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ORIGINAL ARTICLE

A combination of bacterial products and cover crops as an innovative method of weed control in organic spring barley

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Abstract

Field research was conducted at Siedlce University of Natural Sciences and Humanities in 2019–2021. The objective was to determine the effects of bacterial formulations and cover crops on the biomass, number and species composition of dominating weeds prior to spring barley harvest. The field trial involved two factors: A – bacterial formulations: I – control, II – nitrogen-fixing bacteria (*Azospirillum lipoferum Br17, Azotobacter chroococcum*), III – nitrogen-fixing bacteria (*Azospirillum lipoferum Br17*, *Azotobacter chroococcum*) + phosphorus-solubilizing bacteria (*Bacillus megaterium* var*, phosphaticum*, *Arthrobacter agilis*), IV – nitrogen-fixing bacteria (*Azotobacter chroococcum*) + plant growth-promoting rhizobacteria (PGPR) (*Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Pseudomonas fluorescens*); B – cover crops: control without a cover crop, red clover, red clover + Italian ryegrass, Italian ryegrass. Spring barley was harvested in late July. Weed samples were collected just before harvest to determine the fresh and dry matter of weeds as well as their number and species composition. The research demonstrated conclusively that an application of bacterial products combined with cover crops contributed to a significant reduction in the weight and number of weeds including dominating species such as *Chenopodium album*, *Sinapis arvensis*, *Tripleurospermum inodorum* and *Elymus repens*. Superior weed control was achieved in spring barley grown in combination with *Azotobacter chroococcum* + PGPR and a mixture of red clover and Italian ryegrass as a cover crop.

Keywords: cereals, intercropping, microorganisms, organic agriculture, weeds

Introduction

Considerable weed infestation is a drawback of growing cereals in organic agriculture. To counteract this negative phenomenon, alternative solutions involving innovative technologies should be sought. Biofertilizers may offer such a solution as they enhance cereal growth and development and, indirectly, contribute to reduced weed burden. The problem of weed infestation has been of world-wide interest for decades. However, in Poland there has been limited research on weeds despite the fact that proposals of the European Green Deal promote organic agriculture involving the utilization of innovative technologies relying on bacterial formulations to control weeds in cereals. Bacterial products facilitate crop plant growth by supplying them with nutrients through biological nitrogen fixation or enhanced availability of insoluble nutrients in soil due to synthesis of substances stimulating plant growth (Ahmad *et al.* 2017; Mumtaz *et al.* 2018). Microorganisms such as bacteria which fix nitrogen or solubilize phosphates tend to, respectively, convert atmospheric nitrogen into plant-available forms, and produce enzymes and solubilize insoluble phosphorus from organic and inorganic sources (Dalve *et al.* 2009; Iqbal *et al.* 2022). Biofertilizers improve plant growth and stand quality by stimulating direct or indirect release of plant hormones (Mumtaz *et al.* 2019; Khan *et al.*

2021). Also, the mechanism acquired by microorganisms to promote plant growth includes the production of phytohormones such as auxins, gibberellins and cytokinins (Dalve *et al.* 2009). Plant growth promoting rhizobacteria (PGPR) improve root development and plant growth by solubilizing insoluble phosphorus and releasing hormones which ameliorate plant growth (Ahmad *et al.* 2018). Rhizosphere bacteria and endophytic bacteria are free-living bacteria which are facultative rhizosphere inhabitants and plant root colonizers, which are directly correlated with improved plant growth and yield (Kumar *et al.* 2014; Khan *et al.* 2021). In recent years, an application of beneficial microbes in cereal cultivation has revealed meaningful effects in the form of enhanced performance of various crops grown in changeable environments (Mumtaz *et al.* 2019; Hussain *et al.* 2020). *Azotobacter*-based biofertilizers have unique properties such as nodule formation which entail resistance to environmental stresses (Aasfar *et al.* 2021). In Poland, drought-induced stress has increased in recent years and an application of bacteria might relieve the effects of drought. Research by Naseri *et al.* (2013) demonstrated that an application of *Azotobacter chroococcum* + *Pseudomonas putina* in spring barley cultivation improved plant morphological parameters and, indirectly, controlled weed infestation of the field cropped to the cereal. Similarly, Dar *et al.* (2020) found that bacteria of the genus *Pseudomonas* contributed to good weed control and stimulated wheat growth. The aforementioned research needs to be continued as it might lead to the development of bio-herbicides that improve the quality of the environment (Dar *et al.* 2020). Also, Abbas *et al.* (2020), who applied the bacteria *Pseudomonas* in rice cultivation, reported lowered weed burden and improved parameters of crop plant growth. However, there is a lack of such research conducted under temperate conditions. Cultivation with cover crops (CC) which suppress weeds is another way of reducing weed burden in organically managed cereals. As confirmed in the study by Arluskienė *et al.* (2021), a field of oats grown with a CC of red clover was less infested with weeds. The same pattern was observed when spring barley was accompanied by white clover. In the past, in experiments which were conducted to examine the potential of undersown catch crops to minimize nitrogen leaching (Amossé *et al.* 2013), the issue of weed infestation often played only a marginal role in the evaluation (Sardana *et al.* 2017). Thus, further research is warranted on different competitive abilities against weeds of a wide array of CC, especially in organic farming systems (Dar *et al.* 2020) in which significant importance is ascribed to leguminous crops (Kocira *et al.* 2020). Living mulches, as CC are often referred to, may also contribute considerably to programs of non-chemical weed control (Westbrook *et al.* 2022). In the study by Kosinski *et al.* (2011), the living mulches of *Trifolium repens* L. and *Lotus corniculatus* L. significantly reduced weed biomass and density in winter wheat. Mechanisms behind weed suppression by living mulches include inhibition of weed seed germination due to shadowing, competition for light and nutrients, and allelopathies (Salonen and Ketoja 2020). In organic agriculture, it is very important to identify and understand patterns which determine interactions in and between crop plants and weeds. In multi-species stands, the vertical and horizontal position of stems and arrangement of leaves are different, which makes it possible for arable crops to better utilize solar radiation while weeds receive less light and are smothered (Yadollahi *et al.* 2014; Sturm *et al.* 2018). However, there is no research on the combined application of bacterial products and CC in order to achieve weed suppression in organic agriculture. It can be hypothesized that a combination of CC and treatment with bacterial products will reduce weed infestation compared to bacterial products applied alone. This research hypothesis assumed that bacterial formulations and CC significantly affect the weed biomass and species composition, and that their appropriate combination will keep weed infestation to a minimum in organically managed spring barley. The objective of the undertaken research was to determine the effects of bacterial formulations and CC on the biomass, density and species composition of dominating weeds prior to spring barley harvest.

Materials and Methods

A field experiment was carried out in Poland from 2019 to 2021 on an organic farm in the village of Wyłazy (52°12’44”N 22°11’05”E) near Siedlce, located in the Mazowieckie Voivodship. The soil of the experimental site was Stagnic Luvisol (IUSS Working Group WRB 2022). The soil reaction was neutral (pH in KCl 6.1) and the organic carbon content was 1.05% d.m. The content of available mineral elements in the soil was as follows: P, 8.3 mg · 100 g^{-1} soil; K, 12.1 mg · 100 g^{-1} soil; and Mg, 4.2 mg · 100 g^{-1} soil. The experimental design was a split-block arrangement with three replicates. The total number of experimental plots in 1 year of the study was 48. The area of one plot was 20 m^2 (5 × 4 m). Two factors were investigated: A – bacterial formulations: I – control (without bacterial formulations), II – inoculant containing nitrogen-fixing bacteria (*Azospirillum lipoferum Br17, Azotobacter chroococcum*), III – simultaneous inoculation with nitrogen-fixing bacteria (*Azospirillum lipoferum Br17, Azotobacter chroococcum*) and phosphorus-releasing bacteria (*Bacillus megaterium var. phosphaticum, Arthrobacter agilis*), IV – nitrogen-fixing bacteria (*Azotobacter chro-*

ococcum) + plant growth-promoting rhizobacteria (PGPR) which also protect the crop against fungi (*Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorescens*); B – cover crop: control (spring barley grown in a pure stand without a cover crop), red clover, red clover + Italian ryegrass and Italian ryegrass. The applied agrotechnical treatments conducted during the field experiment are shown in Table 1.

Spring barley harvest was performed in late July. Just before the harvest, two areas in each plot were randomly selected, by means of a 1.0 × 0.5 m frame, to collect samples of weeds which were then used to determine the fresh matter, dry matter, number and species composition.

Data for each variant studied were analyzed by means of ANOVA suitable for the split-block arrangement. Comparison of means was achieved by means of Tukey test at a significance level of $p \leq 0.05$. All the calculations were performed with the Statistica PL ver. 13.3 software (TIBCO 2017), and MS Excel.

The course of weather conditions varied in the study years (Tab. 2). The highest average air temperature was recorded in 2021 when the precipitation sum amounted to 155.3 mm and was lower than the average long-term sum by 48.9 mm. The lowest rainfall sum was recorded in 2019 when the average monthly air temperature was higher than the long-term mean

Table 1. Agrotechnical treatments carried out during the field experiment

Treatment date	Treatment	Quantity	Comments
October	goat manure	15 t · ha^{-1}	
April	inoculate of *Azospirillum lipoferum Br17* on barley grain	100 ml · 15 kg^{-1} grain	for specific blocks
	seeding of spring barley	160 kg · ha^{-1}	row spacing of 12.5 cm, a depth of 5–6 cm
	seeding of red clover	18 kg · ha^{-1}	row spacing of 12.5 cm, a depth of 1–2 cm, for specific blocks
	seeding of Italian ryegrass	30 kg · ha^{-1}	
	seeding of red clover + Italian ryegrass	9 + 15 kg · ha^{-1}	
	applied *Azotobacter chroococcum*	1 l/250 l water · ha^{-1}	for specific blocks
	applied *Bacillus megaterium var. phosphaticum, Arthrobacter agilis*	1 l/250 l water · ha^{-1}	
	applied *Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorescens*	1 l/250 l water · ha^{-1}	
April (BBCH 10–15)	applied *Azospirillum lipoferum Br17*	1 l/150 l water · ha^{-1}	
May (BBCH 29–30)	applied *Azotobacter chroococcum*	1 l/250 l water · ha^{-1}	
	applied *Bacillus megaterium var. phosphaticum, Arthrobacter agilis*	1 l/250 l water · ha^{-1}	
	applied *Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorescens*	1 l/250 l water · ha^{-1}	

Table 2. Distribution of temperatures and precipitation during spring barley growing seasons according to the Zawady Meteorological Station

Year	Month				Means/Sum
	April	May	June	July	
Temperature [°C]					
2019	9.8	13.3	17.9	18.5	14.9
2020	8.6	11.7	19.3	19.0	14.7
2021	6.6	12.4	20.4	22.7	15.5
Long-term mean	7.9	11.2	16.7	19.3	13.8
Precipitation [mm]					
2019	5.9	59.8	35.9	29.7	131.3
2020	6.0	63.5	118.5	67.7	255.7
2021	42.0	29.5	33.8	50.0	155.3
Long-term mean	49.6	48.2	60.7	45.7	204.2

by 1.1°C. In 2020, the highest precipitation sum was recorded, and the air temperature, averaged across the growing season, was higher than the long-term mean by 0.9°C.

Results and Discussion

Fresh matter of weeds

The fresh matter of weeds before spring barley harvest differed significantly due to growing season conditions and bacterial formulations (Tab. 3). The lowest weed biomass of 73.5 g · m^{-2} was recorded in the dry 2021, being significantly higher by 7.3 g · m^{-2} in 2019, and the highest in 2020 (higher by 9 g · m^{-2} than in 2021) when precipitation was the highest. Similar to light interception by crop plants, water usage is a likely mechanism of weed suppression. Under conditions of reduced water availability from precipitation, crops use a significant portion of the water thus reducing its availability to weeds. This leads to reduced weed development in the proper crop. Research by Naseri *et al.* (2013) demonstrated that *Azotobacter chroococcum* applied with *Pseudomonas putina* in spring barley cultivation improved plant morphological characteristics and, indirectly, reduced weed burden. *Azotobacter*-based biofertilizers have unique properties such as nodule formation which make the amended crops resistant to environmental stresses such as drought (Aasfar *et al.* 2021). In this study, bacterial formulations significantly affected the fresh matter of weeds prior to spring barley harvest. The highest fresh matter of weeds was determined in the field under the control spring barley grown without bacterial products. The application of nitrogen-fixing bacteria reduced the fresh matter of weeds by 75.3 g · m^{-2}, and the combination of nitrogen-fixing bacteria and phosphorus-solubilizing bacteria by 80 g·m^{-2}. The greatest reduction in fresh matter of weeds by 87.8 g · m^{-2} was found after application of *Azotobacter chroococcum* + PGPR. Also in their work, Dar *et al.* (2020) demonstrated a good ability to suppress weeds by stimulating crop plant growth through the use of PGPR. This stimulation results from the fact that bacterial products assist in crop plant growth by supplying the plants with nutrients through biological nitrogen fixation or enhanced availability of insoluble nutrients in soil due to synthesis of substances promoting plant growth (Ali *et al.* 2017; Ahmad *et al.* 2018). Microorganisms such as nitrogen-fixing bacteria and phosphorus-solubilizing bacteria tend to, respectively, convert atmospheric nitrogen into a plant-available form, and produce enzymes and solubilize insoluble phosphorus from organic and inorganic sources of phosphates (Ahmad *et al.* 2017). Biofertilizers improve crop growth and quality by direct or indirect stimulation of plant hormone release (Mumtaz *et al.* 2018). The mechanism acquired by microbes to promote plant growth also includes production of phytohormones such as auxins, gibberellins and cytokinins (Dalve *et al.* 2009). Plant growth promoting rhizobacteria (PGPR) enhance root development (Kumar *et al.* 2014; Ahmat *et al.* 2017; Khan *et al.* 2021). In our experiment, an interaction was confirmed which indicated that the lowest fresh matter of weeds prior to spring barley harvest was recorded in the dry 2021 following an application of *Azotobacter chroococcum* + PGPR, and was the highest in 2019 in the control unit where no bacterial products had been applied.

An interaction between weather conditions and CC was confirmed (Fig. 1). The lowest fresh matter of weeds was determined in 2021 in spring barley grown with Italian ryegrass, and it was the highest in 2020 in the control unit without a CC.

CC significantly influenced the fresh matter of weeds determined before spring barley harvest (Tab. 4).

Table 3. The fresh matter of weeds in spring barley as affected by bacterial formulations in 2019–2021, g · m^{-2}

Bacterial formulations[1] [A]	Years [Y]			Means
	2019	2020	2021	
I	149.1 ± 72.5 a[2]	139.9 ± 79.0 a	130.0 ± 77.7 a	139.7 ± 74.8 A
II	63.8 ± 29.2 b	69.7 ± 33.3 b	59.8 ± 31.9 b	64.4 ± 30.7 B
III	58.8 ± 26.7 c	65.1 ± 31.1 c	55.2 ± 30.5 c	59.7 ± 28.8 C
IV	51.3 ± 24.4 d	55.4 ± 27.4 d	48.9 ± 27.0 d	51.9 ± 25.6 D
Means	80.8 ± 54.2 B	82.5 ± 54.5 A	73.5 ± 54.2 C	

[1] I – control; II – nitrogen-fixing bacteria (*Azospirillum lipoferum Br17, Azotobacter chroococcum*); III – nitrogen-fixing bacteria (*Azospirillum lipoferum Br17, Azotobacter chroococcum*) + phosphorus-releasing bacteria (*Bacillus megaterium var. phosphaticum, Arthrobacter agilis*); IV – nitrogen-fixing bacteria (*Azotobacter chroococcum*) + PGPR (*Bacillus subtilis, Bacillus amyloliquefaciens, Pseudomonas fluorescens*)
± standard deviation
[2] Values in columns for the interaction followed by the same small letter (a, b) do not differ significantly at $p \leq 0.05$; means for the bacterial formulations in a column followed by the same capital letter (A, B) do not differ significantly at $p \leq 0.05$; means for the years in row followed by the same capital letter (A, B) do not differ significantly at $p \leq 0.05$

Table 4. The fresh matter of weeds in spring barley as affected by bacterial formulations and cover crops in 2019–2021, g · m^{-2}

Bacterial formulations[1] [A]	Cover crops [B]			
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass
I	251.4 ± 16.7 a[2]	118.7 ± 40.6 b	91.4 ± 16.7 c	97.2 ± 15.6 bc
II	104.3 ± 15.6 a	52.7 ± 23.0 b	48.4 ± 16.1 b	52.2 ± 17.2 b
III	97.4 ±15.6 a	48.9 ± 21.2 b	44.3 ± 15.0 b	48.3 ± 15.6 b
IV	83.9 ± 14.6 a	42.7 ± 19.5 b	38.9 ± 15.2 b	42.0 ± 15.1 b
Means	134.3 ± 69.8 A	65.8 ± 34.5 B	55.8 ± 26.1 D	59.9 ± 20.4 C

[1] for explanations see Table 3

[2] Values in rowsfor the interaction followed by the same small letter (a, b) do not differ significantly at $p \leq 0.05$; means for the cover crops in rowfollowed by the same capital letter (A, B) do not differ significantly at $p \leq 0.05$

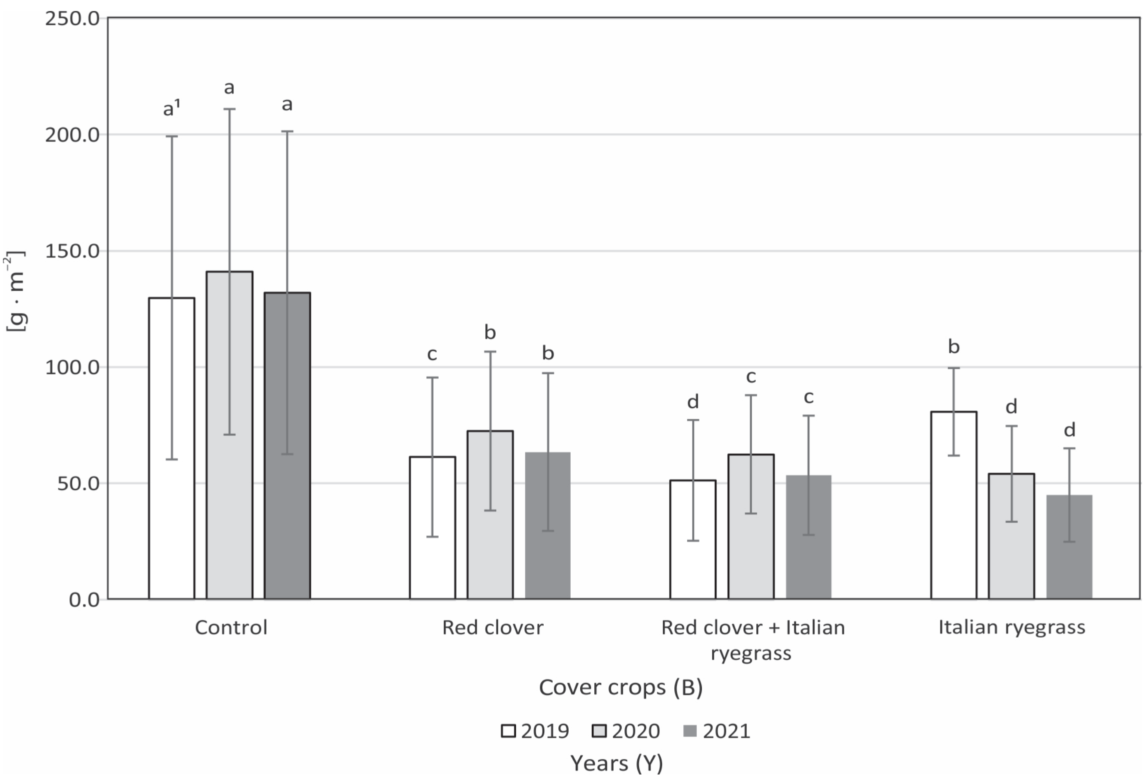


Fig. 1. The fresh matter of weeds in spring barley as affected by cover crops in 2019–2021, g · m^{-2}

The highest fresh matter of weeds was found in spring barley grown without a CC. Growing spring barley accompanied by CC red clover resulted in a 68.5 g · m^{-2} reduction in fresh matter of weeds and with application of CC Italian ryegrass there was a reduction of 74.4 g · m^{-2}. The greatest reduction in fresh matter of weeds of 78.5 g·m^{-2} was found in spring barley accompanied by a mixture of red clover and Italian ryegrass. This finding agrees with research by Bhaskar *et al.* (2014) where white clover contributed to significantly lower weed infestation in organic spring wheat. Kosinski *et al.* (2011), Salonen and Ketoja (2020) as well as Bhaskar *et al.* (2021) claimed that living mulches significantly reduced weed biomass in organically-managed cereals. This, in turn, ensures effective weed control (Cutti *et al.* 2016; Elsalahy *et al.* 2019). Weed biomass production may be curbed by increasing the soil cover with a CC during early growth stages. Moreover, cultivation including a low canopy of CC, whether in a system of mixed cropping or intercrops, is more beneficial for the main crop, in particular if it is a higher species, because of low competition for light between the main crop and the CC (Uchino *et al.* 2011). Achieving a higher soil cover by plant cover from early growth stages, due to an introduction of CC, even if they are low plants, may inhibit weed biomass production (Amossé *et al.* 2013; Lemessa and Wakjira 2015; Cutti *et al.* 2016; Wallace *et al.* 2017; El-salahy *et al.* 2019). This approach is applied in organic cropping (Kocira *et al.* 2020). Although shading by living mulches seems to be an important mechanism of weed suppression, research pertaining to this issue is scarce. Reports concerning reduction of light availability for the main crop (Bartel *et al.* 2020) suggest that weeds experience the shading as well, which was also confirmed in the present study. In the experiment reported here, an interaction between the experimental factors was confirmed indicating that the lowest fresh matter of weeds was recorded in bacterial product--amended plots with CC. It was due to the fact that bio-fertilisers enhance the growth and development of crop plants, including CC, which suppresses weed development in organic spring barley. By contrast, the highest fresh matter of weeds was determined for the control unit where neither bacterial formulations nor CC had been used.

Total number of weeds

Statistical analysis demonstrated a significant effect of growing season conditions, experimental factors and their interaction on the total number of weeds prior to spring barley harvest. The highest number of weeds at 30.5 pcs. · m^{-2} was recorded in 2020 characterized by the highest precipitation sum (Tab. 5). Lower precipitation sums in 2019 and 2021 reduced the number of weeds by 22.1 and 18.7 pcs. · m^{-2}, respectively. Kosinski *et al.* (2011) claimed that both the number and biomass of weeds in barley and triticale were the highest in years with the highest precipitation sums. We found that bacterial products contributed to a significant reduction in the number of weeds before spring barley harvest compared to non-amended control. The highest number of weeds at 34.2 pcs. · m^{-2} was found in the control unit where no bacterial treatments were applied. The greatest reduction of 27.5 pcs. · m^{-2} in the number of weeds was found in the unit treated with *Azotobacter chroococcum* + PGPR. In the case of the nitrogen-fixing bacteria and phosphorus-solubilizing bacteria treatment, the number of weeds was reduced by 25.6 pcs. · m^{-2}. The highest number of weeds among the units on which bacterial preparations were applied was found after the nitrogen-fixing bacteria treatment. However, even then the total number of weeds was 16.2 pcs. · m^{-2} lower than the total number of weeds in the unamended control unit. In their research, Naseri *et al.* (2013) and Iqbal *et al.* (2022) demonstrated that an application of the bacteria *A. chroococcum* accompanied by *Pseudomonas*, as well as new strains of endophytic bacteria and rhizobacteria beneficially affected the development of cereal root systems, and accelerated the growth and development of crop plants, which effectively suppressed weeds. Also, the study by Dar *et al.* (2020) revealed that an application of *Pseudomonas* was an effective method of weed suppression and wheat growth stimulation. Four *Pseudomonas* strains were stimulating in terms of cyanide and siderophore production, phosphorus solubilization, oxidase and catalase activity, and ACC activity *in vitro*. The strains were phytotoxic and caused 73.3% mortality in a biological test of lettuce seedlings. Consortia of compatible *Pseudomonas* strains increased wheat shoot length, root length, fresh biomass, dry biomass and leaf greenness compared to the non-inoculated control. They might play an important part in the weed control of organically grown cereals, as demonstrated in the present research. In the experiment reported here, CC significantly affected the total number of weeds in spring barley (Fig. 2). The lowest number of weeds was recorded in 2019 in spring barley accompanied by a mixture of red clover and Italian ryegrass, being the highest in the control unit (without CC) in 2020 when precipitation was the highest. Regardless of weather conditions during the growing season of cereals, CC significantly reduced weed numbers compared to cereal cultivation without CC (Kosinski *et al.* 2011; Bhaskar *et al.* 2021).

In the present study, the lowest number of weeds was recorded in spring barley accompanied by a mixture of red clover and Italian ryegrass (Tab. 6). The number of weeds was reduced by 18.3 pcs. · m^{-2} in comparison to the control unit. Cultivation of spring barley

Table 5. The number of weeds in spring barley as affected by bacterial formulations in 2019–2021, pcs. · m^{-2}

Bacterial formulations[1] [A]	Years [Y]			Means
	2019	2020	2021	
I	17.5 ± 9.3 a[2]	60.7 ± 21.5 a	24.5 ± 12.4 a	34.2 ± 24.8 A
II	8.6 ± 3.5 b	32.8 ± 11.1 b	12.7 ± 4.7 b	18.0 ± 13.1 B
III	4.4 ± 2.1 c	15.3 ± 9.4 c	6.2 ± 4.1 c	8.6 ± 7.8 C
IV	2.9 ± 1.8 d	13.3 ± 12.2 d	3.9 ± 2.7 d	6.7 ± 8.7 D
Means	8.4 ± 7.2 C	30.5 ± 21.4 A	11.8 ± 12.4 B	

[1, 2] for explanations see Table 3

Table 6. The number of weeds in spring barley as affected by bacterial formulations and cover crops (average 2019–2021), pcs. · m^{-2}

Bacterial formulations[1] [A]	Cover crops [B]			
	Control	Red clover	Red clover + Italian ryegrass	Italian ryegrass
I	58.1 ± 28.2 a[2]	28.4 ± 18.0 b	26.0 ± 18.1 b	24.5 ± 14.1 b
II	28.6 ± 16.7 a	15.3 ± 9.2 b	13.8 ± 9.3 b	14.4 ± 9.5 b
III	17.3 ± 10.2 a	7.0 ± 3.5 b	4.1 ± 2.5 b	6.0 ± 3.5 b
IV	16.2 ± 12.9 a	3.5 ± 2.0 b	3.4 ± 2.2 b	3.7 ± 2.2 b
Means	30.1 ± 24.9 A	13.6 ± 17.8 B	11.8 ± 13.6 D	12.2 ± 11.4 C

[1, 2] for explanations see Tables 3 and 4

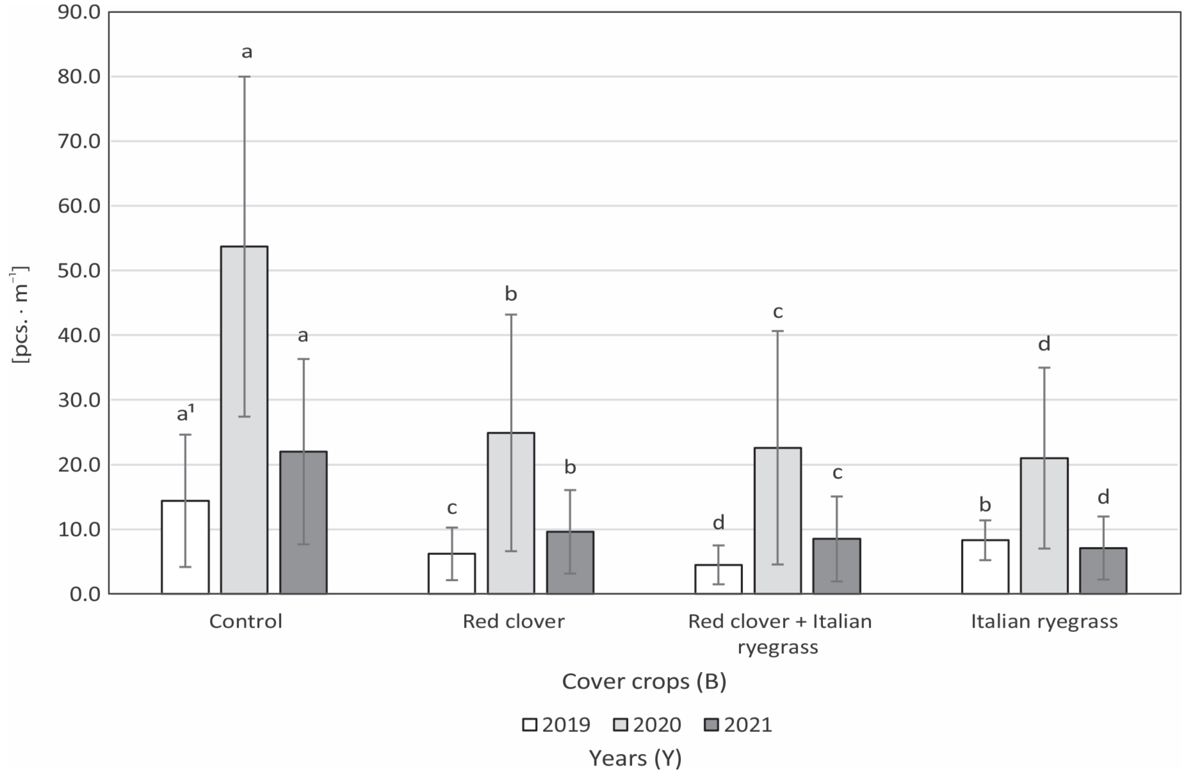


Fig. 2. The number of weeds in spring barley as affected by cover crops in 2019–2021, pcs. · m^{-2}

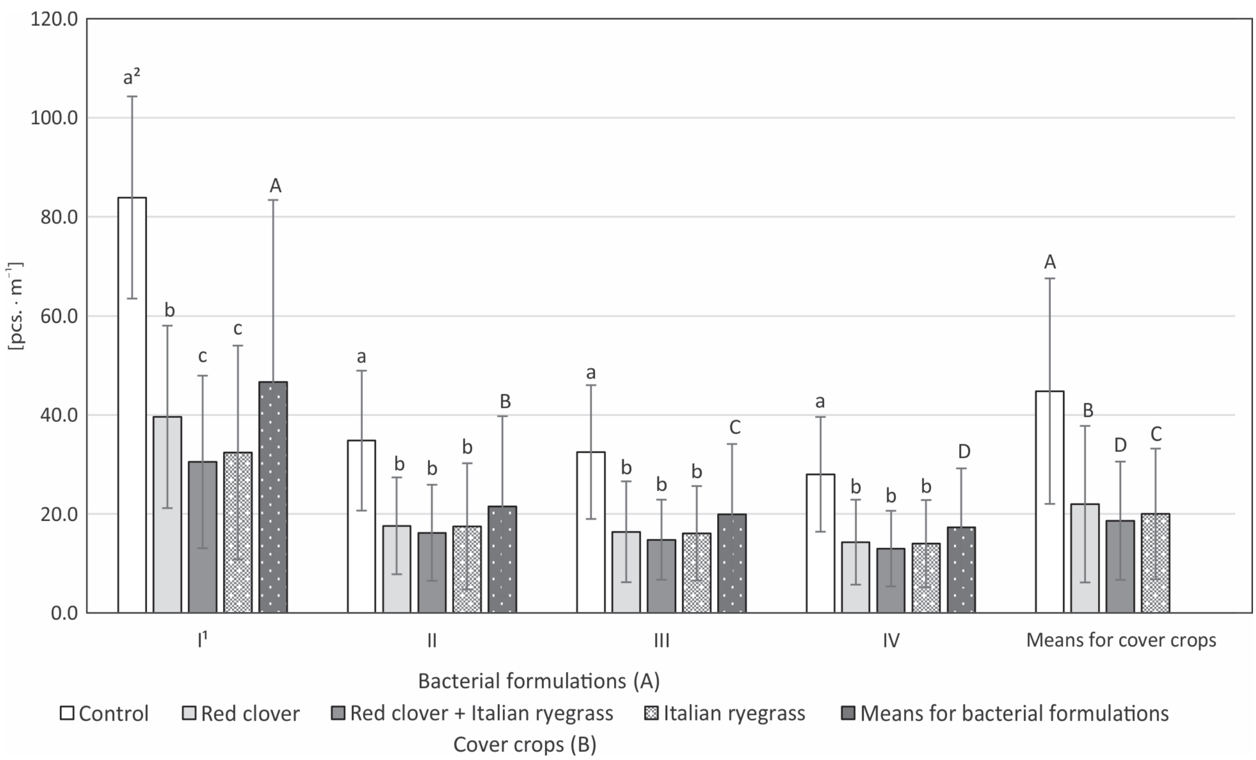
with CC red clover reduced the number of weeds by 16.5 pcs. · m^{-2} and with CC Italian ryegrass by 17.9 pcs. · m^{-2} in relation to the control unit. In order to relieve weed pressure, it is recommended to introduce multi-species cropping systems with the species characterized by varied growing season lengths, and biological and agrotechnological characteristics in one and the same field. Marcinkevičienė *et al.* (2021) emphasized the fact that multiple cultivation, particularly including allelopathic plants, may be used as organic alternatives to control weeds. Altogether, mixed crop stands suffer a lower weed burden than fields cropped with one species. Sowing a plant mixture allows filling more ecological niches, which reduces the chances of and resources for weeds to grow in number (Kocira *et al.* 2020). They should receive more attention, particularly in the context of weed control. CC are believed to suppress weeds but their full potential in this respect has not been adequately investigated in organic cropping systems (Salonen and Koteja 2020; Westbrook *et al.* 2022). There is a paucity of research on the combined application of living mulches and bacterial formulations in organic cereal farming. The study conclusively demonstrated that CC accompanied by *A. chroococcum* + PGPR, or nitrogen-fixing bacteria + phosphorus-solubilizing bacteria contributed to the lowest number of weeds in organic spring barley stands. An application of CC and nitrogen-fixing bacteria was followed by a significant increase in the total number of weeds and yet it was significantly lower than spring barley without CC even if bacterial products had been used.

Dry matter of weeds

Statistical analysis demonstrated a significant effect of experimental factors and their interaction on the dry matter of weeds (Fig. 3). The highest dry matter of 46.6 g · m^{-2} prior to spring barley harvest was determined in the control unit which had not been amended with bacterial products. Formulations applied to organic spring barley contributed to a significant decline in the dry matter of weeds, with the greatest reduction of 29.3 g · m^{-2} in the unit treated with *A. chroococcum* + PGPR. Also in their study, Naseri *et al.* (2013) found that an application of *A. chroococcum* + *P. putina* was followed by a decline in barley weed burden. Research by Dar *et al.* (2020) revealed that selected strains of *Pseudomonas* applied to wheat crop was followed by weed suppression and wheat growth stimulation. In the present work, CC reduced the dry matter of weeds compared to the dry matter of weeds in control spring barley unaccompanied by CC. The lowest dry matter of weeds was recorded for the CC of red clover mixed with Italian ryegrass. In this unit, there was a 26.2 g · m^{-2} reduction in dry matter of weeds compared to the control unit. Bhaskar *et al.* (2014), who examined spring wheat accompanied by white clover, reported lower values of weed dry matter due to the leguminous CC. Also, Kosinski *et al.* (2011), Salonen and Ketoja (2020) as well as Westbrook *et al.* (2022) found that CC substantially contributed to non-chemical weed control as they effectively suppressed weed growth in cereals, which lowered their dry matter. In the experiment reported here an interaction of the experimental factors was confirmed indicating that the lowest dry matter of weeds was harvested in plots amended with bacterial formulations and spring barley with CC.

Dominant weed species

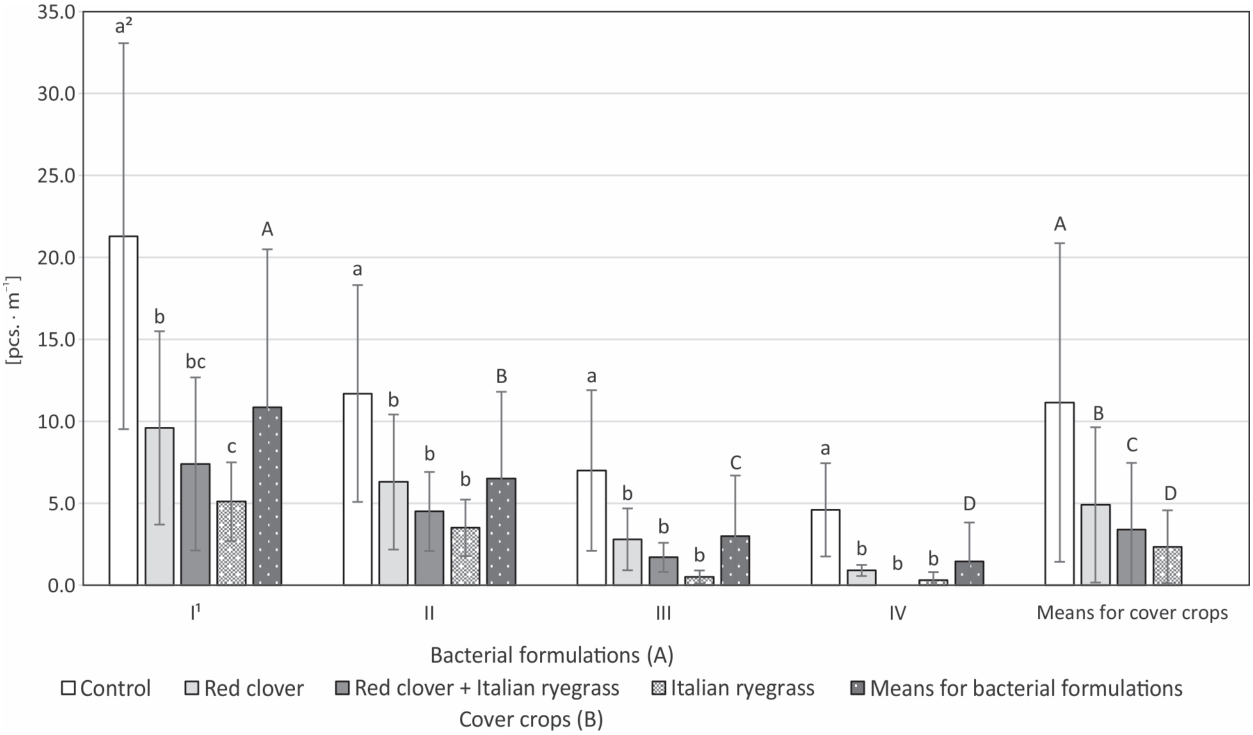
The number of dominant weed species in the spring barley canopy significantly differed with the tested experimental factors and their interactions (Fig. 4–7). Application of bacterial preparations compared to the control unit significantly reduced the occurrence of dominant weed species, *Chenopodium album* by



[1]I – control; II – nitrogen-fixing bacteria (*Azospirillum lipoferum Br17*, *Azotobacter chroococcum*); III – nitrogen-fixing bacteria (*Azospirillum lipoferum Br17*, *Azotobacter chroococcum*) + phosphorus-releasing bacteria (*Bacillus megaterium var. phosphaticum, Arthrobacter agilis*); IV – nitrogen-fixing bacteria (*Azotobacter chroococcum*) + PGPR (*Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Pseudomonas fluorescens*)

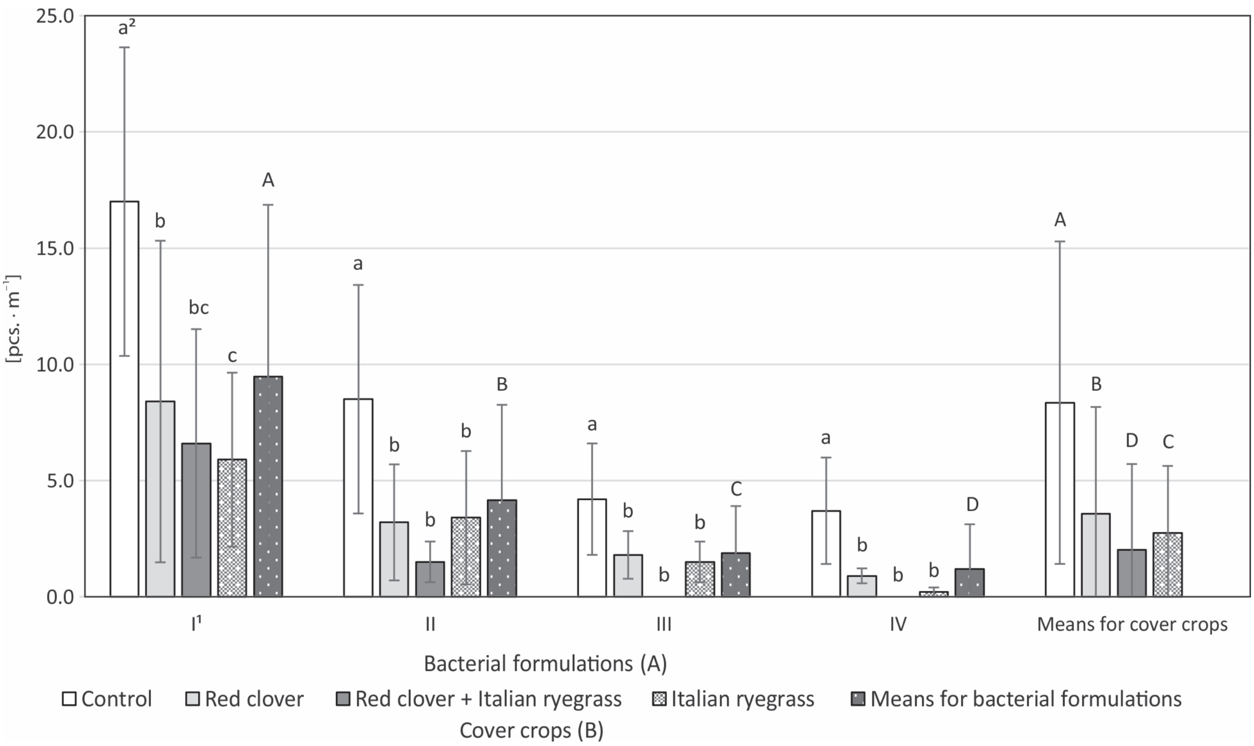
[2] Values in bacterial formulation for the interaction (A × B) followed by the same small letter (a, b) do not differ significantly at $p \leq 0.05$; means for the bacterial formulations and cover crops by the same capital letter (A, B) do not differ significantly at $p \leq 0.05$; ± standard deviation

Fig. 3. The dry matter of weeds in spring barley as affected by bacterial formulations and cover crops (average 2019–2021), g · m^{-2}



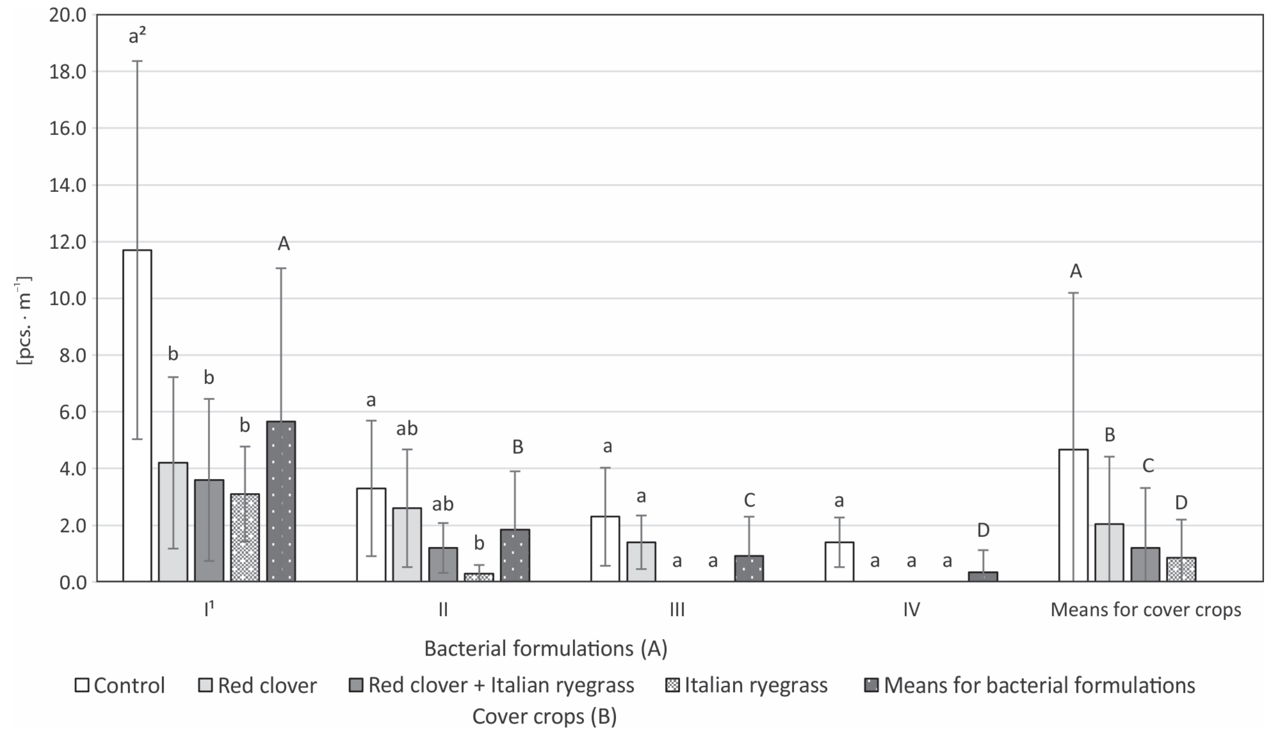
[1,2] for explanations see Figure 3

Fig. 4. The number of *Chenopodium album* plants in spring barley as affected by bacterial formulations and cover crops (average 2019–2021), pcs. · m^{-2}



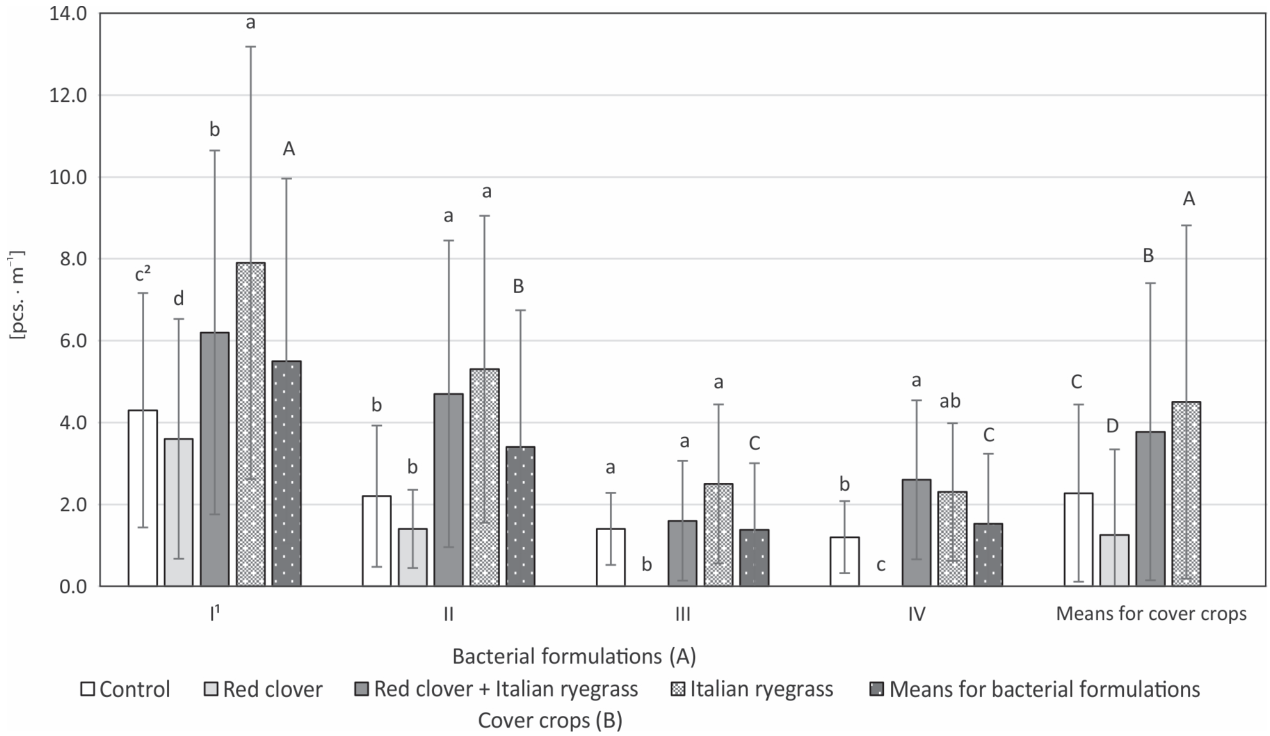
[1,2] for explanations see Figure 3

Fig. 5. The number of *Sinapis arvensis* plants in spring barley as affected by bacterial formulations and cover crops (average 2019–2021), pcs. · m^{-2}



[1,2] for explanations see Figure 3

Fig. 6. The number of *Tripleurospermum inodorum* plants in spring barley as affected by bacterial formulations and cover crops (average 2019–2021), pcs. · m^{-2}



[1,2] for explanations see Figure 3

Fig. 7. The number of *Elymus repens* plants in spring barley as affected by bacterial formulations and cover crops (average 2019–2021), pcs. · m^{-2}

7.2 pcs. · m^{-2}, *Sinapis arvensis* by 7.1 pcs. · m^{-2}, *Tripleurospermum inodorum* by 4.6 pcs. · m^{-2} and *Elymus repens* by 3.4 pcs. · m^{-2}. The lowest number of *Ch. album* 1.5 pcs. · m^{-2}, *S. arvensis* 1.2 pcs. · m^{-2} and *T. inodorum* 0.4 pcs. · m^{-2} were recorded in the unit after applying nitrogen-fixing bacteria + PGPR, and *Elymus repens* 1.5 pcs. · m^{-2} after applying nitrogen-fixing bacteria + phosphorus bacteria and after applying nitrogen-fixing bacteria + PGPR. A study by Naseri *et al.* (2013) showed that the use of *A. chroococum* and *P. putina* in barley cultivation had a beneficial effect on the growth and development of the crop, which indirectly reduced the occurrence of weeds. Also, in studies by Dar *et al.* (2018, 2020) *Pseudomonas* bacterial strains showed good weed suppression ability.

CC also significantly caused differences in the occurrence of dominant weed species. The lowest numbers of *Ch. album* 2.4 pcs. · m^{-2}, *S. arvensis* 2.0 pcs. · m^{-2} and *T. inodorum* 0.9 pcs. · m^{-2} were recorded in the unit with a CC mixture of red clover and Italian ryegrass and *E. repens* 1.3 pcs. · m^{-2} in the unit with a CC red clover. This is due to the fact that in the units with CC, especially with a CC of Italian ryegrass and a mixture of red clover and Italian ryegrass, the soil cover of barley and CC increased, which inhibited the growth of weeds. Also, in a study by Bhaskar *et al.* (2014) CC white clover significantly reduced the number of weeds. This is consistent with the findings of Kosinski *et al.* (2011), Arluskienė *et al.* (2021) and Westbrook *et al.* (2022). In our study, the highest number of *Elymus repens* plants was recorded in spring barley with CC Italian ryegrass 4.5 pcs. · m^{-2}. This is possibly due to the contamination of Italian ryegrass seeds with *E. repens* seeds, especially in seeds from organic agriculture.

In the present experiment, interactions of the tested factors were shown, with the absence of *Ch. album* in the unit after the application of nitrogen-fixing bacteria + PGPR and with a CC mixture of red clover and Italian ryegrass, *S. arvensis* and *T. inodorum* in the units after the application of nitrogen-fixing bacteria + phosphorus bacteria and nitrogen-fixing bacteria + PGPR and with the CC of a mixture of red clover with Italian ryegrass and *E. repens* in the unit after the application of nitrogen-fixing bacteria + phosphorus bacteria and nitrogen-fixing bacteria + PGPR and with the CC of red clover. On the other hand, the highest number of *Ch. album* 21.3 pcs. · m^{-2}, *S. arvensis* 17.0 pcs. · m^{-2} and *T. inodorum* 11.7 pcs. · m^{-2} were recorded in the control unit without the application of bacterial preparations and CC, and *E. repens* 7.9 pcs. · m^{-2} were recorded in the control unit without the application of bacterial preparations with the CC Italian ryegrass.

The results reported in the present paper is decidedly novel. There is a distinct lack of research on the effect of bacterial products and CC on the extent of weed infestation in cereals grown in organic agriculture. The research conclusively indicated that an application of bacterial formulations combined with CC contributed to a significant reduction in the biomass and number of weeds including dominating species. Weeds were the most effectively suppressed in spring barley grown in soils amended with *A. chroococcum* + PGPR and planted with a mixture of red clover and Italian ryegrass as a CC. This type of research needs to be extended to include the organic cultivation of other cereal species and other combinations of bacterial inoculants in order to understand mechanisms employed by microorganisms affecting weeds.

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