

Influence of plant growth-promoting bacteria and green manures on the degree of weed infestation in *Triticum spelta* in organic farming**

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Abstract. The research was conducted on an organic farm in Poland in 2019-2022. The purpose of the research was to determine the effect of plant growth-promoting rhizobacteria and cover crops plowed as green manure on the biomass, number, and species composition of weeds found in *Triticum spelta* grown in organic agriculture. Two factors were researched: I. Bacterial consortium (BC): control without the use of bacterial consortia; BC I: *Bacillus subtilis* + *Bacillus amyloliquefaciens* + *Pseudomonas fluorescens*; BC II: *Bacillus subtilis* + *Bacillus amyloliquefaciens* + *Pseudomonas fluorescens* + *Azotobacter chroococcum*. II. Green manure: control without green manure; *Trifolium pratense* L.; *Trifolium pratense* L. + *Lolium multiflorum* L.; *Lolium multiflorum* L. The research was focused on the combined effect of plant growth-promoting bacteria with green manures on the degree of weed infestation in *Triticum spelta* grown in organic agriculture. The highest efficiency of weed control in *Triticum spelta* was demonstrated after application of BC II together with green manures from a mixture of *Trifolium pratense* with *Lolium multiflorum* and from *Trifolium pratense*.

Keywords: microorganisms, organic amendment, *Triticum spelta*, organic farming

1. INTRODUCTION

At present, there is an increase the cultivation of in cereal varieties grown years ago, whose lower yield is compensated by the high nutritional value of the grain (Sobczyk *et al.*, 2017). An increasingly common crop is *Triticum*

spelta (spelt wheat), known for 5000 years and increasingly grown on organic farms. However, a drawback of its cultivation in organic farming is its high weed infestation. Every year there are large losses in the size and quality of the crop, caused primarily by weed growth. It is estimated that the uncontrolled growth and development of segetal species can cause up to 100% of losses in crops of economic importance. Therefore, it is necessary to counteract this adverse phenomenon. There is an urgent need to look for crop management techniques, including organic crops, which provide opportunities to counteract weed invasion in crops. Around the world, there is growing interest among researchers in innovative crop technologies based on the use of bacterial consortia that reduce weed invasion. In recent years, bacterial preparations containing plant growth-promoting rhizobacteria (PGPR) have increasingly been the subject of research among scientists worldwide (Reed and Glick, 2023). The widespread interest in this group of bacteria in agriculture is in part due to their ability to positively influence the growth and development of crop plants (Glick, 2020). PGPR promote plant growth as a result of increasing the availability of nutrients in the soil, thereby increasing their absorption, synthesis of phytohormones, or secretion of enzymes (Olanrewaju *et al.*, 2017). Thus, the use of PGPR in crops generally leads to more

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intensive development and increased plant yields (Baris *et al.*, 2014; Artyszak and Gozdowski, 2021; Cinkocki *et al.*, 2021). Indirectly, better plant development can increase the level of competitiveness of crops with weeds, thus reducing their occurrence. In addition, as demonstrated by Dar *et al.* (2020), the use of *Pseudomonas* bacterial strains in wheat cultivation has a beneficial effect on wheat growth and development while also demonstrating good weed suppression ability. Mustafa *et al.* (2019) demonstrated that some bacterial strains can act as bioherbicides, reducing the germination and growth of weeds. Ongoing research in this area has also demonstrated that microorganisms can act very selectively, thus eliminating specific weed species. Therefore, this type of research should be continued worldwide, including Poland, because the efficiency of bacterial preparations depends on climate and soil conditions.

Another way to decrease weed infestation in cereals is to introduce cover crops (CCs) for green manure. By plowing the right amount and at the right time, cover crops not only provide nutrients to crops but also catalyze biochemical transformations in the soil, improving soil fertility (Niewiadomska *et al.*, 2020). CCs prevent weed invasion by becoming competitive through allelopathy, among other mechanisms (Liu *et al.*, 2022; Seidel *et al.*, 2019). It has also been indicated that subterranean clover (*T. subterraneum* L.) can significantly decrease weed biomass and decrease soil seed and species richness (Seidel *et al.*, 2019). This research demonstrated that aqueous CC extracts can release phytotoxic substances that inhibit weed growth. Allelochemicals released by CC as a result of natural decomposition of root secretions and leaching induced by rain can inhibit weed growth and development (Yamane *et al.*, 2014; Frabboni *et al.*, 2017). Research has demonstrated that red clover intercrop sown into winter wheat can inhibit weed growth in soybean or corn rotations (Anderson, 2015). The above research shows that the introduction of CC into the crop is associated with the release of phytotoxic substances from the decomposing CC biomass, which can effectively inhibit the development of weeds in the following crop. This is very important in organic cereal cultivation, where herbicides are not used.

The present research aimed to determine the influence of PGPR and CC ploughed as green manure on the number and species composition of weeds found in *Triticum spelta* grown in organic agriculture. The research hypothesis assumed that the applied bacterial consortia and CC ploughed as green manure would differentiate the biomass and species composition of weeds in the *Triticum spelta* canopy, allowing the selection of a treatment with the lowest biomass and number of weeds.

2. MATERIALS AND METHODS

2.1. Experimental design

The field experiment was conducted on an organic farm near Siedlce (52°12'35"N 22°11'05"E), Poland. The field experiment covered three growing seasons in 2019–2022. Two main factors were analyzed in the conducted study: application of bacterial consortia: control without the use of bacterial consortia; BC I (*Bacillus subtilis* + *Bacillus amyloliquefaciens* + *Pseudomonas fluorescens*), BC II (*Bacillus subtilis* + *Bacillus amyloliquefaciens* + *Pseudomonas fluorescens* + *Azotobacter chroococcum*) and plowing green manure: control without green manure; *Trifolium pratense* L. (red clover); *Trifolium pratense* L. + *Lolium multiflorum* L. (Italian ryegrass); *Lolium multiflorum* L. In each year of the field research, three replications of each experimental object were implemented. The area of one experimental object was 20 m². The average soil parameters before the field experiment was established are shown in Table 1. Average temperatures and precipitation totals during the implementation of the field experiment are shown in Fig. 1. The data comes from the Zawady Meteorological Station (52°03'40"N 22°33'26"E).

Table 1. Soil conditions when conducting field research

Levels of available mineral nutrients (mg kg ⁻¹ soil)				pH in KCL	Organic carbon (g kg ⁻¹ soil)
P	K	Mg	N		
83	121	42	8.9	6.1	10.5
Size fraction of soil (mm)					
Granulometric composition of the soil (%)	2.0-0.05		0.05-0.02	0.02-0.002	<0.002
	79.49		9.58	9.57	1.37

2.2. Agrotechnology field experiment

The crop preceding *Triticum spelta* was *Hordeum vulgare* L. (spring barley) grown with a cover crop. Goat manure with average parameters of 0.54% N, 0.28% P, 0.87% K, and 0.15% Mg was applied under *Hordeum vulgare* L. at 15 t ha⁻¹ in late October and early November. In early April, sowing of *Hordeum vulgare* L. was carried out at 160 kg ha⁻¹, along with cover crops: *Trifolium pratense* L. 16 kg ha⁻¹, a mixture of *Trifolium pratense* L. + *Lolium multiflorum* L. 9 + 15 kg ha⁻¹, and *Lolium multiflorum* L. 30 kg ha⁻¹. *Hordeum vulgare* L. was harvested at the end of July, whereas the cover crops were left in the field. In the final days of September, the cover crop left in the field after *Hordeum vulgare* L. cultivation was incorporated into the soil through plowing as green manure. At the beginning of October, sowing of *Triticum spelta* was carried out at the rate of 230 kg ha⁻¹. In late March and early April, double harrowing was carried out perpendicular to each other to control weeds and improve *Triticum spelta* tillering. During

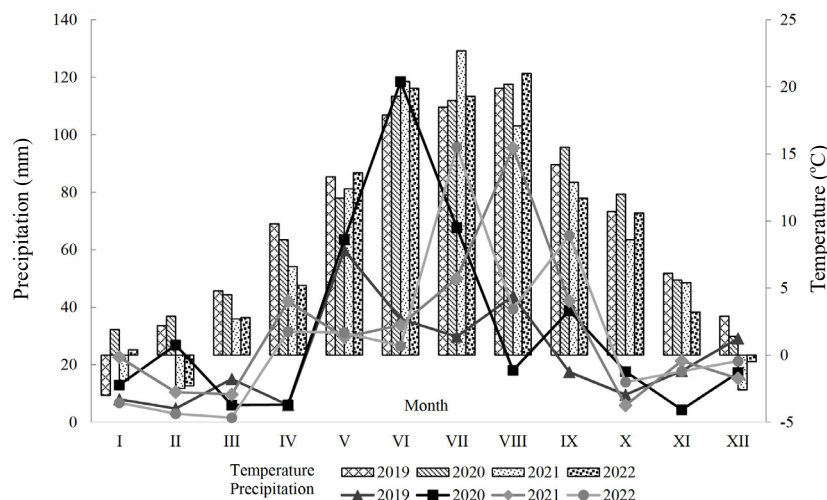


Fig. 1. Distribution of temperatures and precipitation in 2019-2022 recorded by the Zawady Meteorological Station.

the tillering phase, bacterial consortia were applied according to the experimental factor at a rate of 1 L per 150 L of water per hectare. The density of bacterial cells in the applied fertilizer was 10^8 CFU mL⁻¹. The bacterial species used for inoculation came from the collection of the Department of Soil Science and Microbiology of the Poznań University of Life Sciences. They were isolated from under cultivated plants, on selective medium, and then genetically identified on the basis of a fragment of the 16S rRNA gene sequence. *Azotobacter chroococcum* was isolated on Jensen medium, *Bacillus subtilis* on Bacillus ChromoSelect agar, *Bacillus amyloliquefaciens* on starch medium, and King B medium was used for the isolation of *Pseudomonas fluorescens*. The isolates obtained for the study were stored in tubes on agar slants in a refrigerator at 8°C. Before setting up the field experiment, in order for the strains to regain vitality and metabolic activity, the isolates selected for the study were passaged several times onto prepared agar slants with an appropriate medium for the given bacterial species. Then, for each barley inoculation date, liquid cultures of selected inocula were established in 100 mL flasks (five replicates). For this purpose, three-day-old stock cultures of bacteria were used to prepare a suspension by adding 5 ml of saline to each tube on a slant. Subsequently, the microbial cultures were scraped using an eyedropper and the resulting 0.5 ml bacterial suspension was inoculated into 100 mL of liquid medium. The obtained liquid cultures were incubated at 28°C on a shaker at 70 rpm min⁻¹ for 48 h in the case of cultures of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens*, whereas the incubation time in the case of *Azotobacter chroococcum* was 72 h.

2.3. Data collection

Triticum spelta was harvested at the end of July. Immediately before harvesting, two 0.5 x 1 m sites in each experimental object were randomly selected for weed sampling. Immediately after sampling, the fresh matter of

weeds, the number of weeds, and the number of dominant weed species were determined. To determine the dry matter of the weeds, the samples were transported to the laboratory and dried to a constant weight at 65°C in an Ecocell 111 BMT dryer.

2.4. Statistical Analysis

Analysis of variance (ANOVA) was used to assess the significance of differences in the studied traits (fresh and dry matter of weeds, number of weeds, number of dominant weeds) of the variables between the field research factors. Groups with statistically non-different homogeneous mean values of the traits were denoted by the same letter index (Tukey's HSD test; $p < 0.05$). Statistica 13.3 software was used to perform the calculations.

3. RESULTS

3.1. Fresh and dry matter of weeds

The growing season conditions significantly differentiated the fresh matter of weeds before harvesting *Triticum spelta* (Table 1). The highest fresh matter of weeds was recorded in 2022, but significantly lower in 2021 (46% loss of fresh matter of weeds compared to 2022) and in 2020 (38% decrease in fresh matter of weeds compared to 2022). The bacterial consortia also significantly differentiated the fresh matter of weeds before harvesting *Triticum spelta* (Table 1). The lowest fresh matter was recorded in the object exposed to the combined application of BC II (as high as 81% loss of fresh matter relative to the control). Significantly higher values were obtained after the application of BC I, but also in this case, the loss of weed fresh matter relative to the control was 74%. The green manure applied also significantly differentiated the fresh matter of weeds (Table 1). The lowest fresh matter of weeds was recorded after plowing *Trifolium pratense* (48% fresh matter decrease over the control) and a mixture of *Trifolium*

pratense with *Lolium multiflorum* (47% fresh matter loss relative to the control). Although the fresh matter of weeds was significantly higher in the object after plowing *Lolium multiflorum* than in the previously discussed objects, the fresh matter of weeds was also 22% lower than in the control object.

The statistical analysis demonstrated a significant effect of the growing season conditions and the experimental factors on the dry matter of weeds before harvesting *Triticum spelta* (Table 1). The lowest weed dry matter was recorded in 2021 and 2020 (46 and 38% lower, respectively, compared to 2022, with the highest weed dry matter). After the application of BC II and BC I, the weed dry matter was significantly reduced, 81 and 74% lower, respectively, compared to the control. The application of green manures also significantly decreased the dry matter of weeds in the *Triticum spelta* canopy. The lowest weed dry matter was recorded when *Trifolium pratense* and the mixture of *Trifolium pratense* and *Lolium multiflorum* were ploughed as the green manure (48% weed dry matter loss relative to the control). Significantly higher weed dry matter was recorded after plowing *Lolium multiflorum*. However, in this case, too, the weed dry matter loss relative to the control was 22%.

The experiment demonstrated a significant bacterial consortia x green manure interaction for fresh and dry weed matter (Fig. 2). In objects where bacterial consortia were not applied, the significant reduction in weed fresh matter was as follows: Control object (without green manure) > *Lolium multiflorum* > *Trifolium pratense* > mixture of *Trifolium pratense* with *Lolium multiflorum*. On the other hand, the following reduction was observed in the dry matter of weeds: Control object (without green manure) > *Lolium multiflorum* > *Trifolium pratense* and a mixture of *Trifolium pratense* with *Lolium multiflorum*. In the objects where BC I and BC II were applied, the same highest statistical level of fresh and dry weed matter was found in the control object (without green manure) > *Lolium multiflorum* > *Trifolium pratense* and a mixture of *Trifolium pratense* with *Lolium multiflorum*. On the other hand, significantly the lowest levels of fresh and dry weed matter for both bacterial consortia were revealed in objects where *Trifolium pratense* and a mixture of *Trifolium pratense* and *Lolium multiflorum* were ploughed. The year x bacterial consortia interaction was also revealed with regard to fresh and dry weed matter (Fig. 3). In all field research years, the highest fresh and dry weed matter was demonstrated by objects in which bacterial consortia were not applied.

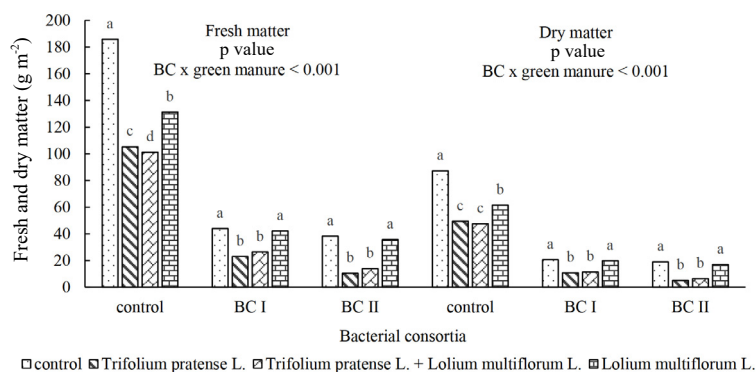


Fig. 2. Fresh and dry matter of weeds and the bacterial consortium x green manure interaction in the field experiment (g m^{-2}). BC I – *Bacillus subtilis* + *Bacillus amyloliquefaciens* + *Pseudomonas fluorescens*; BC II – *Bacillus subtilis* + *Bacillus amyloliquefaciens* + *Pseudomonas fluorescens* + *Azotobacter chroococcum*.

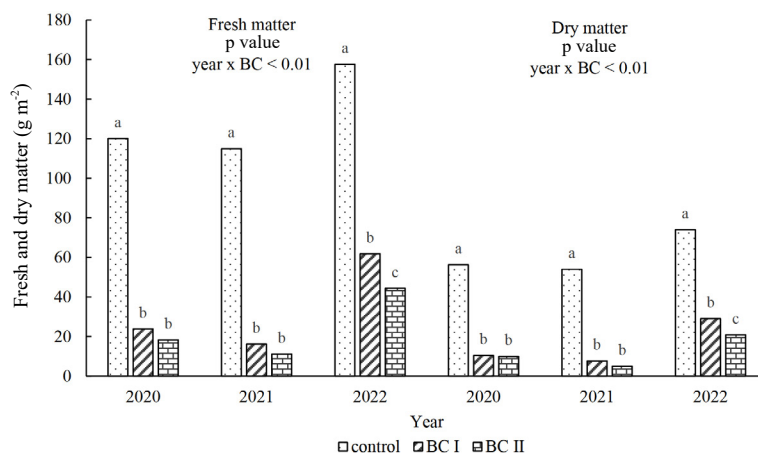


Fig. 3. Fresh and dry matter of weeds and the year x bacterial consortium interaction in the field experiment (g m^{-2}) (means 2020-2022). Explanations as in Fig. 2.

In 2020 and 2021, significantly lower weed matter, but not significantly different, was demonstrated in objects where BC I and BC II were applied. In 2022, on the other hand, significantly lower fresh and dry weed matter was revealed after the application of BC I, while significantly lowest levels were determined after the BC II treatment.

3.2. Number of weeds

The number of weeds before harvesting *Triticum spelta* was significantly differentiated by weather conditions and the experimental factors (Table 2). The smallest number of weeds was recorded in 2021 (78% loss in the number of weeds compared to 2022). A significantly higher number of weeds was recorded in 2020, but also in this case the number of weeds was 58% lower than in 2022. The bacterial consortia used significantly decreased the number of weeds in the *Triticum spelta* canopy. The smallest number of weeds was recorded in the object after the application of BC II (67% decrease in the number of weeds compared to the control). A significantly higher number of weeds was recorded in the object after the application of BC I. However, in this case, too, the decrease in the number of weeds compared to the control was 49%. The applied green manures also significantly differentiated the number of weeds in the *Triticum spelta* canopy. Plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* and *Trifolium pratense* as the green manure resulted in the greatest reduction in weed numbers by 31 and 29%, respectively, compared to the control. Only after plowing *Lolium multiflorum* was the number of weeds significantly higher, and it was not significantly different from that recorded in the control object, without green manure. The field research also found a significant effect of the bacterial consortia x green manure interaction on the number of weeds in *Triticum spelta* (Fig. 4). For all the applied bacterial consortia and the control object (without bacterial consortia), significantly the highest number of weeds was recorded in objects without green manure and in objects where *Lolium*

multiflorum was ploughed. It was significantly lower, however, in the variants with green fertilizer mixtures of *Trifolium pratense* with *Lolium multiflorum* and *Trifolium pratense*. In addition, in objects where BC I was applied, there were no significant differences between the absence of green manure and the introduction of *Lolium multiflorum* and *Trifolium pratense* into the soil.

3.3. Number of dominant weeds

The statistical analysis demonstrated a significant effect of the experimental factors on the number of dominant weed species (Table 3). The application of BC II resulted in a significant reduction in the number of *Galium aparine* L. (cleavers) by 28% compared to the control. In contrast, the application of BC I resulted in a significant increase in the number of *Galium aparine* by 11% compared to the control. The applied green manures also significantly differentiated the incidence of *Galium aparine* in *Triticum spelta*. The highest reduction in the number of *Galium aparine* (by 43% compared to the control) was observed after plowing the mixture of *Trifolium pratense* with *Lolium multiflorum*. The number of *Galium aparine* plants after plowing *Trifolium pratense* (34% decrease vs. the control) as well as *Lolium multiflorum* (22% decrease vs. the control) was at the same level as after plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* and was not significantly different from that recorded in the control object, without green manure.

The applied BC II as well as BC I decreased the number of *Papaver rhoeas* L. (field poppy) plants by 58 and 41%, respectively, compared to the control, without bacterial application. The applied green manures also significantly differentiated the number of *Papaver rhoeas* L. in the *Triticum spelta* canopy. The lowest number of *Papaver rhoeas* L. was recorded in the object after plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* (32% decrease compared to the control). In the other objects, the number of *Papaver rhoeas* L. plants was not significantly

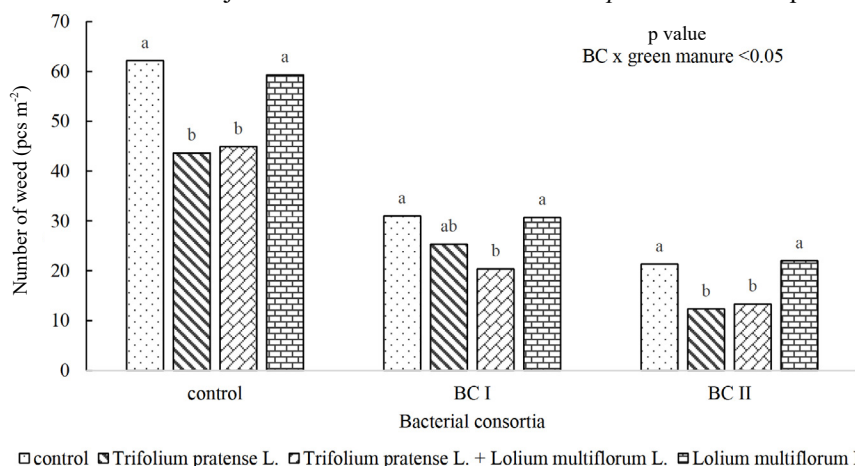


Fig. 4. Number of weeds and the bacterial consortium x green manure interaction in the field experiment (pcs m⁻²). Explanations as in Fig. 2.

Table 2. Mean fresh and dry weeds matter and number of weeds for the main effects of the field experiment

Effect	Fresh matter (g m ⁻²)	Dry matter (g m ⁻²)	Number (pcs m ⁻²)
Bacterial consortia			
Control	130.9 a	61.5 a	52.5 a
BC I	34.0 b	15.7 b	26.8 b
BC II	26.6 c	12.0 b	17.3 c
Green manure			
Control	89.4 a	42.3 a	38.2 a
<i>Trifolium pratense</i> L.	46.3 c	21.9 c	27.1 b
<i>Trifolium pratense</i> L. + <i>Lolium multiflorum</i> L.	47.2 c	21.8 c	26.2 b
<i>Lolium multiflorum</i> L.	69.8 b	32.8 b	37.3 a
Year			
2020	54.1 b	25.6 b	24.7 b
2021	47.4 b	22.2 b	13.0 c
2022	87.9 a	41.3 a	58.9 a
p value			
Bacterial consortia	<0.001	<0.001	<0.001
Green manure	<0.001	<0.001	<0.001
Years	<0.001	<0.001	<0.001

Values in columns for the bacterial consortia, green manure and year followed by the same small letter (a, b, c) do not differ significantly. Other explanations as in Fig. 2.

different from that recorded after plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* and was at the same level as in the control object, without green manure.

The application of BC II and BC I caused a significant decrease in the number of *Tripleurospermum inodorum* L. (scentless mayweed) by 88 and 77%, respectively, compared to the control. The lowest number of *Tripleurospermum inodorum* was recorded after plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* (41% decrease compared to the control). The number of *Tripleurospermum inodorum* plants in the *Trifolium pratense* variant was at the same level as in the aforementioned object (30% decrease relative to the control) and was not significantly different from that recorded after the use of *Lolium multiflorum* (20% decrease relative to the control) and in the control object.

The number of *Apera spica-venti* L. (grain broom) plants after the application of BC II as well as BC I was significantly decreased by 73 and 51%, respectively, compared to the control. Also, the green manures applied decreased the number of *Apera spica-venti* L. plants. The greatest reduction in the number of *Apera spica-venti* L. was observed after plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* (50% decrease compared to the control) and after plowing *Trifolium pratense* (45% decrease compared to the control). After plowing *Lolium multiflorum*, the number of *Apera spica-venti* L. plants was

at the same level as after plowing the other green manures (25% decrease compared to the control) and did not differ significantly from that recorded in the control object.

The greatest decrease in *Elymus repens* (couch grass) (78% decrease relative to the control) was recorded after the application of BC II bacteria. A significantly greater decline (49% decrease compared to the control) was recorded after the application of BC I. However, even in this case, the number of *Elymus repens* was significantly lower than in the control object, without the application of bacteria. Green manures also significantly differentiated the occurrence of *Elymus repens*. The lowest number of *Elymus repens* was recorded in the object where *Trifolium pratense* was plowed (32% reduction compared to the control). The number of *Elymus repens* plants after plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* and in the control object was not significantly different from that recorded after plowing *Trifolium pratense*. In contrast, the highest number of *Elymus repens* plants was recorded in the object where *Lolium multiflorum* was plowed. In this case, there was a 27% increase in the number of *Elymus repens* plants compared to the control.

A significant bacterial consortia x green manure interaction was also demonstrated for the number of *Galium aparine* L. and *Elymus repens* occurring in the *Triticum spelta* crop (Fig. 5). The highest number of *Galium aparine* L. in objects where bacterial consortia were not applied was found in the absence of green manure. On the other hand, the introduction of all types of green manures into

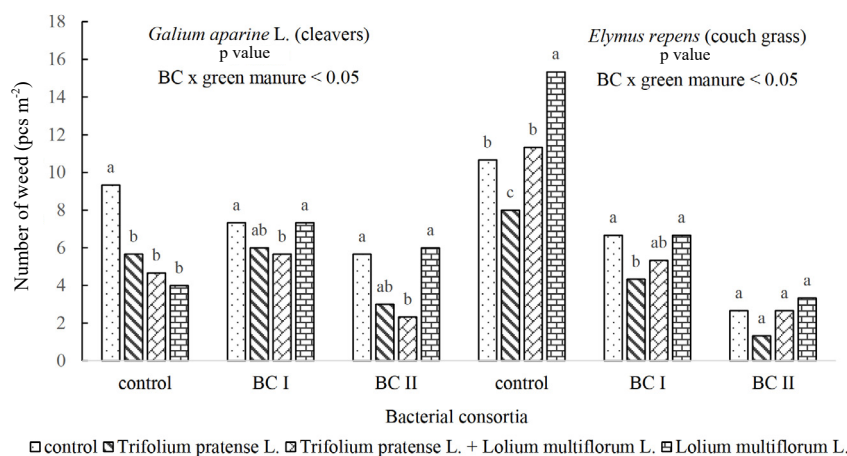


Fig. 5. Number of *Galium aparine* L. (cleavers) and *Elymus repens* (couch grass) for bacterial consortium x green manure interaction of the field experiment (pcs m⁻²). Explanations as in Fig. 2.

Table 3. Mean number of dominant weed the main effects of the field experiment (pcs m⁻²)

Effect	<i>Galium aparine</i> L. (cleavers)	<i>Papaver rhoeas</i> L. (field poppy)	<i>Tripleurospermum inodorum</i> L. (scentless mayweed)	<i>Apera spica-venti</i> L. (grain broom)	<i>Elymus repens</i> (couch grass)
Bacterial consortia					
Control	5.9 a	9.3 a	8.4 a	7.4 a	11.3 a
BC I	6.6 a	5.4 b	1.9 b	3.7 b	5.8 b
BC II	4.3 b	3.9 b	1.0 b	2.0 b	2.5 c
Green manure					
Control	7.4 b	7.6 a	4.9 a	6.2 a	6.7 ab
<i>Trifolium pratense</i> L.	4.9 ab	5.6 ab	3.4 ab	3.4 b	4.6 b
<i>Trifolium pratense</i> L. + <i>Lolium multiflorum</i> L.	4.2 a	5.1 b	2.9 b	3.1 b	6.4 ab
<i>Lolium multiflorum</i> L.	5.8 ab	6.6 ab	3.9 a	4.7 ab	8.4 a
p value					
Bacterial consortia	<0.001	<0.001	<0.001	<0.001	<0.001
Green manure	<0.001	<0.001	<0.001	<0.001	<0.001

Values in columns for the bacterial consortia, green manure and year followed by the same small letter (a, b, c) do not differ significantly. Other explanations as in Fig. 2.

the soil caused a significant reduction in the abundance of this weed. In objects where BC I was applied, the lowest number of *Galium aparine* L. was demonstrated in objects without green manure, lower in objects with *Lolium multiflorum*, and the lowest when green manures were introduced with *Trifolium pratense* and the mixture of *Trifolium pratense* with *Lolium multiflorum*. Additionally, no statistically significant differences were demonstrated in objects where *Trifolium pratense* and *Lolium multiflorum* were used as green manures. On the other hand, after the application of BC II in *Triticum spelta*, the same significantly highest level of *Galium aparine* L. was demonstrated in objects without green manure and objects where *Lolium multiflorum* was plowed. It was significantly

lower in objects where *Trifolium pratense* and mixtures of *Trifolium pratense* with *Lolium multiflorum* were plowed as green manure. With regard to the occurrence of *Elymus repens* in the *Triticum spelta* canopy in the absence of the application of bacterial consortia, the highest number was found in objects with *Lolium multiflorum* green manure, significantly lower in the control object and in those with the mixtures of *Trifolium pratense* with *Lolium multiflorum* as green manure, and significantly lowest with *Trifolium pratense* green manure. However, with BC I and BC II, all objects with and without green manure demonstrated statistically the same *Elymus repens* number.

4. DISCUSSION

Cereal weed infestation in organic farming is a major problem, as herbicides cannot be used in this system of agriculture. Every year, there are huge losses in the size and quality of crops, caused primarily by the growth of weeds. It is estimated that uncontrolled growth and development of segetal species can cause up to 100% of losses in crops of economic importance (Sindhu *et al.*, 2018). Higher weed pressure is recorded during growing seasons with a favorable distribution of precipitation and temperatures (Kosinski *et al.*, 2011; DuPre *et al.*, 2021; Seipel *et al.*, 2022). This is consistent with the results of our research, in which the highest weed pressure was recorded in years with favorable weather conditions.

To decrease the incidence of weeds in organic cereal cultivation, alternative solutions should be sought. One of them is the use of bacterial consortia. This is confirmed by our research, which demonstrated that BC II decreased the growth and development of weeds in *Triticum spelta*. The lowest number and biomass of weeds were recorded after the application of these bacteria. This should be explained by the fact that biofertilizers accelerate plant growth and development, which inhibits weed growth. Interest in this research issue is observed worldwide. Impressive are the findings reported by Dar *et al.* (2020), who demonstrated that the use of *Pseudomonas* spp. bacterial strains in wheat cultivation have a beneficial effect on its growth and development, while at the same time demonstrating a good ability to suppress weeds. Our research and that conducted by other authors on the use of PGPR bacteria in cereal cultivation confirm this relationship (Baris *et al.*, 2014; Artyszak and Gozdowski, 2021; Cinkocki *et al.*, 2021). In addition, Mustafa *et al.* (2019) demonstrated that certain groups of bacteria can act as bioherbicides, reducing the germination and growth of weeds. Research by Abbas *et al.* (2020) demonstrated a reduction in weed infestation in rice as a result of the use of *Pseudomonas fluorescens*. Analogous results after the application of four strains of *Pseudomonas* spp. in wheat crops were obtained by Dar *et al.* (2020). In addition to reducing the number of weeds, these authors also obtained a significant reduction in the length of weed roots (Dar *et al.*, 2020). Also, *Bacillus* ssp. applied in wheat cultivation induced reduction of weeds (Dahiya *et al.*, 2019). This coincides with the results of our research, which used *Bacillus subtilis* + *Bacillus amyloliquefaciens* + *Pseudomonas fluorescens* + *Azotobacter chroococcum*. In this way, the main plants become more competitive than the weeds, limiting their access to resources (Górski *et al.*, 2024). However, some of the available results from field research did not demonstrate any effect of applied bacteria on crop weed infestation (Marcinkevičienė *et al.*, 2023). The different results may have been due to the species composition of the weeds. Weissmann *et al.* (2003) demonstrated a differential effect of *Serratia plymuthica* on the presence

of a number of weeds, including *Chenopodium album*, *Stellaria media*, *Polygonum convolvulus*, and *Galeopsis speciosa*. On the other hand, Li and Kremer (2006) demonstrated that *Pseudomonas fluorescens* inhibits the growth of *Ipomoea* spp. and *Convolvulus arvensis* in wheat crops. In turn, Dahiya *et al.* (2019) demonstrated inhibition of *Avena fatua* growth in wheat crops after application of *Bacillus* spp. These findings confirm the results of our research, in which the greatest reduction in dominant weed species (*Galium aparine* L., *Papaver rhoeas* L., *Tripleurospermum inodorum* L., *Apera spica-venti* L.) was achieved after the application of BC II. The promotion of plant growth as a result of PGPR application is most often attributed to the synthesis of phytohormones and enzymes, mineral solubilization, and biological nitrogen fixation (Hashem *et al.*, 2019; Ladha *et al.*, 2022; Minuț *et al.*, 2023; Sati *et al.*, 2023). However, the efficacy of bacterial preparations can vary depending on the climate, soil conditions or application site (Herrera *et al.*, 2016). Therefore, such research should be conducted worldwide, including Poland.

Another way to decrease weed infestation of cereals in organic farming is to introduce cover crops for plowing as green manure. They prevent weed invasion and become competitive through the mechanism of allelopathy (Liu *et al.*, 2022; Seidel *et al.*, 2019). Researchers have found that *Trifolium subterraneum* L. can significantly decrease weed biomass and decrease the size of the soil seed bank and species richness (Restuccia *et al.*, 2020). Also, in our research, plowing the mixture of *Trifolium pratense* with *Lolium multiflorum* and *Trifolium pratense* as green manure under *Triticum spelta* significantly decreased the biomass and number of weeds compared to the control, without green manure. Research demonstrated that aqueous extracts of cover crops can release phytotoxic substances that inhibit weed growth. On the one hand, sowing cover crops can control weeds, giving crops a greater advantage (Yamane *et al.*, 2014; Mishchenko and Mazik, 2017; Carlesi *et al.*, 2020). On the other hand, allelochemicals released by cover crops as a result of natural decomposition of root secretions and leaching by rain can inhibit weed growth and development (Yamane *et al.*, 2014; Frabboni *et al.*, 2017). The above research demonstrated that red clover intercrop sown into winter wheat can inhibit weed growth in soybean or maize rotations (Anderson, 2015). In addition, legume cover crops can initiate seed germination, leading to faster depletion of the soil weed seed bank, especially in long-term crops (Moonen and Bàrberi, 2004). Better weed reduction in succession crops was also found after plowing legume mixtures with other species compared to non-legume monocultures (Döring *et al.*, 2017; Ranaldo *et al.*, 2019; Wells *et al.*, 2016; Saucke and Ackermann, 2006). The occurrence of the dominant weed species (*Galium aparine* L., *Papaver rhoeas* L., *Tripleurospermum inodorum* L., *Apera spica-venti* L., *Elymus repens*) in the in-field tests was the lowest after the mixture of *Trifolium*

pratense with *Lolium multiflorum* and *Trifolium pratense* was plowed as the green manure. It should be clarified that species grown as cover crops should produce substantial amounts of biomass, which is important for evenly covering the soil surface. In addition, their C:N ratio should be balanced and thus resistant to rapid biomass decomposition, which ensures good growth and development of the successor plant, thus reducing the occurrence of weeds (Kocira *et al.*, 2020). Research demonstrated that annual weeds were suppressed to a greater extent by legume cover crop residues (Kocira *et al.*, 2020; Mohler and Teasdale, 1993; Teasdale and Mohler, 2000; Bhowmik, 2003). The aqueous leachates of the investigated legumes showed strong phytotoxic effects on the growth of *Echinochloa crus-galli* or *Amaranthus hypochondriacus* roots (Caamal-Maldonado *et al.*, 2001). Similar correlations were noted in our study when controlling *Papaver rhoeas* and *Tripleurospermum inodorum*. The greatest loss of *Apera spica-venti* in our study was recorded after plowing *Trifolium pratense* and the mixture of *Trifolium pratense* with *Lolium multiflorum*. In the case of legume cover crops (*T. hybridum*), their allelopathic properties were decisive for the efficiency of weed control (Kocira *et al.*, 2020). In contrast, Ross *et al.* (2001) found that the competitiveness of *Trifolium* spp. is determined not only by morphological characteristics but also by the cultivation method. The inhibitory effect of *M. sativa* cultivars on weeds was proportional to the number and amount of growth inhibitors (phenolic compounds), which showed strong allelopathic activity. Chemicals from allelopathic plants incorporated into the soil are toxic and cause growth inhibition in some species; hence, they can be used as biological tools for weed control (Xuan *et al.*, 2005). Analogous relationships were confirmed by our research after the application of green manure from *Trifolium pratense*, even to control *Elymus repens*. Also, research by other authors (Kocira *et al.*, 2020; Blaser *et al.*, 2011) demonstrated that legumes effectively decrease the number of weeds, including the troublesome perennial weed *Elymus repens*. In our study, the highest number of *Elymus repens* was recorded in the object after plowing *Lolium multiflorum*. This is due to contamination of *Lolium multiflorum* seeds with *Elymus repens* seeds, especially in the organic farming system.

5. CONCLUSIONS

The research conducted presents the combined effect of PGPR with green manures on the degree of weed infestation in *Triticum spelta* grown in organic agriculture. The highest efficiency of weed control in *Triticum spelta* was demonstrated after the BC II bacterial consortium was applied together with the green fertilizer from the mixture of *Trifolium pratense* with *Lolium multiflorum* and *Trifolium pratense*. The efficiency of the bacterial consortium and green manure in varying weather conditions is

also an important finding. The results obtained in the field research may provide an important direction for the development of the weed control technology in organic farming.

Conflicts of Interest: The Authors do not declare any conflict of interest.

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