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ORIGINAL ARTICLE

Sweet alyssum (*Lobularia maritima* L.) enhances aphidophagous insects and increases yield in field broad bean – agronomic aspects

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Abstract

Sweet alyssum (*Lobularia maritima* L.) is known as an insectary plant with great potential in enhancing the occurrence and diversity of beneficial insects in different crops. However, agronomic aspects of the introduction of this plant are still not fully recognized. Field studies aimed at assessing entomological relationships in the quasi-coordinate system focused on evaluating the impact of sweet alyssum as a companion plant in broad bean (*Vicia faba* L.) cultivation on the prevalence of the black bean aphid (*Aphis fabae* Scop.) and its natural enemies. It was also sought to determine the optimal row spacing for broad beans when introducing an additional plant between the rows. A 3-year field experiment involved various row spacings for broad beans: 50 cm, 65 cm, and 80 cm, with a control group at a 50 cm row spacing representing conventional cultivation, and another group with standard chemical pest protection as a reference. The results indicated that using sweet alyssum as a companion plant significantly reduced the black bean aphid population. It was comparable to the effect of chemical pest control. This companion planting also considerably increased the population of natural enemies of the black bean aphid, including hoverfly eggs and larvae, as well as various stages of ladybirds, particularly the adult stage. Sweet alyssum contributed to a reduced aphid-to-predator ratio, leading to a significant decrease in black bean aphid numbers and an earlier colonization of aphids by hoverflies and ladybirds on broad bean plants. In summary, sweet alyssum has the potential to effectively decrease black bean aphid occurrences, particularly on ecological farms. Notably, sweet alyssum's competitiveness with broad beans and the different row spacing had minimal impact on predator occurrence, eliminating the need to increase standard row spacing for this plant.

Keywords: black bean aphid, broad bean, hoverflies, intercropping, spacing, sweet alyssum

Introduction

One of the natural methods of limiting the harmfulness of pests is increasing the biodiversity of cultivations through introducing companion plants to the main plant. The correct selection of such plants may contribute to an increase in yield quality and volume, and to the improvement of production properties of soil. It also provides benefits resulting from abandoning plant protection chemical agents. The use of multi-species cultivations may be regarded as the safest (in terms of safety for the environment and human health) manner of crop pest control. It makes the cultivation field as close as possible to natural biocenosis. When selecting plants for companion cultivation, it is necessary to define the scope of competitiveness between species.

The correct selection of plants excludes their mutual, adverse, allelopathic impact and it limits maximally the mutual competition concerning access to water, nutritional substances and light (Tesio and Ferrero 2010; Kaur *et al.* 2018).

Broad bean (*Vicia faba* L.) is a very valuable crop characterized by high nutritional value. It is an excellent alternative to meat. The grains contain approximately 7% balanced protein and it is a very rich source of folic acid. Therefore, its consumption is recommended for pregnant women (Carpino *et al.* 2020). Furthermore, it was discovered to have a high content of natural L-dopa amino acid, leading to its use in Parkinson therapy (Apaydin *et al.* 2000). It is distinguished by a low sodium content and its seeds are rich in proteins, carbohydrates (14%), significant quantities of B and C vitamins, organic acids and mineral salts, mostly iron, phosphorus, copper and calcium. It is also rich in potassium and soluble fiber (Akinci *et al.* 2009; Jiang *et al.* 2020). Furthermore, it is a useful plant in research on the impact of various protection strategies on the occurrence of harmful and useful entomofauna, because it is attacked annually by pests which are important in economic terms, such as black bean aphid (*Aphis fabae* Scop.), pea leaf weevils (*Sitona* spp.), and broad bean seed weevils (*Bruchus rufimanus* Boh.). On the other hand, sweet alyssum (*Lobularia maritima* L.) is an annual plant belonging to *Brassicaceae* (*Brassicaceae* Burnett) family (Legaspi *et al.* 2020; Renkema and Smith 2020) with a very long blooming period (it blooms continuously for a few months). It grows and blooms very quickly after planting, and may be planted mechanically in a very simple way with the use of seedling planters. It is resistant to drought and does not have any high soil demands. In literature, it is mentioned among the four most popular species of plants luring useful insects (so called insectary plants) (Brennan 2013; Aparicio *et al.* 2018; Shrestha *et al.* 2019; Hayashi *et al.* 2020). Among plants such as: buckwheat, *Fagopyrum esculentum* (Moench), phacelia, *Phacelia tanacetifolia* (Bentham), calendula, *Calendula officinalis* (L.) and ammi, *Ammi majus* (L.), alyssum had the longest bloom period and attracted the most syrphids (Harris-Cypher *et al.* 2023). The pollen and nectar of *L. maritima* flowers contribute to an increase in the number of hymenopteran parasitoids and the viability of these insects (Patt *et al.* 1997; Johanowicz and Mitchell 2000; Legaspi *et al.* 2020).

Black bean aphid (*Aphis fabae* Scop.) is one of the most dangerous polyphagous pests which attack not only broad bean crops but also other cultivation plants with high economic significance (Basedow *et al.* 2006; Almogdad and Semaškienė 2021). The occurrence of black bean aphid in broad bean crops is most dangerous in its effects and is most visible during dry and very warm seasons. Attacks of this pest and then its feeding on plants, in each case, results in lowering the quantity and quality of crops by more than 50% (Hansen *et al.* 2008; Webster *et al.* 2008). Seeds from plants attacked by aphids are characterized by lower protein content (Shannag 2007; Shannag and Ababneh 2007). Black bean aphid is also a vector of many plant viral diseases (Neeraj *et al.* 1999; Wamonje *et al.* 2020). The most important predators of black bean aphid are flies from the hoverfly family (Diptera, Syrphidae) and beetles from the coccinellidae family (Coleoptera, Coccinellidae).

The practical aspects of introducing plants that favor beneficial fauna are still rarely the subject of research. Choosing the right spacing in mixed crops is crucial for proper exposure of the insectary plant (which is responsible for its attracting properties) as well as avoiding competition with the main plant. So far, very little is known about the choice of spacing between main plants when using *L. maritima* as plants for biological control of aphids. When grown with lettuce, this plant caused a slight reduction in lettuce heads (Brennan 2013).

The aim of this paper was to determine the impact of *L. maritima* as a companion plant in broad bean cultivation on the occurrence of black bean aphid and its natural enemies, as well as to establish the optimal broad bean spacing when introducing an additional plant between the rows. Furthermore, the quantitative value of aphids in relation to its natural enemies was also analyzed, as well as the influence of *L. maritima* on the date of colonization of aphids by hoverflies and ladybirds in relation to different spacing. Finally, we assessed yield and selected morphological parameters of broad bean.

Materials and Methods

Field research was carried out in Poland (50°06’45”N 20°05’03”E) between 2014 and 2016. The climatic conditions throughout the study period were moderate and typical for the region. The soil at the experiment site was degraded chernozem formed from loess, with particle size distribution of a loamy dust. Soil analysis carried out before starting the experiment demonstrated that it was characteristic for a reaction close to neutral (pH in H_2O = 6.54; pH in KCl = 5.71) with a humus content of 2.29%. The content of available elements was as follows: 22.0 mg $P_2O_5 \cdot 100\ g^{-1}$ of soil, 27.0 mg $K_2O \cdot 100\ g^{-1}$ of soil, 8.5 mg Mg $\cdot\ 100\ g^{-1}$ of soil and the contents of other elements were: 1.65 mg B $\cdot\ kg^{-1}$ of soil, 176.40 mg Mn $\cdot\ kg^{-1}$ of soil, 4.40 mg Cu $\cdot\ kg^{-1}$ of soil, 27.73 mg Zn $\cdot\ kg^{-1}$ of soil and

1081 mg Fe · kg^{-1} of soil. Soil analysis was carried out in the District Chemical-Agricultural Station in Cracow. The experiment was started by means of a method of randomized blocks in four repetitions for each plot (20 plots in total), according to the field research methodology (Naraghi *et al.* 2010). The size of a single plot was 25 m^2 (5 m × 5 m). Depending on the spacing, there were 10, 7, or 6 rows of broad beans in a single plot. Cereal plants were a pre-crop every year. Bartek variety broad bean was cultivated together with sweet alyssum Capri variety, with varied spacing between the rows (Fig. 1).

The smallest spacing between broad bean rows in the experiment with *L. maritima* was 50 cm, medium – 65 cm and the largest – 80 cm. Homogeneous broad bean cultivation with a spacing of 50 cm was a control cultivation; whereas, the same cultivation was subjected to standard chemical protection with the use of chemical insecticides Decis 2.5 EC (active substance content: deltamethrin (a compound belonging to the group of pyrethroids) – 25 g per 1 liter of the product (2.8%) and Fastac 100 EC (active substance content: alfa – cypermethrin (a product from the group of pyrethroids) – 100 g per 1 liter of the product (10.87%) was a reference to conventional protection. The use of both preparations in the experiment under chemical protection took place twice. Pea leaf weevil beetles were controlled by means of Fastac 100 EC at the dose of 0.09 l · ha^{-1} when the first damage caused by these pests was noted. This procedure was repeated after 7 days. Broad bean seed weevils and black bean aphids were controlled by means of Decis 2.5 EC at the dose of 0.25 l · ha^{-1}. The first treatment with the use of this preparation was carried out as with aphids occurring on the broad bean. The preparation was used again against broad bean weevils at the end of the blossoming of the first inflorescence level of broad bean. Broad bean was sown annually in the third week decade of March. Spacing (15 cm) between the broad bean plants in a row was based on cultivation recommendations for this plant. The seeds were placed in the soil at the depth of 6 cm. Seedlings of sweet alyssum were prepared in a greenhouse by sowing seeds into plugtrays (15 seeds per one plugtray cell with the dimensions of 2.5 cm × 2.5 cm). Then the sweet alyssum tufts were planted after the broad bean plants germinated in the middle of the main plant between the rows, at the distance of 25 cm in a row (Fig. 1). Weed control during the whole experiment was conducted mechanically. The respective plots with companion planting, controlled cultivations and cultivations under chemical protection were separated with 3 m wide oat belts. Similar buffer zones with the use of cereal (1.25 m wide belts), on account of their neutrality for broad bean pests, were used by other authors in preceding research on the impact of companion planting on the pests' natural enemies (Seidenglanz *et al.* 2011).

As a result, the following experimental treatments were created:

- broad bean with sweet alyssum, 50 cm between broad bean rows (S50);
- broad bean with sweet alyssum, 65 cm between broad bean rows (S65);
- broad bean with sweet alyssum, 80 cm between broad bean rows (S80);
- broad bean in the homogeneous cultivation, 50 cm between the rows - control;
- broad bean in the homogeneous cultivation, 50 cm between the rows, subject to standard chemical protection against pests (CH).

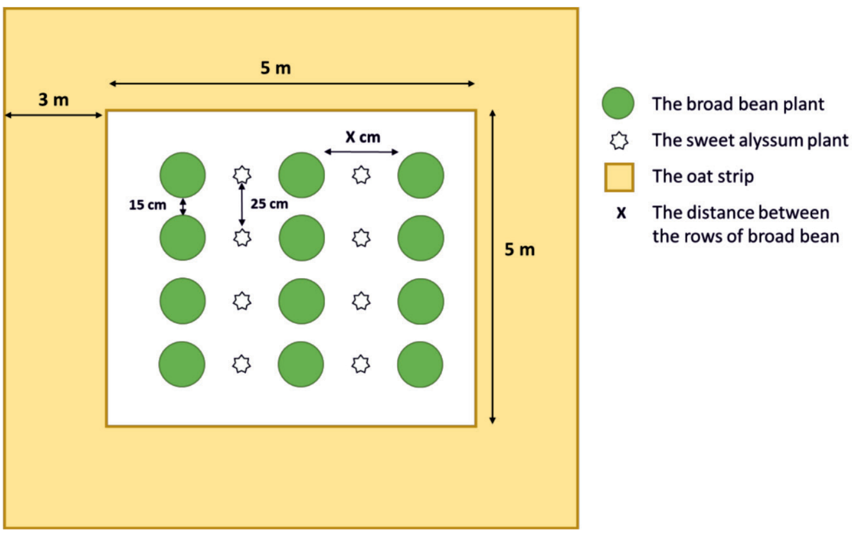

Fig. 1. Distribution of intercropping in the experiment – scheme. X-distance between the rows of broad beans, depending on the combination of cultivation (50 cm – S50, 65 cm – S65, 80 cm – S80 and 50 cm – chemical protection and control)

Black bean aphid

Observations were carried out when winged migrants on the plants were first noticed until the end of their feeding period, at intervals of approx. 5 days, on 30 randomized and labelled plants from each plot. The observations involved the quantity of the respective morphotic forms of the aphid (wingless females, winged females, nymphs) and the location of the colony on the plant (apex, shoot, pod, flower, leaf). With a maximum number of 100 specimens of the aphids in the colony on a given plant, all the specimens were counted carefully. With a higher number of aphids in the colony, their quantity was estimated roughly by counting aphids on the length of 1 cm of the shoot/pod and multiplying it by the length of the colony on the shoot/pod. In the case of aphids feeding on leaves or flowers, all specimens were counted per one randomly selected and attacked leaf/flower and multiplied by the number of leaves/flowers attacked by aphids. Aphids on the apexes were counted carefully even if their number was >100. The resulting values were then summed to obtain the total number of aphids on each plant.

Black bean aphid's predators and broad bean yield

As far as Coccinellidae are concerned, the occurrence and quantity of developmental stages was analyzed (egg clutches, larvae and imago) and a predator species was defined. As far as hoverflies are concerned, the analysis involved the number of eggs and larvae. Observation of predator occurrence was done on the same plants as aphids and with the same frequency. In order to specify the composition of hoverflies, the pupae were carried to the laboratory and placed on Petri dishes at 22–24°C and the air relative humidity of 70%. The species of the reared Syrphidae were identified based on Veen's (2004) key, while Coccinellidae were identified according to Bieńkowski's (2018) key. Pupae of Coccinellidae on the plant were counted, but due to their developmental period and non-predatory stage, their quantity was not included in the analysis. They were not collected from the experiment to avoid disrupting the results. Selected growth parameters of the broad bean plant were also analyzed (number and weight of pods and seeds) as well as broad bean yield. These analyses were performed once a year in the stage of milk maturity of broad bean plants.

Statistical analysis

The results obtained were analyzed with the use of STATISTICA 12.5 PL. The data analysis was preceded with a normality test (Shapiro-Wilk with a correction by Lilliefors) and the variance homogeneity test (Levene's test). The significance of differences between the means was assessed through one or two factor variance analysis (year, protection method or year x protection method as factors) which we treated: cultivation of broad beans with *L. maritima* at different spacings (50, 65 and 80 cm), chemical treatment and control as protection methods), and the means were differentiated by the NIR Fisher test at the significance level α = 0.05. Statistical differences in the form of letters were marked on the basis of Anova results and post hoc test performed on physical data, while the graphs in Figures 2, 3 and 4 present normalized values (converted in relation to the control). In the present study, normalizing the results to the control represents the presentation of data showing what portion of the control the obtained result represents. The obtained result is divided by the average value of the control from all four repetitions, and multiplied by 100%. This allows for comparison of observation values in individual years, during which the resulting values differed significantly, for example, between seasons. In order to analyze the quantitative ratios between the team components of the aphid predators, the number of victims (aphids) per one predator (Syrphidae or Coccinellidae larva, Coccinelidae imago) was also calculated. During the statistical analysis, there was no need for logarithmic transformation.

Results

Aphids and predators – life stages

Two factor variance analysis (year and protection method as factors) of the occurrence of aphids and selected life stages of Syrphidae and Coccinellidae showed that both the year of the experiment and the protection method significantly influenced these parameters (Table 1). In the case of predators there was also significant interaction between the year of study and the protection method.

Aphis fabae Scop.

The observation results normalized to the control conditions (the values in the control treatment as a reference were set at 100%) are shown in Figures 2–4. In each year of observations, the number of *A. fabae* was significantly lower in treatments with sweet alyssum than in the control, however there were no significant differences between treatments with *L. maritima* at different spacings (Fig. 2).

Table 1. Anova results for year (Y) and protection method (PM) of the number of aphids and selected life stages of Syrphidae and Coccinellidae on the broad bean

	SS	df	MS	F	p
			Aphids		
PM	486.6	4	122.6	112.06	0.00
Y	357.5	2	179.5	16.44	0.00
PM*Y	132.5	8	165.4	1.53	0.14
			Syrphidae eggs		
PM	1592	4	398.0	245.93	0.00
Y	1875	2	937.7	579.44	0.00
PM*Y	1599	8	199.8	12348	0.00
			Syrphidae larvae		
PM	48.51	4	12.13	44.13	0.00
Y	9.87	2	4.93	17.95	0.00
PM*Y	16.12	8	2.01	7.33	0.00
			Coccinellidae eggs		
PM	2.417	4	604	15.27	0.00
Y	1.387	2	693	17.52	0.00
PM*Y	1.068	8	134	3.37	0.00
			Coccinellidae larvae		
PM	1741	4	435	27.63	0.00
Y	3586	2	1793	113.78	0.00
PM*Y	2378	8	297	18.86	0.00
			Coccinellidae imago		
PM	362.2	4	90.55	131.40	0.00
Y	78.2	2	39.09	56.72	0.00
PM*Y	65.6	8	8.21	11.91	0.00

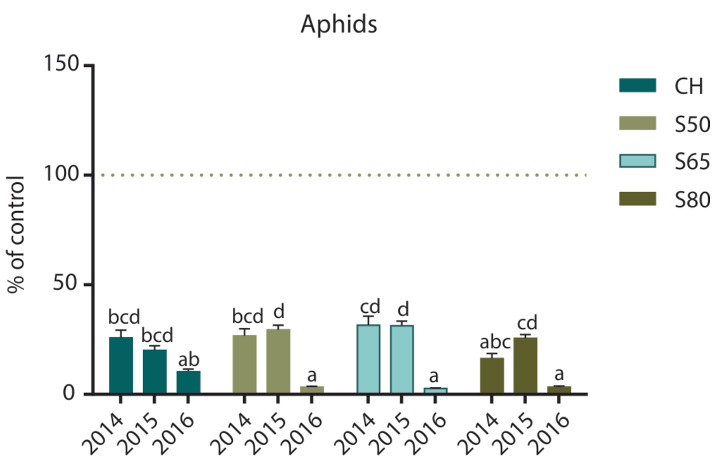

Fig. 2. The number of *Aphis fabae* Scop – normalization to control. Values marked with different letters differ significantly from each other at α = 0.05, factor: protection method × year. S80 – broad beans cultivated with sweet alyssum at a distance of 80 cm between the rows of beans; S65 – broad beans cultivated with sweet alyssum at a distance of 65 cm between the rows of beans; S50 – broad beans cultivated with sweet alyssum at a distance of 50 cm between the rows of beans; Control – broad beans in homogeneous cultivation, CH – broad beans in homogeneous cultivation, subjected to standard chemical protection.
Explanation – marking statistical differences in the form of letters was presented based on the results of Anova and post hoc test performed on physical data, while the graphs present normalized values (converted in relation to the control)

Predators

Egg-laying period

To check whether the presence of sweet alyssum in the inter-rows of broad beans significantly influenced the date of colonization of aphids by hoverflies and ladybirds, the dynamics of the appearance of the eggs of these predators in individual treatments was statistically analyzed (Fig. 3–4, Tab. 2–3). In the first year of observations, large numbers of Syrphidae eggs first appeared on the plants in the treatment with *L. maritima* at the smallest broad bean row spacing (Fig. 3).

In 2015, no such relation was found, however the number of Syrphidae eggs 10 days after colonizing aphid colonies (May 25, 2015) was significantly higher in treatments with sweet alyssum than in the control or chemical treatment and this state continued during the next 10 days. In the last year of the study, all the targets with the sweet alyssum were first attracted by predatory Syrphidae that deposited eggs on broad beans in their vicinity (Tab. 2). We did not find a significant influence of different spacing on the date of colonization of aphids by hoverflies. During the first observation period in 2014, the eggs of ladybirds appeared most frequently in the treatment with *L. maritima* in the smallest broad bean row spacing and on two subsequent dates their presence was visible only in the treatments with companion plants (Fig. 3). In 2015, the first Coccinellidae eggs appeared in the treatment with sweet alyssum with the largest row spacing and again on the next date of observation Coccinellidae eggs were observed only on broad bean plants in the vicinity of *L. maritima*. Also in 2016, Coccinellidae eggs appeared first in treatments with sweet alyssum as a companion plant (in the greatest amount in S65 treatment). So, again there was no clear response of egg lying by ladybirds to different spacing (Tab. 3).

Syrphidae

In the case of Syrphidae eggs (Fig. 5A) and larvae (Fig. 5B), the presence of companion plants stimulated their increased settlement on broad bean plants, except for 2014, when differences were not statistically significant in comparison to the control. In the case of Syrphidae eggs, the greater the inter-row spacing, the smaller the number of this predator stage (statistically significant differences in 2016), while in the case of larvae, there were no differences between the plots

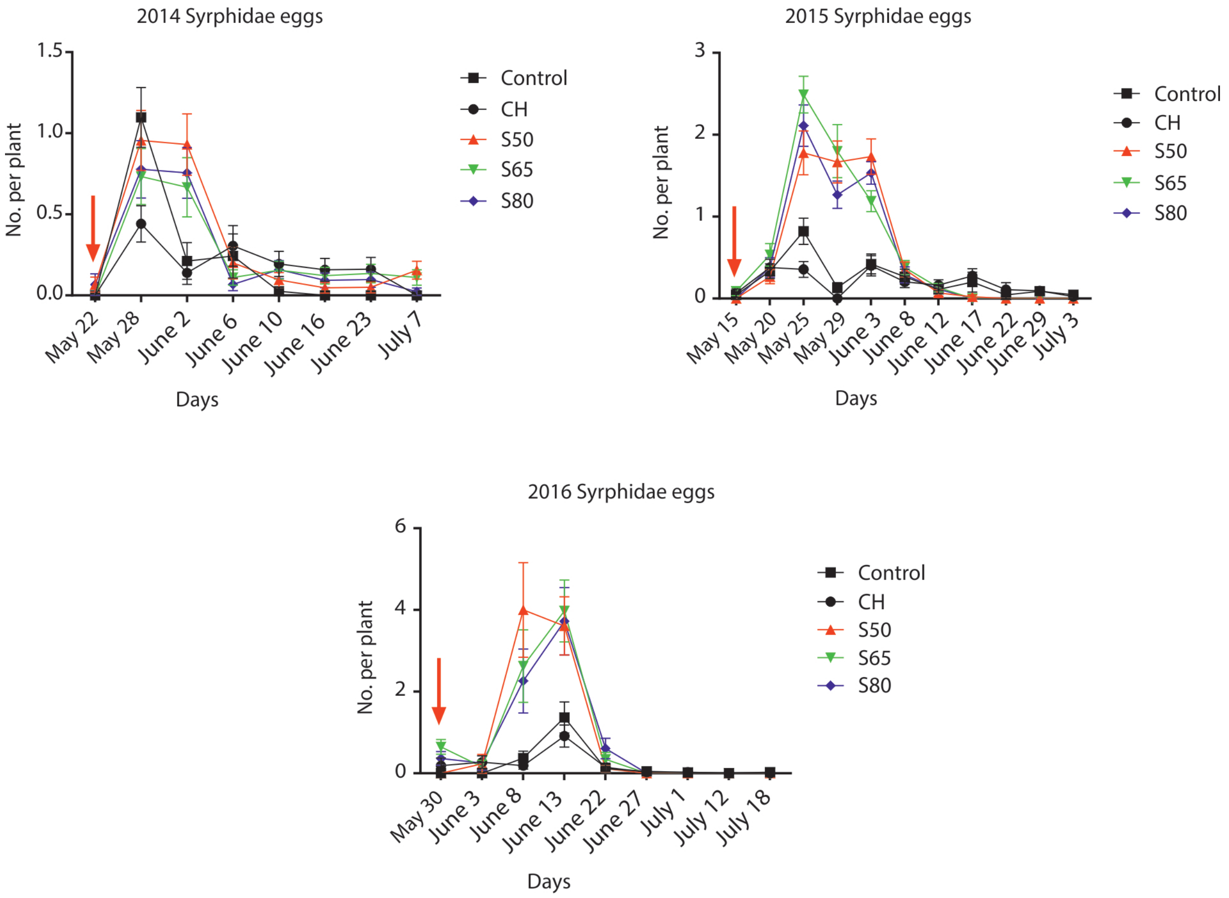

Fig. 3. The dynamics of Syrphidae egg occurrence on specific dates and seasons of observation. For treatments see Figure 2. Vertical bars mean standard errors

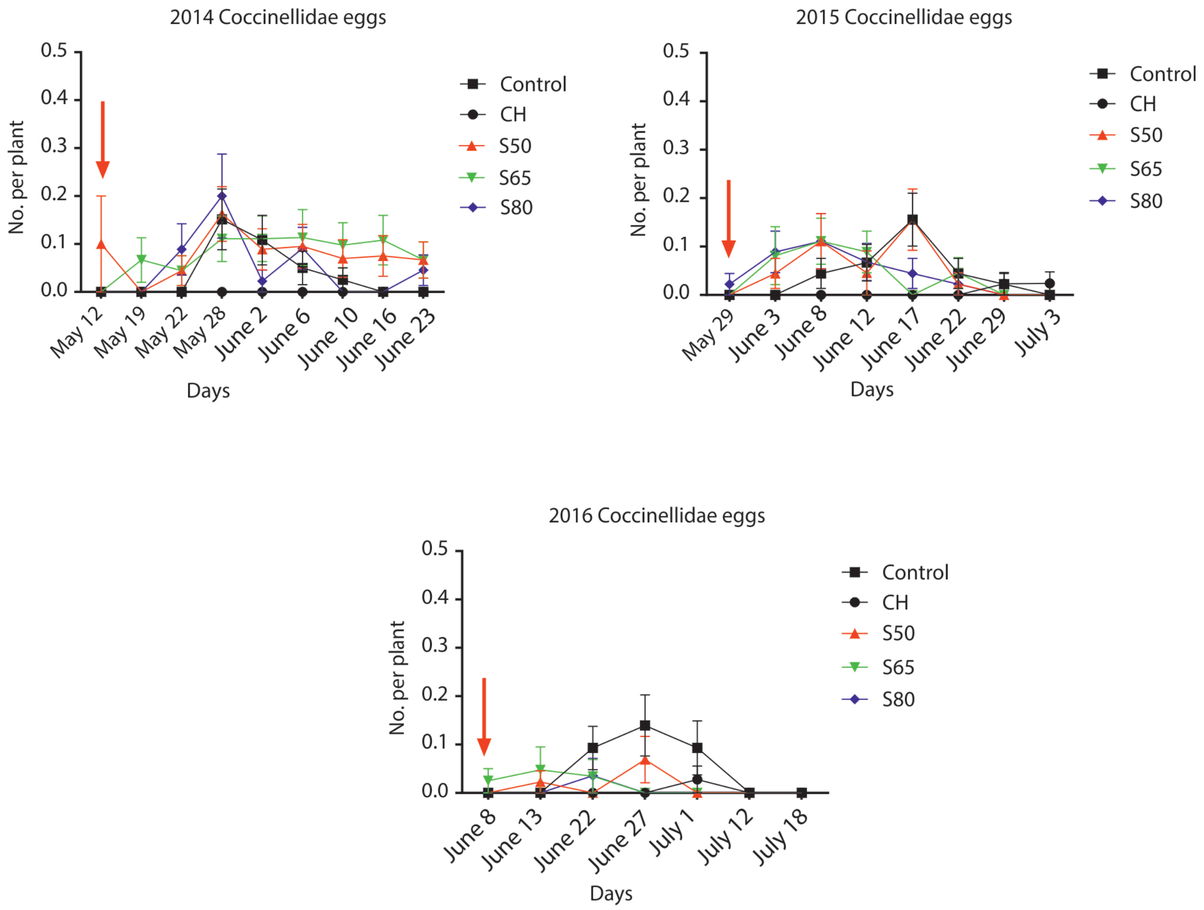

Fig. 4. The dynamics of Coccinellidae egg occurrence on specific dates and seasons of observation. For treatments see Figure 2. Vertical bars mean standard errors

Table 2. Statistically significant differences calculated for the dynamics of Syrphidae eggs occurrence in 2014–2016. For treatments see Figure 2

Protection method	Dates of measurement in 2014									
	May 19	May 22	May 28	June 2	June 6	June 10	June 16	June 23	July 7	–
Control	–	a	a	a	a	a	a	a	–	–
CH	–	a	a	a	b	b	b	a	–	–
S50	–	b	b	a	ab	ab	ab	c	–	–
S65	–	ab	b	a	ab	ab	ab	bc	–	–
S80	–	ab	b	a	ab	ab	ab	ab	–	–
	Dates of measurement in 2015									
	May 15	May 20	May 25	May 29	June 3	June 8	June 12	June 17	June 22	June 29
Control	a	a	a	a	a	a	a	b	a	b
CH	a	a	a	a	a	a	a	c	a	b
S50	a	a	b	b	c	a	a	a	a	a
S65	a	a	c	b	b	a	a	a	a	a
S80	a	a	bc	b	bc	a	a	a	a	a
	Dates of measurement in 2016									
	–	May 5	June 3	June 8	June 13	June 22	June 27	–	–	–
Control	–	a	a	a	a	a	a	–	–	–
CH	–	a	a	a	a	a	a	–	–	–
S50	–	b	b	b	b	a	a	–	–	–
S65	–	b	b	b	b	ab	a	–	–	–
S80	–	b	b	b	b	b	a	–	–	–

Table 3. Statistically significant differences calculated for the dynamics of Coccinellidae egg occurrence in 2014–2016. For treatments see Figure 2

Protection method	Dates of measurement in 2014									
	May 12	May 19	May 22	May 28	June 2	June 6	June 10	June 16	June 23	–
Control	a	a	ab	ab	b	a	ab	a	a	–
CH	a	a	a	a	a	a	a	a	a	–
S50	b	a	ab	ab	ab	a	ab	ab	a	–
S65	a	b	ab	ab	ab	a	b	b	a	–
S80	a	a	b	b	ab	a	a	a	a	–
	Dates of measurement in 2015									
	May 29	June 3	June 8	June 12	June 17	June 22	June 29	July 3	–	–
Control	a	a	a	a	b	a	a	a	–	–
CH	a	a	a	a	a	a	a	a	–	–
S50	a	a	a	a	b	a	a	a	–	–
S65	a	a	a	a	a	a	a	a	–	–
S80	b	a	a	a	ab	a	a	a	–	–
	Dates of measurement in 2016									
	June 8	June 13	June 22	June 27	July 1	July 12	July 18	–	–	–
Control	a	a	a	b	b	a	–	–	–	–
CH	a	a	a	a	ab	a	–	–	–	–
S50	a	b	a	ab	a	a	–	–	–	–
S65	b	b	a	a	a	a	–	–	–	–
S80	a	a	a	a	a	a	–	–	–	–

Different letters for a given observation date mean that values differ significantly at α = 0.05, factor: protection method

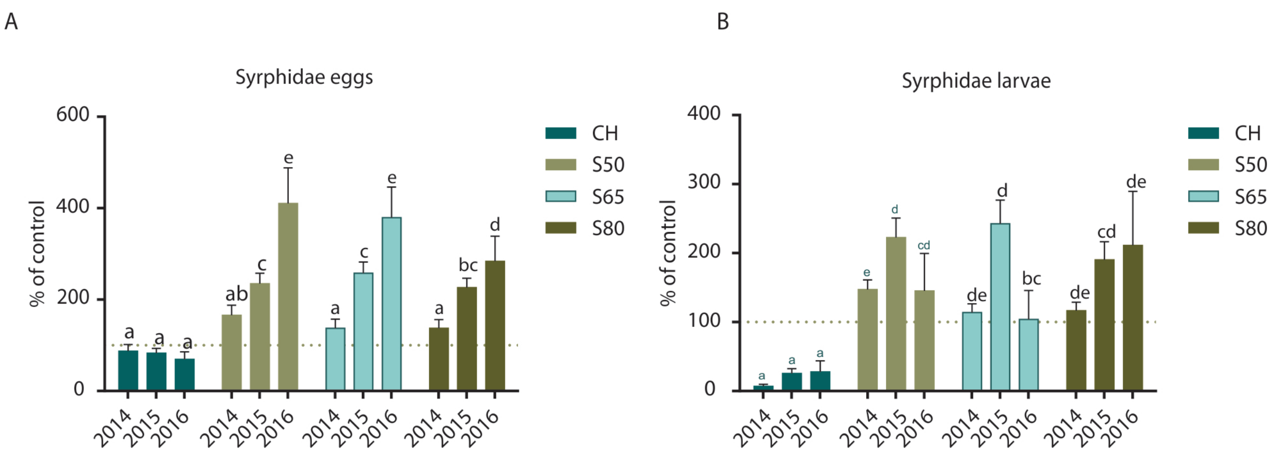

Fig. 5. The number of Syrphidae eggs (A) and larvae (B) – normalization to control. Values marked with different letters differ significantly at α = 0.05, factor: protection method x year. For treatments see Figure 2.
Explanation – marking statistical differences in the form of letters were based on the results of Anova and post hoc test performed on physical data, while the graphs present normalized values (converted in relation to the control)

with a different row spacing, except for 2016, when they were most frequently recorded in the treatment with the largest spacing (S80) (differences significant in comparison to S65 in this year).

Coccinellidae

On the other hand, eggs and larvae (Figs. 6A and 6B) (especially in 2015) of Coccinellidae were most often recorded in treatments with sweet alyssum with medium spacing (S65), and imago (Fig. 6C) in the years 2014–2015 were feeding more frequently in treatments with *L. maritima* in broad bean inter-rows with a spacing of 50 and 65cm. Each row spacing favored the appearance of the examined black bean aphid predators.

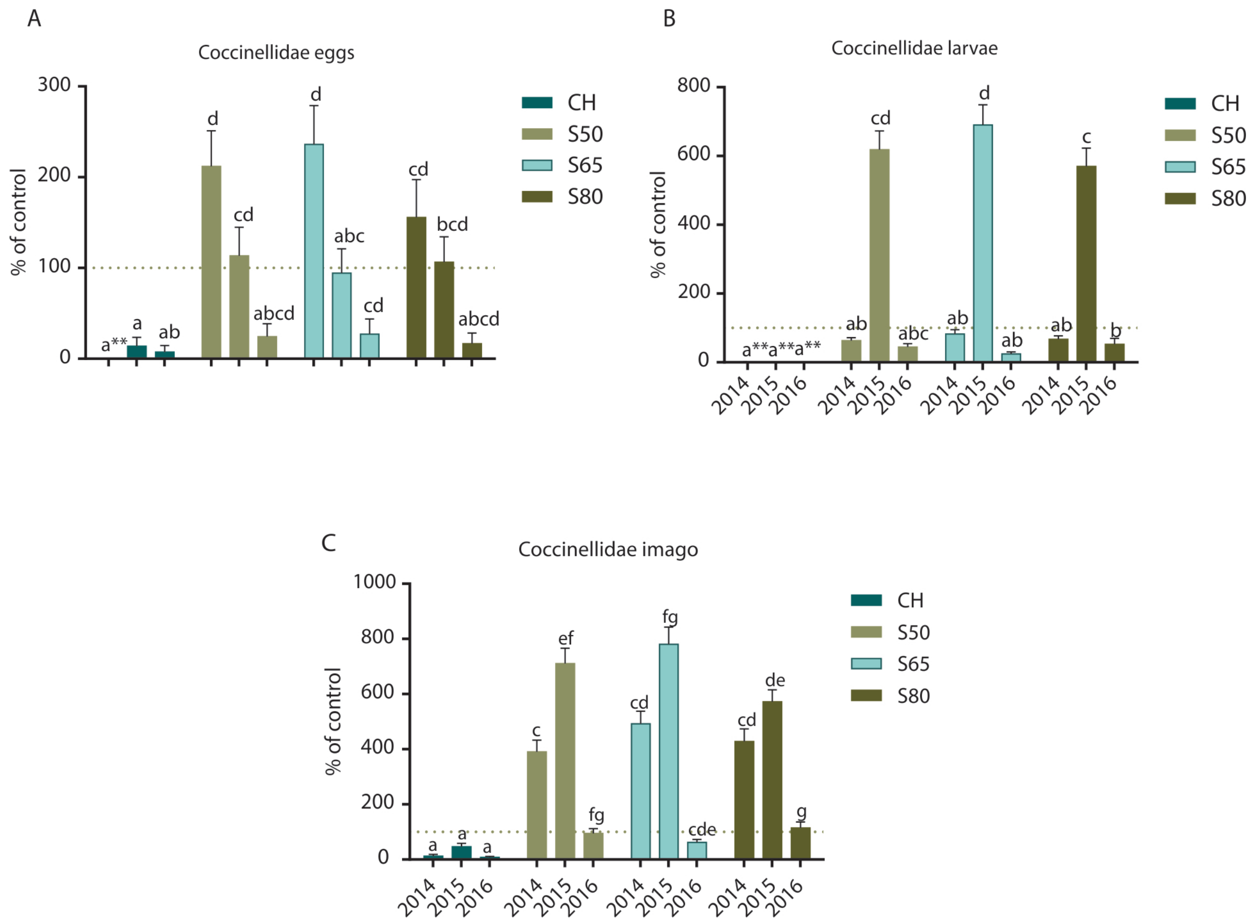

Fig. 6. The number of Coccinellidae eggs (A), larvae (B) and imago (C) – normalization to control. Values marked with different letters differ significantly at α = 0.05, factor: protection method x year. For treatments see Figure 2. ** – lack of the tested parameter in a given treatment.

Explanation – marking statistical differences in the form of letters were based on the results of Anova and post hoc test performed on physical data, while the graphs present normalized values (converted in relation to the control).

Quantitative ratio of the predator – prey

The species composition of *A. fabae* predators in all the observation seasons are included in Table 4. The representatives of Syrphidae were dominated by *Syrphus ribesii* L. (48.23%) and *Episyrphus balteatus* Deg. (33.66%). The remaining species, such as *Scaeva pyrastrii* L., *Syrphus corollae* Fabr. and *Meligramma triangulifera* Zetterstedt occurred very rarely. Equally, Coccinellidae were dominated by the following two species: *Coccinella septempunctata* L. (52.95%) and *Harmonia axyridis* Pallas (43.25%), *Adalia bipunctata* L. and *Propylea quatuordecimpunctata* L. occurred occasionally.

Tables 5 and 6 show the effect of *L. maritima* on the quantitative ratio of the predator (Syrphidae larva predatory stage and larva and imago of Coccinellidae) to the black bean aphid. It shows that the largest number of aphids per one larva of Syrphidae were in treatments with chemical protection and in the control. In

Table 4. Species composition of predatory hoverflies (Syrphidae) and ladybirds (Coccinellidae) in the *Aphis fabae* Scop. colonies on broad bean.

Syrphidae		Coccinellidae	
Syrphus ribesii (L.)	48.23%	*Coccinella septempunctata* (L).	52.95%
Episyrphus balteatus (Deg.)	33.66%	*Harmonia axyridis* (Pallas)	43.25%
Scaeva pyrastrii (L.)	6.05%	*Adalia bipunctata* (L.)	0.16%
Syrphus corollae (Fabr.)	9.30%	*Propylea quatuordecimpunctata* (L.)	3.64%
Meligramma triangulifera (Zetterstedt)	2.76%		

Table 5. Anova results for year (Y) and protection method (PM) of the quantitative ratio of the predator (Syrphidae larvae, larvae and imago Coccinellidae) – prey (black bean aphid) on broad bean in 2014–2016

	Number of black bean aphid / 1 Syrphidae larva		Number of black bean aphid / 1 Coccinellidae larva		Number of black bean aphid / 1 Coccinellidae imago	
	F	p	F	p	F	p
Y	1.39	0.25	178.43	0.00	2.04	0.13
PM	63.12	0.00	29.15	0.00	80.23	0.00
Y*PM	10.38	0.00	30.65	0.00	11.40	0.00

Table 6. Quantitative ratio of the predator (Syrphidae larvae, larvae and imago Coccinellidae) - prey (black bean aphid) on broad bean in 2014–2016. For treatments see Figure 2

Protection method	Number of black bean aphid / 1 Syrphidae larva	Number of black bean aphid / 1 Coccinellidae larva	Number of black bean aphid / 1 Coccinellidae imago
2014			
Control	3861 a* ± 308.73**	2239 a ± 855.71	11840 b ± 2851.40
CH	17278 b ± 3776.50	26840 b ± 5311.76	12168 b ± 1657.88
S50	798 a ± 222.05	1095 a ± 428.15	908 a ± 488.16
S65	1171 a ± 283.36	1272 a ± 515.62	976 a ± 563.51
S80	612 a ± 124.25	535 a ± 154.16	363 a ± 72.77
2015			
Control	10053 b ± 2362.77	1724 a ± 344.06	11366 b ± 4063.29
CH	9336 b ± 3427.37	25176 b ± 13919.04	8521 b ± 3884.21
S50	1546 a ± 514.40	110 a ± 42.18	553 a ± 213.52
S65	1763 a ± 806.28	97 a ± 29.75	496 a ± 137.97
S80	1438 a ± 259.69	115 a ± 52.84	573 a ± 196.73
2016			
Control	20090 b ± 18470.39	1304 b ± 99.57	2860 b ± 1093.92
CH	7649 b ± 4065.86	–*** ± –***	6010 b ± 2283.63
S50	1957a ± 1569.89	147 a ± 74.27	138 a ± 23.65
S65	1429 a ± 627.77	151 a ± 6.51	168 a ± 34.04
S80	511 a ± 254.94	394 a ± 172.84	103 a ± 7.65

*values marked with different letters for a given year of observation differ significantly at α = 0.05, factor: protection method; ** – ± standard error; *** – no predator presence detected

the case of treatments with *L. maritima*, the number of aphids per one larva of Syrphidae was significantly lower than in the control (except for 2014, when the difference was not statistically significant). The largest disproportion in the number of aphids inhabiting broad bean plants per this predator was recorded in 2016 – the values were 3–10 times lower in treatments with *L. maritima* than in the control. In each year of observation, the largest cultivation spacing (S80) favored the favorable proportions of the Syrphidae larva - aphid. In the case of Coccinellide the effect of different spacing was not so clear and depended on year and predator stage (larva or adult). In 2016, in areas under chemical protection, the presence of Coccinellidae larvae was not recorded.

The broad bean yield

In each of the observation seasons, the presence of sweet alyssum in the inter-rows of broad beans significantly increased the yield of broad beans compared to unprotected control and in 2 years of study even compared to chemical protection (Fig. 7, Tab. 7). The distance between broad bean rows did not influence the yield except for 2016 when it was significantly higher in S50 than in S65 and S80.

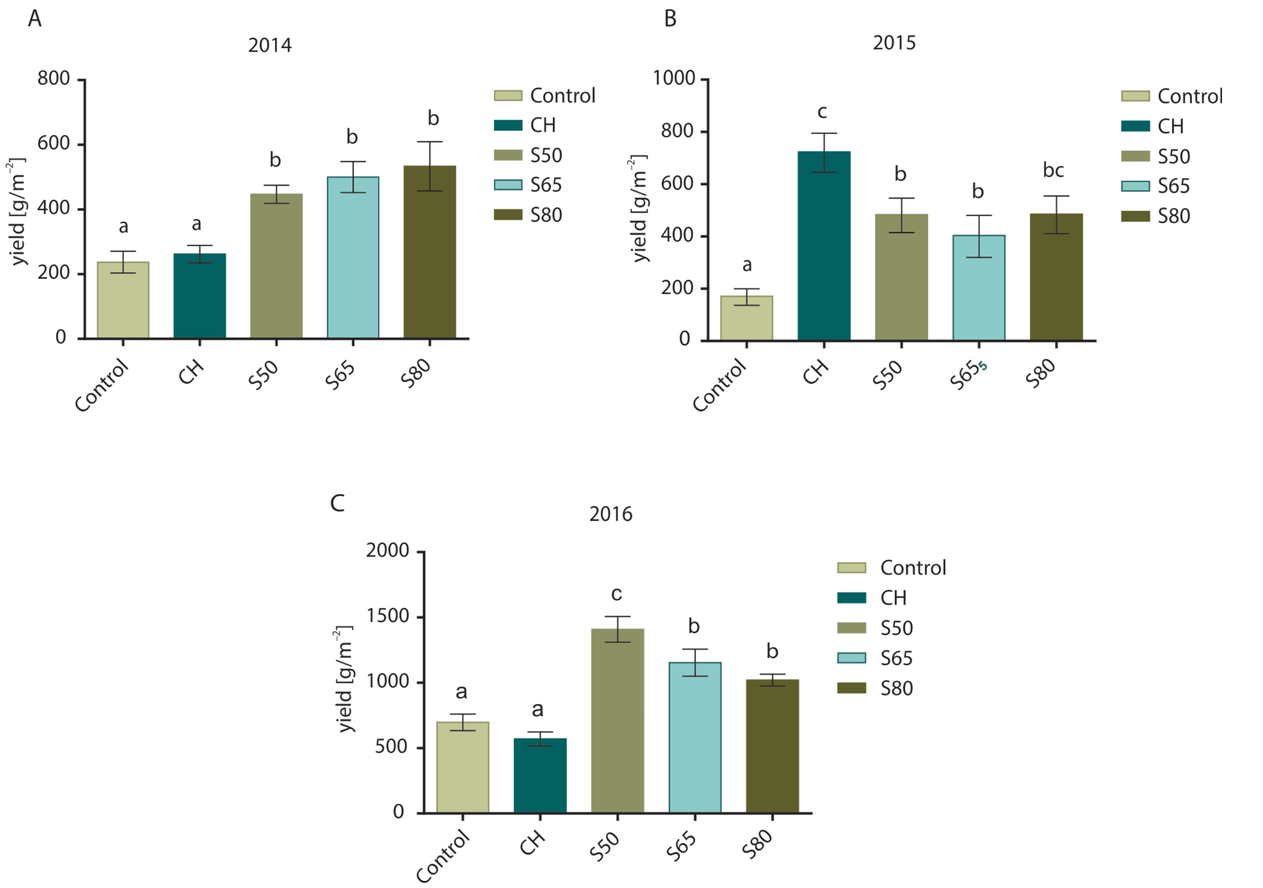

Fig. 7. The broad bean seeds yield in 2014–2016. For treatments see Figure 2. Values marked with different letters for a given year differ significantly at α = 0.05, factor: protection method

Table 7. Anova results for year (Y) and protection method (PM) on the selected growth parameters and yield of broad bean

	Number of seeds		Mass of seeds		Number of pods		Mass of pods with seeds		Yield	
	F	p	F	p	F	p	F	p	F	p
Y	125.45	0.00	134.84	0.00	66.60	0.00	121.84	0.00	138.86	0.00
PM	2.94	0.00	33.10	0.00	15.21	0.00	52.58	0.00	18.41	0.00
Y*PM	12.61	0.00	11.64	0.00	5.36	0.00	15.11	0.00	9.17	0.00

The morphological parameters of broad bean

Lobularia maritima in cultivation coordinated with broad bean in two of three seasons had a positive effect on the growth parameters of broad bean, such as: weight of pods with seeds and weight of seeds (Fig. 8). This effect was also significant in comparison to chemical protection. In all observation seasons, each of the applied spacing (especially S80) of broad bean cultivation with sweet alyssum resulted in a significantly higher weight of pods with seeds than in unprotected treatment. the case of the remaining morphological parameters, a general tendency of their growth was noticed along with an increase of plant spacing in cultivation.

Discussion

The presence of sweet alyssum between the rows of broad bean contributed to a noticeable reduction in the black bean aphid occurrence, comparable to chemical protection. The number of *A. fabae* on the broad bean in all the treatments with *L. maritima* was at average 3.5 times lower; whereas in the season 2016 it was even 24 times lower than in the control treatment. In available literature, the results concerning the effectiveness of companion plants in companion planting in relation to aphids are not so clear. Sweet alyssum in companion planting with lettuce contributed to the protection

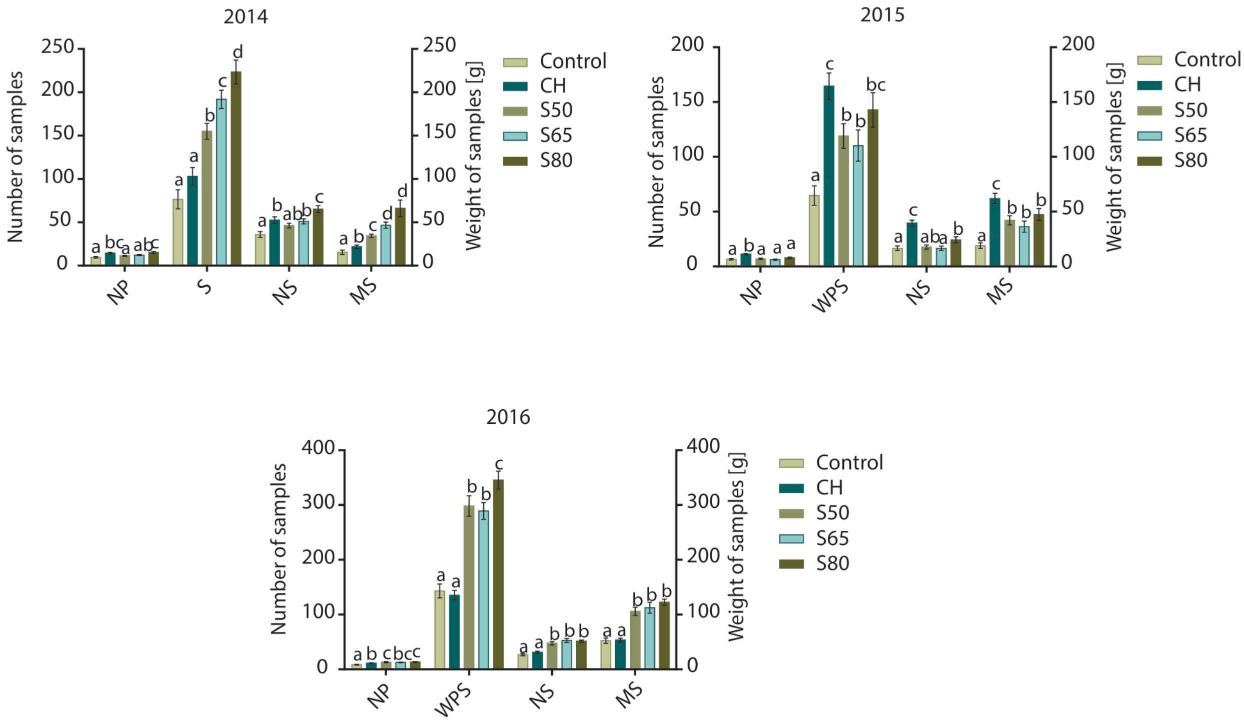

Fig. 8. The selected morphological parameters of broad bean in 2014–2016, where: NP – the number of pods on the plant, WPS – weight of pods with seeds from the plant (g), NS – the number of seeds per plant, MS – the mass of seeds from the plant (g). For treatments see Figure 2. Values marked with different letters for a given year and parameter differ significantly at α = 0.05, factor: protection method

of the crop through reducing the number of current-lettuce aphid by 40% (Brennan 2013). Summer savory (*Satureja hortensis* L.) cultivated between the rows reduced significantly the number of *A. fabae* on *V. faba* (max. by 35%) (Basedow *et al.* 2006). Similarly, basil (*Ocimum basilicum* L.) cultivated between the rows reduced the number of black bean aphids on broad bean; nevertheless, its effect was significantly lower (5%–23% of the effectiveness of reducing the number of this pest). Leguminous plant cultivation with cereals also contributed considerably to the limitation of *A. fabae* occurrence. In homogeneous bean cultivation, the count of *A. fabae* was on average 96 specimens / plant; whereas, the combination of bean and wheat cultivation resulted in the reduction of the pest quantity to 38 specimens/plant (Hansen *et al.* 2008).

The decrease in the number of *A. fabae* under the influence of sweet alyssum noted in our experiment can be associated with a high number of predators.

Sweet alyssum in our experiment generally contributed to an increase in the number of Syrphidae eggs and larvae on the broad bean plants. Nonetheless, significant differences were recorded between the research seasons (Fig. 5). In 2016, the quantity of hoverfly eggs in the treatments with sweet alyssum was at some moments of *A. fabae* occurrence even 40-times higher than the control treatment and under chemical protection (Fig. 3). In 2015, this trend was similar. However, the effect was not so strong despite the significant differences with the control. These results confirm the luring function of *L. maritima* towards Syrphidae adult specimens for which the pollen and nectar of this plant is a valuable energy source. Sweet alyssum in other research also contributed to the limitation of the aphid quantity on broccoli (*Brassica oleracea* L.) increasing their number on average from 3 to 13 times (Brennan 2016). The *L. maritima* had a statistically significant effect on the reduction of *Aphis nerii* in oleanders (*Nerium oleander* L.) by a three-fold higher number of Syrphidae (Madeira *et al.* 2022). A positive change in the population of natural enemies of the black aphid was observed due to the introduction of companion crops, the detailed analysis of which should be performed in the next stages of field experiments.

Coccinellidae larvae and imago, like Syrphidae, preferred broad bean plants cultivated together with sweet alyssum. The quantity of laid eggs was on average 0–0.19 pcs. · plant^{-1}, 0 – 3.75 larvae · plant^{-1} and 0.001–1.5 imago · plant^{-1}. In other authors' research, the number of Coccinellidae developmental stages was at a similar level or lower. For instance, the quantity of adult Coccinellidae ranged from 1.2–4.3 specimens · sorghum plant^{-1} (Phoofolo *et al.*

2010), 0–0.49 larvae · broad bean plant^{-1} (Gospodarek 2012) and 0.075–0.275 specimens · cabbage plant^{-1} (Nawrocka 2008).

The diversified spacing between the rows of broad bean did not significantly affect the number of aphids and, in general, most developmental stages of predators. In some years, predators preferred a higher density of plants, e.g., Coccinellidae imago in 2015 were more numerous at 50 and 65 cm spacing than at 80 cm spacing, and Syrphidae eggs in 2016 were most numerous at the smallest spacing (50 cm). In the latter case, however, this did not convert into the highest number of larvae of these predators in this season and at this spacing (Fig. 5). In the available literature from recent years, there are very few reports on the impact of spacing on the occurrence of aphids and their natural enemies. In particular, Malaquias *et al.* (2017) showed that the occurrence of predators in colonies of *Aphis gossypii* under the impact of different row spacing in cotton crop was closely correlated with the number of aphids and was independent of the spacing between plants. Earlier studies also generally indicate no significant effect of this factor. In potato cultivation, varied spacing had no effect on the abundance of lady beetles (Boiteau 1984), as well as on the occurrence of *Aphis craccivora* Koch. and hoverfly larvae on groundnuts (Booker 1963). Only studies by Mayse (1978) on different spacing in soybean cultivation showed a higher concentration of predators with a higher density of plants, which was also partly noticeable in our experiment.

Lobularia maritima in cultivation with broad bean created favorable predator-prey proportions, made it possible to naturally regulate the number of the pest to a level that does not pose a threat to the plant. The voracity of Syrphidae larvae depends on the predator species (Verheggen *et al.* 2008; Amiri-Jami and Sadegi-Namaghi 2014) as well as the species and age of an aphid (Sadeghi 2008). For instance, *S. pyrastrii* larva eats in its whole life not more than 72–231 specimens of black bean aphid. Whereas, cabbage aphid is eaten in the quantity of more than six times higher. *E. balteatus* eats ca. 200–255 specimens of black bean aphid or nearly twice as many cabbage aphids (Wnuk and Fuchs 1977; Wnuk 1979). The voracity of *E. balteatus* in relation to sour cherry aphid (*Myzus cerasi* L.) fluctuates from 400 to 500 specimens, and as far as *S. ribesii* is concerned, it is more than 20% higher (Wnuk 1979). The representatives of Syrphidae in our research were dominated by *S. ribesii* and *E. balteatus*. In the case of *E. balteatus* larvae, the colony of *A. fabae* may be eliminated exclusively by them during 1.5–4 days, provided that the quantity proportions of the predator – victim are maintained at the level 1 : 15 – 1 : 50. In larger colonies of aphids, i.e., when this ratio is 1 : 300 and higher, *E. balteatus* larva was not able to destroy the entire colony but its feeding impeded significantly the development rate of this pest. Taking into account the results of our experiment (the mean values of predator to prey ratio shown in Table 4.), the conditions observed in 2016 in treatments with sweet alyssum allowed Syrphidae larvae to eliminate aphids almost exclusively (without the help of other predators). In the remaining years the values of the above parameter were several times higher. However, a very strong positive effect of sweet alyssum in improving the predator-victim ratio was observed.

The voracity of Coccinellidae larvae, depending on the developmental stage, ranged on average from 8 to 13 aphid specimens · day^{-1} for L1 larvae, 15–26 aphid specimens · day^{-1} for L2 larvae and 16–25 and 22–42 aphid specimens · day^{-1} for L3 and L4 stages (Iperti 1999). Adult specimens eat daily 15–65 aphids (Gallo 2020), and larvae during their 10 day development may eat from 90 to 370 aphids (Undurraga *et al.* 2020). Hodek *et al.* (1965) provided the value of 1 : 50 as the minimum quantity ratio of a predator to a victim as the one enabling the impediment of aphid colony development on the plants. Such predator – victim proportions, maintained for at least 10–14 days, enable the Coccinellidae to limit considerably the number of aphids on plants. On the other hand, Cabral *et al.* (2006) revealed that the values of the predator – victim quantitative ratio at which they can prevent the development of aphid colonies in plants and nearly completely destroy them change together with the larva stage development. In this present investigation, the quantitative ratio of Coccinelidae larvae was on average for the entire season from 1 : 23 to 1 : 26840 (depending on the treatment and the season). The value of this parameter for Coccinellidae imago in relation to aphids was at the average level of 1 : 20 – 1 : 11840. The values of this parameter in 2016 and to some extent also in 2015 in treatments with *L. maritima* allows one to state that the presence of Coccinellidae could contribute to the fast elimination of aphids on broad bean.

A condition for the effective limitation of aphids is not only the appropriate number of predators but also a sufficiently early moment of their occurrence on plants. The mechanisms linking aphids with Syrphidae predators have long been the treatment of interest of scientists dealing with aspects of natural plant protection (Davidson 1922). All of them underline the fact that the reason for the insufficient role of predators is a delay in their occurrence in relation to the preys and their insufficient quantity. In our experiment, initially, the number of hoverfly eggs was low, the maximum of their laying was usually from the second half of May to the beginning of June (2014–2015) and from the end of May to the second half of June (2016) (Fig. 3). However, the presence of sweet alyssum was clearly conducive to the appearance of a significant number of Syrphidae eggs already in the initial period of aphid colony development (especially in seasons 2015 and 2016, Fig. 3). Moreover, no clear trend was found regarding the impact of different spacing between rows of broad beans. *L. maritima* is mentioned by many researchers as a plant used for luring aphid predators, mainly hoverflies (Al Doghairi and Cranshaw 1999). Nonetheless, its luring effect as regards Coccinellidae is also known. This plant lured 23% more Coccinellidae in the vicinity of woolly apple aphid colonies (*Eriosoma lanigerum* L.) than white mustard (Gontijo *et al*. 2013). Nevertheless, previous research did not confirm the impact of *L. maritima* on the frequency of Coccinellidae visits to cabbage (Laurenz and Meyhöfer 2016). To compare, coriander in kale (*Brassica oleracea* L.) cultivation increased the number of adult Coccinellidae on average by 30–50% (Resende *et al.* 2010), whereas, in carrot cultivation (as in the case of summer savory) it increased the number of imago 2–3 times (Jankowska and Wojciechowicz-Żytko 2016). As far as these data are concerned, sweet alyssum, in this research, demonstrates a very strong luring effect for ladybirds. As in the case of Syrphidae, Coccinellidae eggs in the initial period of development of aphid colonies were more numerous on broad bean in the vicinity of sweet alyssum than in the control treatment. However, no clear tendency was observed related to different spacing between plant rows (in each season during the first observation, significantly more Coccinellidae eggs than in the control were recorded in treatments with *L. maritima* at different spacings, Table 3).

Conclusions

1. The presence of *L. maritima* as a companion plant in intercropping with broad bean contributed to a significant reduction in black bean aphid levels and the obtained effect was similiar to broad bean chemical protection.
2. Generally, this plant species contributed to a considerable increase in the number of natural enemies of black bean aphid, i.e., the eggs, larvae of hoverflies, as well as all the development stages of Coccinellidae (especially imago).
3. Sweet alyssum, in intercropping with broad bean, contributed to the decrease in the value of black bean aphid quantitative ratio both with reference to hoverfly larvae as well as the larvae and imago of Coccinellidae to a level which enabled a significant reduction in the number of black bean aphid. In addition, it accelerated the moment of laying eggs by Syrphidae and Coccinellidae in large numbers on the broad bean plants.
4. Moreover, the introduction of sweet alyssum into cultivation was not competitive for the main plant - the broad bean, and it did not adversely affect its yield. Furthermore, the occurrence of aphid predators was generally not influenced by different spacing between broad bean rows.
5. Due to the considerable, prospective abilities of sweet alyssum to reduce the occurrence of the most harmful pest of broad beans, black bean aphid, this plant may be recommended for implementation, particularly in ecological broad bean crops, without the need to increase standard spacing.

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