literature presents facts confirming a 50% increase in eggplant (*Solanum melongena* L.) yield following fertilization with calcium cyanamide at 150 g·m^{-2} compared to sites not fertilized with any form of nitrogen (control) [51]. Tembley et al. [52] reported a 50% increase in the yield of cauliflower (*Brassica oleracea* var. *botrytis* L.) after calcium cyanamide fertilization at 500 kg·ha^{-1} compared to plants not fertilized with nitrogen (control). Sabatino et al. [53] observed a statistically significant increase in the yield and quality traits of strawberry (*Fragatia x ananassa* Duchesne) after applying calcium cyanamide fertilization at 400 kg·ha^{-1}. Di Gioia et al. [54] claim that calcium cyanamide fertilization at 120 kg·ha^{-1} increased the dry matter content of lettuce (*Lactuca sativa* L.) by 50% compared to the control. Kaushal et al. [55] documented the positive effect of calcium cyanamide fertilization (100 kg·ha^{-1}) on soybean—an increase in the number of pods by 330 per unit area and seed yield by 1.49 t·ha^{-1} compared to control.

The herbicides used in this study did not affect buckwheat's yield and yield components. Sakaliene et al. [3] also showed no differences in buckwheat seed yield after the application of an herbicide containing chlorpyralid. On the other hand, Hasanuddin et al. [56] obtained a 35.4% higher seed yield in soybeans on sites protected with pendimethalin at 1.5 kg·ha^{-1} compared to unprotected sites. In our study, we showed a negative effect of herbicides on the field emergence capacity of buckwheat. The application of linuron reduced the plant density after emergence by 5% compared to unprotected objects. This decrease is low in comparison to its effect in a study on sage (*Salvia hispanica* L.), where it reduced the post-emergence plant density by 41% compared to controls [57].

Biostimulants can be an integral part of agrotechnology. Their use can positively influence the morphological traits of crops, but this is not the only known effect of these preparations [58–62]. They can also induce changes in plant physiology [30,63], even at the molecular level, e.g., in protein biosynthesis (transcriptomes) [64,65]. Finally, the use of biostimulants can improve crop yields [66–69].

In this study, the application of a biostimulant containing daminozide had a positive effect on the thousand-seed weight of the buckwheat, resulting in its increase compared to the use of *Ecklonia maxima* algae extract or nitrophenols. In addition, daminozide, as a regulator of plant growth and development, has been shown to initiate inflorescence growth and development in ornamental plants [70,71]. Plants treated with daminozide are characterized by a higher number of flowers in the inflorescence, which may indirectly result in an increase in the number and weight of seeds [72].

The applied nitrophenols and daminozide maintained the yield of buckwheat achenes treated with calcium cyanamide at the level of objects fertilized with ammonium nitrate. The use of nitrophenols in combination with calcium cyanamide fertilization resulted in a tendency to increase the number of seeds per plant compared to the use of nitrophenols combined with ammonium nitrate fertilization. Calcium cyanamide shows toxic properties in crop plants, similar to some active substances contained in herbicides [73]. The literature provides examples of biostimulants reducing the negative effects of active substances contained in herbicides on crop plants [74–76]. The study by Neschev et al. [77] on the use of biostimulants containing free amino acids (Amino Expert® Impuls, Aminozol® and Terra-Sorb®) in the cultivation of musk pumpkin (*Cucurbita moschata* Duchesne ex Poir.) showed a reduction in the negative impact of imazamox on fruit yield.

4.2. Buckwheat Vegetation Indices

The LAI varies between years and depends on the crop species and phenological phase [78–80]. In this study, the LAI in 2017 (5.87 $m^{-2}\cdot m^{-2}$) was influenced by plant density (155 pcs$\cdot m^{-2}$), the assimilation area of a single leaf (12.01 cm^2), and the number of leaves per plant (16.9 pcs$\cdot$plant^{-1}). On the other hand, the LAI did not correspond to the yield of buckwheat achenes in that year.

The NDVI was the highest in 2018 in the BBCH 50–59 stage of buckwheat. The highest NDVI of the buckwheat canopy was also recorded in the BBCH 50–59 stage by Witkowicz et al. [30], and its level did not differ significantly from that presented in our study (0.650).

The largest LAIs were found for Kora (5.45 $m^2\cdot m^{-2}$) and Smuga (5.41 $m^2\cdot m^{-2}$), which, at the same time, translated into higher achene yields for these cultivars. Bavec et al. [49] reported a higher LAI for the Darja cultivar relative to locally occurring buckwheat genotypes by 47%. The presented reduction in SPAD in the tested buckwheat cultivars correlated with NDVI. This means that as SPAD decreased, the NDVI of the buckwheat canopy also decreased. Evaluating the NDVI of individual leaves of buckwheat cultivars Rubra, SuQiao, YuQiao, and NingQiao harvested in field conditions, Sytar et al. [81] recorded higher levels than those presented in their study (0.800).

In this study, we found a negative effect of calcium cyanamide on buckwheat vegetation indices. It decreased the LAI compared to the use of ammonium nitrate, which was associated with reduced plant density after emergence. Di Gioia et al. [54] noted an increase in LAI in *Lactuca sativa* (L.) crops after the application of calcium cyanamide compared to non-nitrogen-fertilized plants (control). The applied calcium cyanamide reduced the SPAD and NDVI in relation to these indices observed in plants fertilized with ammonium nitrate. The reduction in LAI, SPAD, and NDVI adversely affected the yield of buckwheat achenes (Table 5). It is worth noting that the negative effects of calcium cyanamide on crops are associated with its decomposition products in the soil, including free cyanamide and dicyandiamide [8,12].

The measurement of buckwheat canopy vegetation indices documented the negative effect of active herbicide substances on the LAI. As a result, the LAI of buckwheat leaves protected with metazachlor and clomazone decreased from 5.43 $m^2\cdot m^{-2}$ (in the chemically unprotected site) to 5.23 $m^2\cdot m^{-2}$. Sharma et al. [82] observed a more than 50% reduction in the LAI of selected *Lens culinaris* (Medik.) genotypes following the use of metribuzin. Biostimulants applied to herbicide-protected buckwheat plants had various effects on the plants vegetation indices. Daminoside application was associated with a tendency to increase SPAD in chemically protected sites compared to chemically unprotected sites (control). NDVI also increased in herbicide-protected buckwheat plants after the application of daminozide compared to chemically unprotected sites (control). The applied nitrophenols maintained the NDVI and SPAD of herbicide-protected buckwheat at the level of the NDVI and SPAD of control.

4.3. The Segetal Flora of the Buckwheat Field

Calcium cyanamide fertilization proved to be an effective means of reducing weed infestation in buckwheat crops. The number of dicotyledonous weeds and the dry weight of weeds per unit area in a buckwheat canopy fertilized with this form of nitrogen fertilizer decreased compared to the use of ammonium nitrate. Leytur et al. [83] showed a greater efficiency of reducing dicotyledonous weeds of 67% after fertilization with calcium cyanamide (150 $g \cdot m^{-2}$) in a pot experiment with *Impatiens valeriana* (Hook.) compared to a control plant not fertilized with calcium cyanamide. Chohura and Kołota [84] observed a 46% reduction in the abundance of dicotyledonous weeds in a white cabbage plantation after fertilization with calcium cyanamide (150 $kg \cdot ha^{-1}$) compared to sites fertilized with ammonium nitrate.

In addition to the undesirable effects of herbicides, we also observed their high effectiveness. The use of metazachlor and clomazone proved to be the most effective method of reducing weeds in buckwheat cultivation. The selection of these chemical compounds as an effective method of eliminating segetal flora in buckwheat cultivation was also confirmed by Podolska [6]. Studies on weed control are very difficult due to the strong phytotoxic reaction of buckwheat to active compounds contained in herbicides [85].

5. Conclusions

In this study, we found no statistically significant effect of calcium cyanamide fertilization on the yield of buckwheat. However, it resulted in a decrease in vegetation indices (LAI, NDVI, and SPAD). Fertilization with calcium cyanamide significantly reduced the abundance of dicotyledonous weeds and the dry weight of weeds. We also found no negative effect of herbicides on the yield of buckwheat; they only reduced the plant density after emergence. Metazachlor and clomazone also reduced the LAI index of buckwheat, but on the other hand, they were most effective in eliminating weeds in the buckwheat canopy.

The use of biostimulants did not increase buckwheat achene yield, but an increase in thousand-seed weight was observed after the application of daminozide. The application of biostimulants containing nitrophenols reduced the negative effect of calcium cyanamide on buckwheat plants while maintaining the yield of achenes at the level of buckwheat following fertilization with ammonium nitrate. The application of biostimulants increased the NDVI of buckwheat fertilized with calcium cyanamide in relation to ammonium nitrate fertilization. Biostimulants did not reduce the negative effect of herbicides on the LAI, in particular in plants protected with metazachlor and clomazone in relation to chemically unprotected objects. The positive effect of the application of daminozide on chemically protected objects was evident in the form of increased SPAD and NDVI compared to the control site (chemically unprotected).

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Appendix A

Block 3	Buffer Plot	51 Panda F2 H2 B-Nine	20 Smuga F1 H1 Asahi	50 Panda F0 H2 B-Nine	54 Panda F1 H2 Asahi	19 Kora F1 H1 Asahi	23 Panda F0 H2 B-Nine	49 Smuga F1 H0 Asahi	48 Kora F1 H0 Asahi	26 Panda F2 H2 Kelpak	46 Kora F1 H1 Asahi	27 Panda F1 H2 Asahi	25 Panda F0 H2 Kelpak	24 Panda F2 H2 B-Nine	22 Smuga F1 H0 Asahi	47 Smuga F1 H1 Asahi	21 Kora F1 H0 Asahi	53 Panda F2 H2 Kelpak	52 Panda F0 H2 Kelpak	Buffer Plot
Block 2	Buffer Plot	42 Panda F2 H1 Asahi	40 Smuga F1 H2 Kelpak	13 Smuga F1 H2 Kelpak	45 Panda F1 H2 Asahi	15 Panda F2 H1 Asahi	39 Kora F1 H2 Kelpak	16 Panda F0 H0 Asahi	44 Panda F2 H0 Asahi	41 Panda F0 H1 Asahi	10 Kora F1 H2 B-Nine	38 Smuga F1 H2 B-Nine	11 Smuga F1 H2 B-Nine	17 Panda F2 H0 Asahi	12 Kora F1 H2 Kelpak	43 Panda F0 H0 Asahi	14 Panda F0 H1 Asahi	37 Kora F1 H2 B-Nine	18 Panda F1 H2 Asahi	Buffer Plot
Block 1	Buffer Plot	1 Kora F0 H2 Asahi	5 Panda F1 H1 B-Nine	36 Panda F1 H2 Asahi	3 Kora F2 H2 Asahi	6 Panda F1 H0 B-Nine	33 Panda F1 H0 B-Nine	29 Smuga F0 H2 Asahi	2 Smuga F0 H2 Asahi	28 Kora F0 H2 Asahi	9 Panda F1 H2 Asahi	4 Smuga F2 H2 Asahi	7 Panda F1 H1 Kelpak	34 Panda F1 H1 Kelpak	31 Smuga F2 H2 Asahi	8 Panda F1 H0 Kelpak	35 Panda F1 H0 Kelpak	32 Panda F1 H1 B-Nine	30 Kora F2 H2 Asahi	Buffer Plot

Explanation of the abbreviation in the experiment scheme:

Common buckwheat cultivars: Kora, Panda and Smuga

Fertilizer:
F0 – 0 kg $CaCN_2 \cdot ha^{-1}$ (50 kg $N \cdot ha^{-1}$ – NH_4NO_3, 0 kg $N \cdot ha^{-1}$ – $CaCN_2$, 126.5 kg $CaO \cdot ha^{-1}$ – calcium oxide, 0 kg $CaO \cdot ha^{-1}$ – Perlka)
F1 – 78.5 kg $CaCN_2 \cdot ha^{-1}$ (25 kg $N \cdot ha^{-1}$ – $CaCN_2$, 25 kg $N \cdot ha^{-1}$ – NH_4NO_3, 63.5 kg $CaO \cdot ha^{-1}$ – calcium oxide, 63.5 kg $CaO \cdot ha^{-1}$ – Perlka)
F2 – 157 kg $CaCN_2 \cdot ha^{-1}$ (50 kg $N \cdot ha^{-1}$ – $CaCN_2$, 0 kg $N \cdot ha^{-1}$ – NH_4NO_3, 0 kg $CaO \cdot ha^{-1}$ – calcium oxide, 126.5 kg $CaO \cdot ha^{-1}$ – Perlka)

Herbicide:
H0 – No herbicide
H1 – Metazachlor (Metazanex 550 SC) + clomazone (Command 480 EC)
H2 – Linuron (Linurex 500 SC)

Biostimulants:
B-Nine – daminozide (B-Nine 85 SG)
Asahi –Nitrophenols (Asahi SL)
Kelpak – Algae extract *Ecklonia maxima* (Kelpak SL)

Figure A1. Scheme of the field experiment.

Figure A2. The experiment with the common buckwheat (BBCH 15).

Figure A3. The experiment with the common buckwheat (BBCH 51).

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