

## ORIGINAL ARTICLE

## Combination of cover crops and bacterial consortia reduce weediness in organic spelt wheat in Central Europe

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### Abstract

Cultivation technologies based on the use of microbiological preparations or the introduction of cover crops in organic farming are alternatives to chemical plant protection products. To confirm this hypothesis, field studies were conducted in central Poland in 2019–2022 to determine the effect of bacterial consortia and green fertilizers from cover crops on the dry mass, abundance and species composition of dominant weed species occurring in spelt wheat grown in organic farming. Two factors were researched: I. Bacterial consortia: control treatment (no bacteria), bacterial consortium I (*Azotobacter chroococcum* + *Azospirillum lipoferum* Br17), bacterial consortium II (*Bacillus megaterium* var. *phosphaticum* + *Arthrobacter agilis*), bacterial consortium III (*Azotobacter chroococcum* + *Azospirillum lipoferum* Br17 + *Bacillus megaterium* var. *phosphaticum* + *Arthrobacter agilis*), II. Cover crops: control treatment (no cover crops), red clover, red clover + Italian ryegrass, and Italian ryegrass. Spelt wheat was harvested in late July. Just before harvesting, weeds were sampled to determine their dry matter, number, and species composition. The research clearly demonstrated that the application of bacterial consortia with cover crops significantly reduced the dry matter and number of weeds, including the dominant species. The greatest reduction in weed number was recorded in treatments after the application of bacterial consortium III in combination with plowing cover crops of red clover and a mixture of red clover and Italian ryegrass.

**Keywords:** bacterial consortia, cover crop, weed matter, weed number, winter cereal

## Introduction

The ban on synthetic herbicides in organic cereal cultivation requires exploring alternative solutions. One of them is an innovative technology of cereal cultivation based on the use of bacterial preparations. Bio fertilizers accelerate plant growth and development, which inhibits the growth of weeds. Today, interest in this research issue is observed all over the world. The findings of Dar *et al.* (2020) are impressive. They demonstrated that the use of *Pseudomonas* bacterial strains in wheat cultivation has a beneficial effect on its growth and development. At the same time it showed

good weed suppression ability. Other research on the use of plant growth-promoting rhizobacteria (PGPR) in cereal crops confirms this relationship (Baris *et al.* 2014; Artyszak and Gozdowski 2021; Cinkocki *et al.* 2021). Mustafa *et al.* (2019) found that some bacterial strains can act as bioherbicides, reducing weed germination and growth. Ongoing research in this area has shown that microorganisms can act very selectively, thus eliminating specific weed species (Dahiya *et al.* 2019). However, the effectiveness of microbial formulations can vary depending on climate, soil conditions

or application site (Herrera *et al.* 2016). Therefore, it is important to conduct this type of research under different environmental conditions.

Another way to reduce weed infestation in cereals is by introducing plowed cover crops (CC). They prevent weed invasion and become competitive through an allelopathy mechanism (Seidel *et al.* 2019; Liu *et al.* 2022). Researchers have found that *Trifolium subterraneum* L. can significantly decrease weed matter and reduce soil seed bank size and species richness (Restuccia *et al.* 2020). This research demonstrated that aqueous extracts of CC can release phytotoxic substances that inhibit weed growth. CC seeding can control weeds, giving crops a significant advantage (Yamane *et al.* 2014; Mishchenko and Masik 2017; Carlesi *et al.* 2020). The allelochemicals released by CC through natural decomposition of root secretions and leaching by rain can inhibit weed growth (Yamane *et al.* 2014; Frabboni *et al.* 2019). Red clover intercrop sown into winter wheat can inhibit weed growth in soybean or maize rotations (Anderson 2015). The above research suggests that the introduction of the red clover CC into the crop is associated with the release of phytotoxic substances from decomposing red clover matter, which can effectively inhibit weed growth in the following crop. These properties can be used in organic cereal cultivation. In Poland, research results from this area are limited. An attempt to partially fill this gap was the present research aimed at determining the influence of bacterial consortia and CCs on the dry matter, number and species composition of dominant weed species found in spelt wheat grown in organic agriculture.

The research hypothesis assumed that the application of bacterial consortia together with a CC would cause significant differences in the dry matter, number and species composition of dominant weeds. This would allow for the selection of a suitable combination that would most effectively decrease weeds in spelt wheat grown in organic agriculture.

## Materials and Methods

Field research was conducted in Poland from 2019 to 2022 on an organic farm located near Siedlce. The experiment was set up in a split-block arrangement, in three replicates each year. Two factors were researched: I. bacterial consortia: control treatment (no bacteria), nitrogen-fixing bacteria (*Azotobacter chroococcum* + *Azospirillum lipoferum* Br17), phosphorus-solubilizing bacteria (*Bacillus megaterium* var. *phosphaticum* + *Arthrobacter agilis*), and nitrogen bacteria + phosphorus bacteria. II. Cover crops: control treatment (without cover crops), red clover, red clover + Italian ryegrass, and Italian ryegrass. The area of one treatment was 20 m<sup>2</sup> (4 × 5 m). The soil on which the crops were grown was characterized by: pH 6.1, organic carbon 1.05%, P 8.3 mg · 100 g<sup>-1</sup> soil, K 12.1 mg · 100 g<sup>-1</sup> soil and Mg 4.2 mg · 100 g<sup>-1</sup> soil. The weather conditions prevailing throughout the field experiment, obtained from the Zawady Meteorological Station, are presented in Table 1.

In late October and early November, goat manure was applied under spring barley grown in cover crops as a crop preceding spelt wheat at a rate of 15 t · ha<sup>-1</sup>. The average content of individual components in the manure was as follows: 0.54% N, 0.28% P, 0.87% K, and 0.15% Mg. At the beginning of April, sowing of spring barley was carried out together with cover crops, which served as a forecrop for spelt wheat. Sowing was carried out on the same day for all crops. Sowing standards were as follows: spring barley 160 kg · ha<sup>-1</sup>, cover crops: red clover 18 kg · ha<sup>-1</sup>, red clover + Italian ryegrass 9 + 15 kg · ha<sup>-1</sup> and Italian ryegrass 30 kg · ha<sup>-1</sup>. The spring barley was harvested in late July. In late September and early October, the CC was plowed. Then spelt wheat was sown at 230 kg · ha<sup>-1</sup> in early October. In late March and early April, double cross harrowing was carried out to control weeds and improve

**Table 1.** Distribution of temperatures and precipitation in 2019–2022 to the Zawady Meteorological Station

Years	Month												Means/ Sum
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Temperature [°C]													
2019	-3.0	2.2	4.8	9.8	13.3	17.9	18.5	19.9	14.2	10.7	6.1	2.9	9.8
2020	1.9	2.9	4.5	8.6	11.7	19.3	19.0	20.2	15.5	12.0	5.6	1.4	10.2
2021	-1.9	-2.5	2.7	6.6	12.4	20.4	22.7	17.1	12.9	8.6	5.4	-2.6	8.5
2022	0.4	-2.3	2.8	5.2	13.6	19.9	19.3	21	11.7	10.6	3.2	-0.5	8.7
Precipitation [mm]													
2019	7.9	4.7	15.0	5.9	59.8	35.9	29.7	43.9	17.4	9.5	17.8	29.1	276.6
2020	12.9	26.8	5.9	6.0	63.5	118.5	67.7	18.0	38.8	17.6	4.3	17.2	397.2
2021	22.6	10.4	9.6	42.0	29.5	33.8	50.0	95.4	42.1	5.8	21.3	15.2	377.7
2022	6.7	2.9	1.5	31.5	31.1	26.5	95.7	39.3	64.9	13.9	17.7	21.2	352.9

tillering of spelt wheat. During the spelt wheat tillering period, bacterial consortia were applied according to the experimental factor at a rate of 1 l per 150 l water · ha<sup>-1</sup>. The bacterial species used for inoculation were from the collection of the Department of Soil Science and Microbiology at the University of Life Sciences in Poznań, Poland. The density of bacterial cells in the applied fertilizers was 10<sup>8</sup> CFU · ml<sup>-1</sup>.

Spelt wheat was harvested in late July. Just before the harvest, two areas in each plot were randomly selected. A 1.0 × 0.5 m frame was used to collect samples of weeds. The number of weeds and the number of dominant species for each experimental treatment were determined. To determine the dry weight yield of the weeds, the obtained samples were dried in an Eco-cell 111 BMT dryer (BMT Medical Technology, Brno, Czech Republic) at 65°C to a constant weight.

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

## Results

The dry matter of weeds before harvesting the spelt wheat significantly differed between the researched experimental factors and their interaction (Table 2). The lowest weed dry matter was recorded after the application of bacterial consortium I containing nitrogen-fixing bacteria and from bacterial consortium III with nitrogen-fixing bacteria and phosphorus-solubilizing

bacteria (weed dry matter decreased by as much as 75% compared to the control). A significantly higher dry matter of weeds was recorded after the application of bacterial consortium II that included phosphorus-solubilizing bacteria (weed dry matter decreased by 63% compared to the control), and the highest was found in the control treatment without using the bacterial consortium. In the present study, the application of the CC of a mixture of red clover with Italian ryegrass and red clover had the greatest decrease of dry matter of weeds by 44–43% compared to the control. On the other hand, after the application of Italian ryegrass, the dry matter of weeds was significantly higher. However, the decrease of dry matter of weeds was 17% greater than the control, without a CC. There was an interaction of the researched factors after the application of bacterial consortium I. The smallest dry matter of weeds in spelt wheat was recorded after plowing the mixture of red clover and Italian ryegrass and red clover CCs. It was significantly higher after plowing the CC of Italian ryegrass. However, even in this case the dry matter of weeds was lower than in the control treatment without nitrogen-fixing bacteria. After the combined application of nitrogen-fixing bacteria with phosphorus-solubilizing bacteria and after the application of phosphorus-solubilizing bacteria, the smallest dry matter of weeds was recorded after plowing the red clover CC. It was significantly higher after plowing the CC of the mixture of red clover and Italian ryegrass, and it was the highest after plowing the Italian ryegrass CC, but it was significantly lower than in the control treatment without CC. Weather conditions during the growing season of spelt wheat significantly affected the dry matter of weeds (Table 3). The lowest weed dry matter was recorded in 2021. It was significantly higher in 2020, and it was the highest in 2022. In the present study there was significant interaction between growing season conditions and the

**Table 2.** The dry matter of weeds in spelt wheat as affected by bacterial consortia and cover crops (average 2020–2022) [g · m<sup>-2</sup>]

Bacterial consortia*	cover crops				Means
	control	red clover	red clover + italian ryegrass	italian ryegrass	
control	93.6 a**	53.7 c	50.5 d	64.2 b	65.5 A
I	21.1 a	12.8 c	11.3 c	19.7 b	16.3 C
II	32.1 a	15.1 d	18.7 c	30.3 b	24.1 B
III	19.2 a	10.7 d	13.3 c	23.0 b	16.6 C
Means	41.5 A	23.1 C	23.5 C	34.3 B	
P values	bacterial consortia: <0.001; cover crops: <0.001; bacterial consortia x cover crops <0.001				

\*I – *Azotobacter chroococcum* + *Azospirillum lipoferum* Br17; II – *Bacillus megaterium* var. *phosphaticum* + *Atrobacter agilis*; III – *Azotobacter chroococcum* + *Azospirillum lipoferum* Br17 + *Bacillus megaterium* var. *phosphaticum* + *Atrobacter agilis*;

\*\*values in rows for the interaction bacterial consortia x cover crops followed by the same small letter (a, b) do not differ significantly; means for the bacterial consortia in a column followed by the same capital letter (A, B) do not differ significantly; means for the cover crops in row followed by the same capital letter (A, B) do not differ significantly

**Table 3.** The dry matter of weeds in spelt wheat as affected by bacterial consortia in 2020–2022 [ $\text{g} \cdot \text{m}^{-2}$ ]

Bacterial consortia*	Years		
	2020	2021	2022
control	60.7 a**	57.1 a	78.7 a
I	11.6 c	7.7 c	29.4 c
II	19.5 b	15.5 b	37.3 b
III	11.7 c	8.5 c	29.5 c
Means	25.9 B	22.2 C	43.7 A
P values	years: < 0.001; years $\times$ bacterial consortia < 0.05		

\*see Table 2;

\*\*values in columns for the interaction years  $\times$  bacterial consortia followed by the same small letter (a, b) do not differ significantly; means for the years in row followed by the same capital letter (A, B) do not differ significantly

bacteria consortia used. In 2020–2022, the lowest dry matter of weed in the spelt wheat canopy was recorded after the application of bacterial consortiums I and III. After the application of bacterial consortium II, the dry matter of weeds was significantly higher, but lower than that recorded in the control treatment without bacterial consortia. There was also interaction between weather conditions and the applied CC (Table 4). In each year of research, the smallest dry matter of weeds

**Table 4.** The dry matter of weeds in spelt wheat as affected by cover crops in 2020–2022 [ $\text{g} \cdot \text{m}^{-2}$ ]

Cover crops	Years		
	2020	2021	2022
Control	36.9 a*	33.0 a	54.7 a
Red clover	18.3 c	15.0 c	36.0 c
Red clover + italian ryegrass	18.8 c	15.0 c	36.6 c
Italian ryegrass	30.7 b	25.8 b	47.5 b
P values	years $\times$ cover crops: < 0.05		

\*values in columns for the interaction years  $\times$  cover crops followed by the same small letter (a, b) do not differ significantly

was recorded after the application of red clover and a mixture of red clover and Italian ryegrass CCs. It was significantly higher after plowing the Italian ryegrass CC, and it was the highest with the control treatment without CC.

Statistical analysis demonstrated a significant effect of the researched experimental factors and their interaction on the total number of weeds present in the spelt wheat canopy (Table 5). The smallest number of weeds was recorded after the application of bacterial consortium III (there was a 60% decrease in the number of weeds compared to the control). A significantly higher number of weeds was recorded after the application of bacterial consortium I (there was a 46% decrease in the number of weeds relative to the control). In turn, the highest number of weeds among the treatments in which bacterial consortia were applied was recorded after the application of phosphorus-solubilizing bacteria. Also, in this case the decrease in the number of weeds compared to the control was 26%. The conducted studies also showed that the application of CC had a significant influence on reducing the number of weeds in spelt wheat. The greatest reduction in the number of weeds was recorded after the application and plowing of the CCs made up of a mixture of red clover with Italian ryegrass and red clover (the decrease in the number of weeds compared to the control was 32 and 30%, respectively). On the other hand, after the application of the Italian ryegrass CC, the number of weeds in the spelt wheat canopy was at the same level as in the control, without CC. However, there was a 5% decrease of weeds. The analyses also showed that the weather conditions during the growing season of spelt wheat significantly affected the number of weeds (Table 6). The greatest decrease in the number of weeds, by as much as 77%, was recorded in 2021 compared to 2022, the year with the highest number of weeds. In 2020, the number of weeds was 52% less than in 2022. The present study demonstrated the interaction between growing season conditions with bacterial consortia and the number of pre-harvest weeds in spelt wheat.

**Table 5.** The number of weeds in in spelt wheat as affected by bacterial consortia and cover crops (average 2020–2022) [ $\text{pcs} \cdot \text{m}^{-2}$ ]

Bacterial consortia*	Cover crops				Means
	control	red clover	red clover + italian ryegrass	italian ryegrass	
Control	66.7 a**	47.7 c	47.7 c	61.7 b	55.9 A
I	37.3 a	25.3 b	22.8 b	36.3 a	30.4 C
II	47.7 a	36.0 b	35.0 b	46.7 a	41.3 B
III	28.3 a	17.0 b	17.7 b	27.2 a	22.6 D
Means	45.0 A	31.5 B	30.8 B	43.0 A	
P values	bacterial consortia: < 0.001; cover crops: < 0.001; bacterial consortia $\times$ cover crops < 0.05				

\*, \*\*see Table 2



**Table 6.** The number of weeds in spelt wheat as affected by bacterial consortia in 2020–2022) [pcs. · m<sup>-2</sup>]

Bacterial consortia*	Years		
	2020	2021	2022
Control	47.0 a**	24.8 a	96.0 a
I	23.8 c	11.3 c	56.3 c
II	34.5 b	17.8 b	71.8 b
III	20.5 c	7.7 c	39.5 d
Means	31.4 B	15.4 C	65.9 A
P values	years: < 0.001; years × bacterial consortia < 0.001		

\*see Table 2; \*\*see Table 3

In 2020 and 2021, the lowest number of weeds was recorded after the application of bacterial consortium III and after the application of bacterial consortium I. A significantly higher number of weeds was recorded after the application of bacterial consortium II, and the highest occurred with the control treatment, without the application of bacteria. On the other hand, in 2022, the lowest number of weeds was recorded after the application of bacterial consortium III. It was significantly higher after the application of bacterial consortium I, followed by bacterial consortium II. The highest number of weeds was recorded with the control treatment without the application of bacteria. An interaction of weather conditions with CC was also seen (Table 7). In 2020–2021, the smallest number of weeds was recorded after the application of the red clover CC and the mixture of red clover and Italian ryegrass CC. It was significantly higher after the application of Italian ryegrass and in the control treatment, without CC. On the other hand, in 2022, the smallest number of weeds was recorded after the application of CC made up of a mixture of red clover and Italian ryegrass. It was significantly higher after the application of the red clover CC, followed by the Italian ryegrass CC, and it was the highest with the control treatment, without CC.

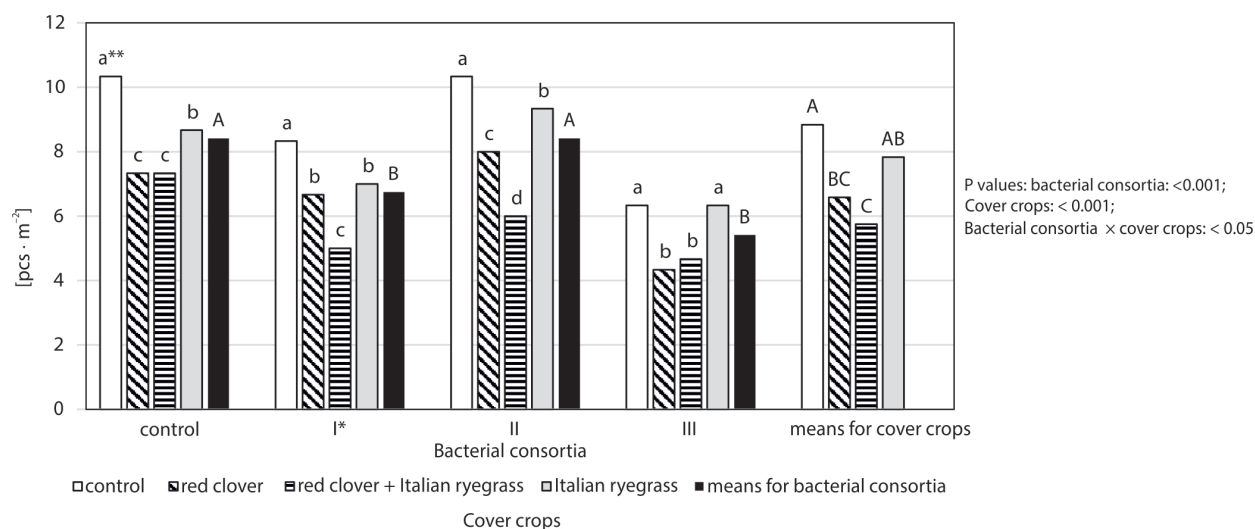
**Table 7.** The number of weeds in spelt wheat as affected by cover crops in 2020–2022) [pcs. · m<sup>-2</sup>]

Cover crops	Years		
	2020	2021	2022
Control	37.5 a*	20.0 a	77.5 a
Red clover	25.0 b	10.5 b	59.0 c
Red clover + italian ryegrass	26.8 b	10.3 b	55.3 d
Italian ryegrass	37.5 a	20.7 a	71.8 b
P values	years × cover crops: < 0.001		

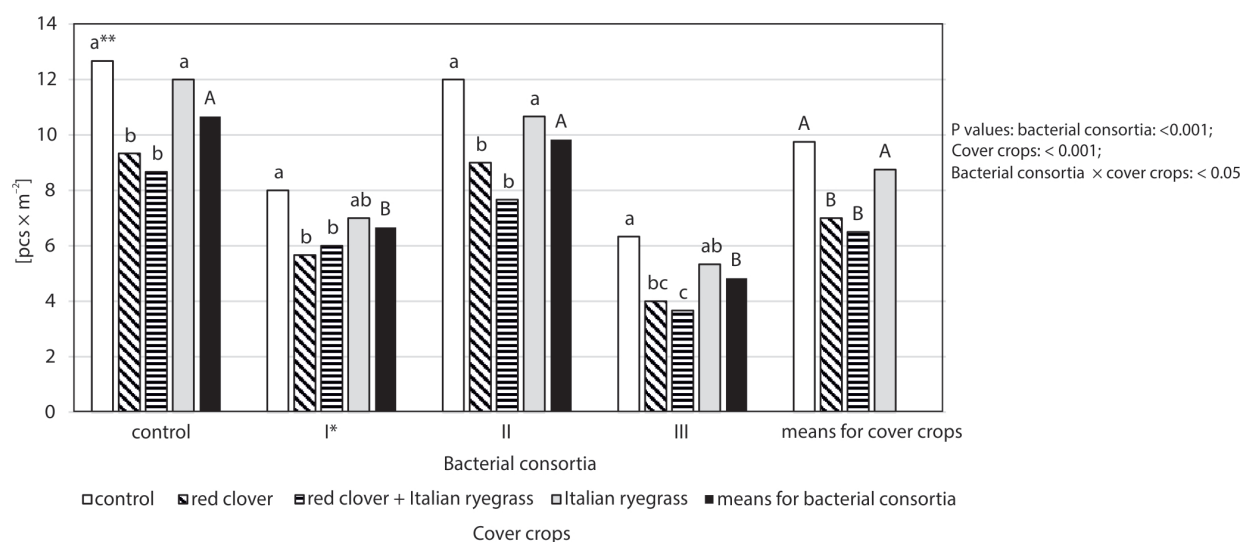
\*see Table 4

The number of *Galium aparine* L. occurring in the pre-harvest spelt wheat canopy significantly differed as a result of the research experimental factors and their interaction (Fig. 1). The lowest number of *G. aparine* L. was recorded after the application of bacterial consortium III and I, and it was significantly higher after the application of bacterial consortium II and with the control treatment without the application of bacteria. CC application also caused significant differences in the occurrence of *G. aparine* L. in the spelt wheat canopy. The lowest number of *G. aparine* L. was recorded after plowing the mixture of red clover and Italian ryegrass CC. After the application of the red clover CC, the number of *G. aparine* L. was not significantly different from that recorded with the aforementioned treatment and was at the same level as the Italian ryegrass CC. In this research, interaction between the studied experimental factors on the occurrence of *G. aparine* L. in the spelt wheat canopy was seen. After the application of nitrogen-fixing bacteria, the lowest number of *G. aparine* L. was recorded after the application of the mixture of red clover and Italian ryegrass CC. It was significantly higher after the application of the red clover and Italian ryegrass CC, and it was the highest with the control treatment without CC. After the application of phosphorus-solubilizing bacteria, the lowest number of *G. aparine* L. was recorded with the application of the of mixture of red clover and Italian ryegrass CC. It was significantly higher after the application of the Italian ryegrass CC, followed by the red clover CC, and the highest was in the control treatment, without CC. On the other hand, after the combined application of nitrogen-fixing bacteria with phosphorus-solubilizing bacteria, the lowest number of *G. aparine* L. was recorded after the application of red clover and a mixture of red clover and Italian ryegrass CCs. It was significantly higher after the Italian ryegrass CC and with the control treatment, without CC.

Statistical analysis demonstrated a significant effect of the researched experimental factors and their interaction on the number of *Papaver rhoeas* L. present in the spelt wheat canopy (Fig. 2). The greatest reduction in the number of *P. rhoeas* L. was observed after the application of bacterial consortium III (the decrease in the number of *P. rhoeas* L. relative to the control was 55%) and I (the decrease in the weed relative to the control was 38%). A significantly higher number of *P. rhoeas* L. was recorded after the application of bacterial consortium II (the loss of *P. rhoeas* L. relative to the control was only 8%). In this case, the number of *P. rhoeas* L. was at the same level as the control. The conducted studies also showed that CC caused significant differences in the occurrence of *P. rhoeas* L. in the spelt wheat canopy. The smallest number of *P. rhoeas*



**Fig. 1.** The number of *Galium aparine* L. in spelt wheat as affected by bacterial consortia and cover crops (average 2020–2022) [pcs · m<sup>-2</sup>]



**Fig. 2.** The number of *Papaver rhoeas* L. in spelt wheat as affected by bacterial consortia and cover crops (average 2020–2022) [pcs · m<sup>-2</sup>]

L. was recorded after plowing the CCs of red clover and a mixture of red clover and Italian ryegrass (the decrease in the number of *P. rhoeas* L. compared to the control treatment was 28%), and it was significantly higher after plowing the Italian ryegrass CC and in the control treatment. Interaction of the researched factors of the experiment showed that the smallest number of *P. rhoeas* L. was recorded after the application of bacterial consortium III and I after plowing a mixture of red clover and Italian ryegrass and after red clover CCs. In this case, the number of *P. rhoeas* L. plants was not significantly different than that recorded in Italian ryegrass. The number of *P. rhoeas* L. plants after plowing the Italian ryegrass CC was at the same level as the control treatment. On the other hand, after application of bacterial consortium II, the number of *P. rhoeas* L. in the red clover and a mixture of red clover and Italian

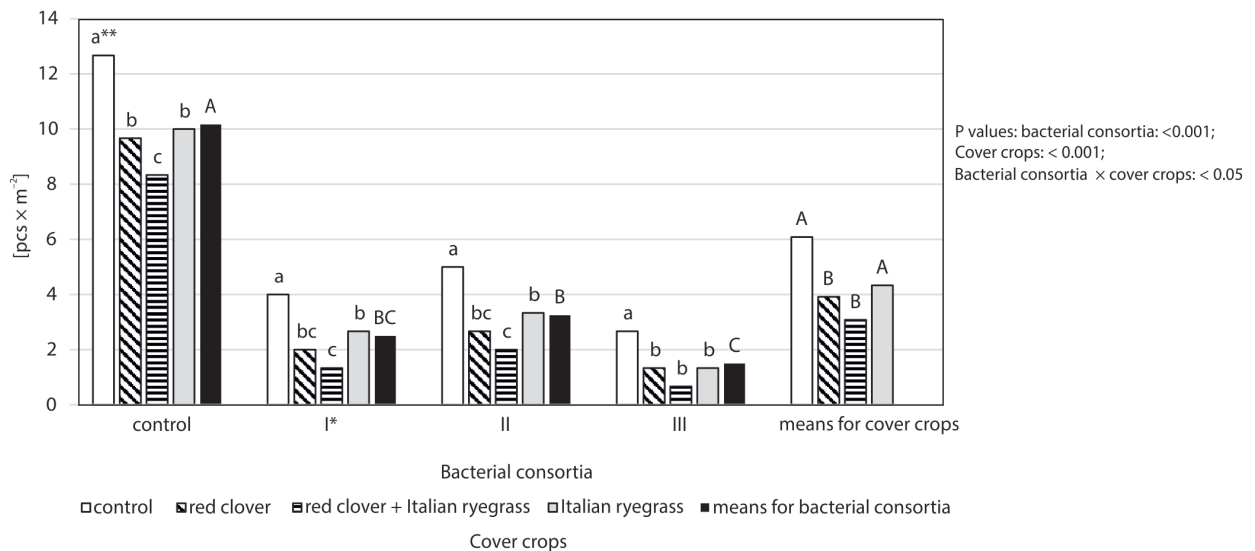
ryegrass CCs was the lowest. It was significantly higher with the CC of Italian ryegrass and in the control treatment without CC.

The number of *Tripleurospermum inodorum* L. in the spelt wheat canopy was significantly influenced by the experimental factors researched and their interaction (Fig. 3). The lowest number of *T. inodorum* L. was recorded after the combined application of bacterial consortium III (weed decrease of 85% compared to the control). After the application of bacterial consortium I (a decrease of the number of *T. inodorum* L. by 75% compared to the control treatment) and after bacterial consortium II (a decrease of the weed by 68% compared to the control), the number of *T. inodorum* L. plants was at the same level as with the lowest number of the weed. Only with the control treatment was the number of *T. inodorum* L. significantly higher. In

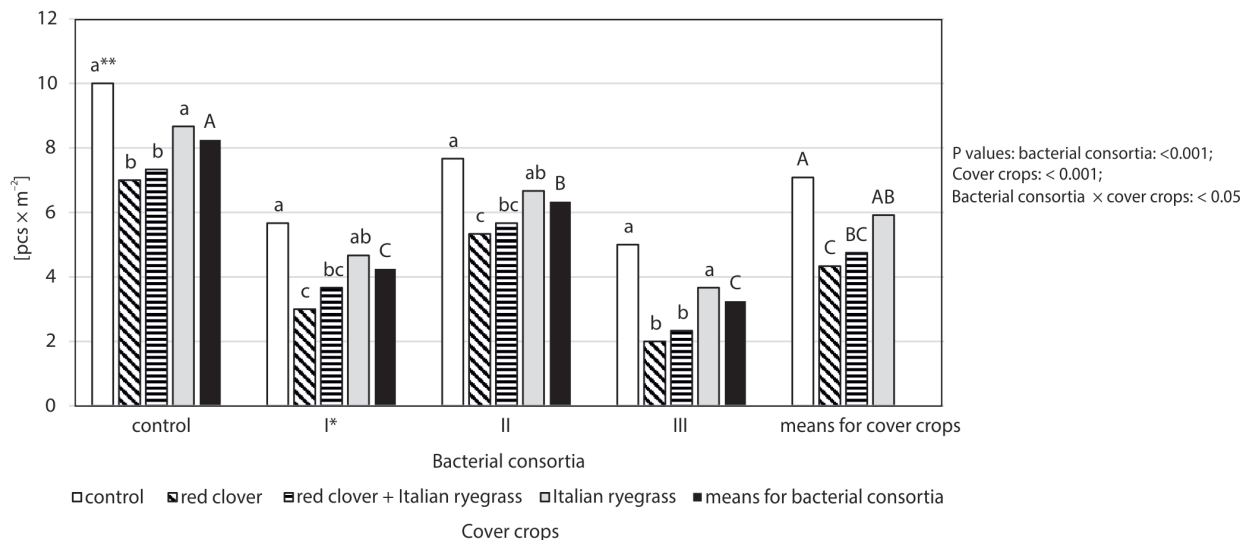
the experiment in question, CC also caused significant differences in the occurrence of *T. inodorum* L. The lowest number of this weed (49% decrease compared to the control) was recorded after plowing the CC of the mixture of red clover and Italian ryegrass. The number of *T. inodorum* L. after the application of red clover (36% decrease compared to the control) was at the same level as the aforementioned treatment and was not significantly different from that recorded on the CC of Italian ryegrass (29% decrease of this weed compared to the control). An interaction of the experimental factors studied was demonstrated, with the result that the lowest number of *T. inodorum* L. was recorded after the combined application of nitrogen-fixing bacteria with phosphorus-solubilizing bacteria

and after the application of all the CCs tested. In contrast, the highest number of weeds was recorded with the control treatment, without the application of bacterial consortia and without CC.

The number of *Apera spica-venti* L. plants significantly differed with the research experimental factors and their interaction (Fig. 4). The lowest number of this weed was recorded after application of bacterial consortium III (61% decrease of the weed compared to the control) and after application of bacterial consortium I (48% decrease of *A. spica-venti* L. compared to the control). A significantly higher number of *A. spica-venti* L. was recorded after the application of bacterial consortium II (a decrease of this weed by 23% compared to the control). In contrast, the highest



**Fig. 3.** The number of *Tripleurospermum inodorum* L. in spelt wheat as affected by bacterial consortia and cover crops (average 2020–2022) [ $\text{pcs} \cdot \text{m}^{-2}$ ]



**Fig. 4.** The number of *Apera spica-venti* L. in spelt wheat as affected by bacterial consortia and cover crops (average 2020–2022) [ $\text{pcs} \cdot \text{m}^{-2}$ ]

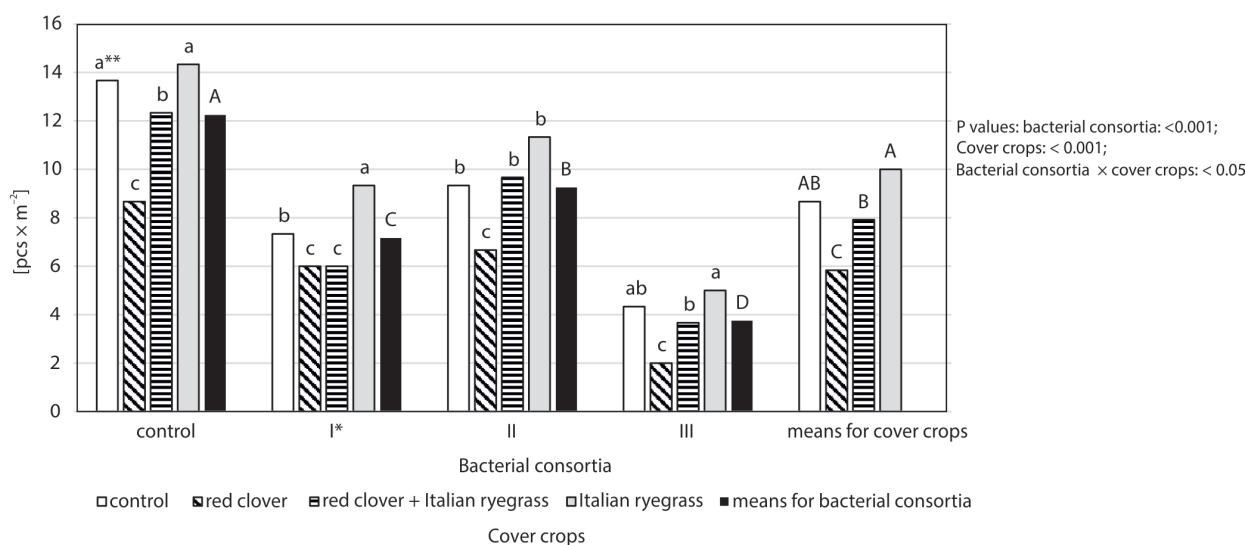
number of *A. spica-venti* L. was recorded with the control treatment without bacterial application. CC also significantly caused differences in the occurrence of *A. spica-venti* L. in the spelt wheat canopy. The greatest decrease in the number of *A. spica-venti* L. was recorded after plowing the CC of red clover (the decrease of this weed relative to the control was 39%) and after plowing the CC of a mixture of red clover and Italian ryegrass (a decrease of *A. spica-venti* L. by 33% relative to the control). In this case, the number of *A. spica-venti* L. plants was not significantly different than that recorded after Italian ryegrass was plowed (16% decrease of this weed compared to the control). The highest number of *A. spica-venti* L. was recorded with the control treatment, without the application of CC. An interaction of the researched factors of the experiment was demonstrated, showing that the lowest number of *A. spica-venti* L. was recorded after the application of bacterial consortium III after plowing the red clover and a mixture of red clover and Italian ryegrass CCs. In contrast, the highest number of this weed was recorded with the control treatment, with no bacterial treatment and no CC.

Statistical analysis demonstrated a significant effect of the researched experimental factors and their interaction on the number of *Elymus repens* L. in the spelt wheat canopy (Fig. 5). The lowest number of this weed was recorded after the application of bacterial consortium III (a decrease of *E. repens* L. by 69% compared to the control). Significantly more of this weed was recorded after the application of bacterial consortium I (a decrease of *E. repens* L. by 41% relative to the control), followed by the application of bacterial consortium II (a decrease of this weed by 24% relative to the control). The most weeds were seen with the control treatment without the application of bacterial

preparations. Application of a CC also significantly affected the number of *E. repens* L. Plowing the CC of red clover most effectively controlled *E. repens* L. (33% decrease in this weed compared to the control). After plowing the mixture of red clover and Italian ryegrass CC, the number of *E. repens* L. was significantly higher (a decrease of this weed by only 9% compared to the control), while after plowing the CC of Italian ryegrass, the number of *E. repens* L. was significantly higher (15% increase in the number of weed plants compared to the control). The experiment in question demonstrated the interaction of the factors researched. The lowest number of *E. repens* L. was recorded after the application of bacterial consortium III and after plowing the red clover CC, and the highest with the control treatment without the application of bacteria and CC, except for Italian ryegrass.

## Discussion

Studies were conducted using cultivation technologies based on the use of microbiological preparations and/or the introduction of CCs in organic farming, confirming the possibilities of their use in organic farming as an alternative to chemical protection products. The reduction of weed infestation in cereal crops after application of PGPR obtained in the field research conducted is analogous to the results of other authors (Baris *et al.* 2014; Artyszak and Gozdowski 2021; Cinkocki *et al.* 2021). In addition, research by Dar *et al.* (2020) indicates that the use of *Pseudomonas* bacterial strains in wheat cultivation has a beneficial effect on wheat growth and development, while showing good weed suppression ability. As reported by Mustafa



**Fig. 5.** The number of *Elymus repens* L. in spelt wheat as affected by bacterial consortia and cover crops (average 2020–2022) [pcs. · m<sup>-2</sup>]



*et al.* (2019), some bacterial strains can act as bioherbicides, reducing the germination and growth of weeds. Therefore, inhibiting weed growth can increase the competitive advantage of desirable plants. Rhizosphere bacteria, identified as exhibiting bioherbicide activity, include the following species: *Acinetobacter*, *Achromobacter*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia* and *Rhizobium* (Ahemad and Kibret 2014; Sindhu *et al.* 2018; Phour and Sindhu 2019). Abbas *et al.* (2020) found a reduction in rice weed infestation due to *Pseudomonas fluorescens*. Similar research results after applying four strains of *Pseudomonas* spp. to wheat crops were also obtained by Dar *et al.* (2020). Some rhizosphere bacteria colonize the roots of weeds and can inhibit their growth and development (Radhakrishnan *et al.* 2017; Sindhu *et al.* 2018). The main mechanism of biocontrol of weeds by bacteria is the production of phytotoxins, phytohormones and antibiotics (Kremer and Souissi 2001; Sindhu *et al.* 2018; Dahiya *et al.* 2019; Phour and Sindhu 2019). Furthermore, Abbas *et al.* (2017) reported that some bacteria produce hydrogen cyanide, which inhibits weed growth by blocking many enzymes involved in the normal metabolic pathway. In the present study, in addition to a reduction in weed dry matter, there was also a reduction in the number of weeds after the application of bacterial consortia. Also, Abbas *et al.* (2020) demonstrated a decrease in the number of weeds in rice as a result of *Pseudomonas fluorescens*. Analogous results after the application of four strains of *Pseudomonas* spp. bacteria in wheat crops were obtained by Dar *et al.* (2020). As well as a decrease in the number of weeds, these authors also obtained a significant decrease in weed root length. *Bacillus* spp. applied in wheat cultivation also influenced the decrease of weed numbers (Dahiya *et al.* 2019). Very importantly, in the previously mentioned studies by other researchers, in addition to reducing weeds, there were positive effects of bacterial preparations on the condition and yield of the main crop. Thus, an indirect result of reducing weeds may be improved grain growth due to bacterial preparations (Reed and Glick 2023). Ongoing research in this area also indicates that microorganisms can act very selectively, thus eliminating specific weed species (Dahiya *et al.* 2019), as confirmed by the present research. Weissmann *et al.* (2003) observed different effects of *Serratia plymuthica* on the occurrence of a number of weeds, including *Chenopodium album*, *Stelaria media*, and *Polygonum convolvulus*. In contrast, Li and Kremer (2006) noted that *Pseudomonas fluorescens* inhibits the growth of *Ipomea* spp. and *Convolvulus arvensis* in wheat crops. Also, in this study, application of a consortium of nitrogen-fixing bacteria with phosphorus-solubilizing bacteria most strongly reduced the occurrence of the nuisance weed *Elymus repens* L. in the spelt wheat

canopy. Therefore, it is appropriate to use bacterial preparations in organic cereal cultivation, which decrease weed invasion (Mustafa *et al.* 2019). However, the effectiveness of bacterial preparations can vary greatly depending on the climate, soil conditions or site of application (Herrera *et al.* 2016). In this research and that of others, higher weed pressure was recorded during growing seasons with a favorable distribution of precipitation and temperatures (Kosinski *et al.* 2011; DuPre *et al.* 2022; Seipel *et al.* 2022; Płaza *et al.* 2023). The production of more matter by weeds under such conditions may be due to the increased availability of moisture in the soil, and thus less competition for this resource. Therefore, this type of research should be carried out in different soil and climatic conditions in Poland as well.

Kocira *et al.* (2020) demonstrated that CCs are an important tool in controlling weeds even after they have been plowed into the following crop. They provide a competitive advantage by contributing to good soil health and inhibit the growth of weeds, significantly reducing their matter (Balbinot and Fleck 2005; Lemessa and Wakjira 2015; Wiggins *et al.* 2015; Smith *et al.* 2020). To attain these advantages, CC crops should produce abundant matter, which is important for even coverage of the soil surface. In addition, their C:N ratio should be balanced and thus resistant to rapid matter decomposition (Kocira *et al.* 2020). Plant residues from CCs continue to release other allelochemicals contained in dead plant material (Tabaglio *et al.* 2013; Schappert *et al.* 2019). Therefore, CCs can affect weed populations from the date of sowing to rotation (Falquet *et al.* 2015). According to Kruidhof *et al.* (2008) suppression of spring weed emergence by CC plant residues may be related to the release of allelochemicals (saponins, flavonoids and phenolic acids) into the soil. In addition, CCs from legumes can initiate weed 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 (Saucke and Ackermann 2006; Wells *et al.* 2016; Ranaldo *et al.* 2019). This is confirmed by the results of the present research. It was found that *Trifolium subterraneum* L. can significantly decrease the size of the soil seed bank and species richness (Restuccia *et al.* 2020). In this research aqueous extracts from cover crops released phytotoxic substances that inhibit weed growth. Weed growth has been inhibited by CCs with various complex factors caused by their joint action. CC seeding can control weeds, giving the crop a significant advantage (Yamane *et al.* 2014; Mishchenko and Masik 2017; Carlesi *et al.* 2020). The allelochemicals released by CCs through their natural decomposition can inhibit weed growth (Yamane *et al.* 2014; Frabboni

*et al.* 2019). Also, the use of *Eucalyptas globulus* leaves as a green manure contains phenolic substances and volatile organic compounds that inhibit weed growth (Carolina *et al.* 2018). In the above research the intercropping of red clover with winter wheat inhibited the growth of weeds including the number of weeds in the soybean – winter wheat – maize rotation. Research has demonstrated that annual weeds were suppressed to a greater extent by legume CC residues (Bhowmik 2003; Kocira *et al.* 2020; Teasdale and Mohler 2000). Aqueous leachates from the legumes studied showed strong phototoxic effects on the root growth of *E. crus-gali* or *Amaranthus hypochondriacus* (Caamal-Maldonado *et al.* 2001).

The research conducted in Poland is entirely innovative, as there is a lack of research on the effect of bacteria consortia and CCs on the degree of weed infestation in cereals grown in organic farming. The presented research clearly showed that the use of consortia bacteria with CC significantly reduced the dry matter and the number of weeds, including the dominant species. The greatest reduction in weeds was recorded after the combined application of nitrogen-fixing bacteria with phosphorus-solubilizing bacteria and after plowing CCs of red clover and a mixture of red clover and Italian ryegrass. This type of research needs to be continued in organic cereal cultivation, using different consortia of bacteria with CC, as well as learning more about the mechanisms of their effects on weeds.

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