

RESPONSE OF *Rhopalosiphum padi* L. (HEMIPTERA: APHIDIDAE) TO THE CONTENT OF FLAVONOIDS IN WINTER WHEAT TREATED WITH A BIOSTIMULANT AND BENEFICIAL MICROORGANISMS

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ABSTRACT

Background. The bird cherry-oat aphid (*Rhopalosiphum padi* L.) is one of the most important pests of cereal plants. Limiting its number by using pro-ecological methods such as biostimulants does not result in environmental contamination.

Material and methods. Winter wheat plants were grown under laboratory conditions and treated with the following preparations: EM Naturalnie Aktywny, which is based on beneficial microorganisms, and/or the Asahi SL biostimulant. In the BBCH 32 phase some plants were harvested and the percentage flavonoid content, calculated as quercetin, was determined, while the remaining plants were used to assess the level of feeding and development of the bird cherry-oat aphid.

Results. Wheat plants treated with biostimulants containing beneficial microorganisms contained on average 0.641% dry matter flavonoids. The pre-reproductive period of bird cherry-oat aphids was 8 days. Daily aphid fecundity was slightly more than three larvae per day. The length of the reproductive period of bird cherry-oat aphids on wheat plants ranged from 8 to 9 days. After 5 days of feeding, 2 adult aphids caused on average 15% damage to the plant surface. Correlation coefficients between the flavonoid content in wheat plants and development parameters and the extent of damage caused by bird cherry-oat aphids were insignificant.

Conclusion. The use of a biostimulant and beneficial microorganisms in wheat cultivation was found to be not statistically significant, but a reduction in the feeding and development of *R. padi* was observed.

Key words: bird cherry-oat aphid, flavonoids, insect feeding and development, pro-ecological measures

INTRODUCTION

Cereal plants under natural conditions are exposed to attack from a wide range of phytophagous entomofauna. The most important of them are cereal leaf beetles (*Oulema melanopus* L. and *Oulema gallaeciana* Heyden) and cereal aphids, mainly the bird cherry-oat aphid (*Rhopalosiphum padi* L.) and

the grain aphid (*Sitobion avenae* F.). A number of methods are used to combat them and include chemical, agrotechnical and biological methods (Bereś *et al.*, 2007; Kaniuczak and Bereś, 2008).

For many years methods based on pro-ecological methods of limiting the number of pests have been promoted in order to minimize the use of the chemical method. Recommended methods are

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treatments that have been of interest to people for several years, increasing plant resistance to biotic and abiotic stress factors. Beneficial microorganisms (known also as effective microorganisms), the Asahi SL biostimulant, and other products, are known to increase plant yield and health and also to improve soil properties so as to be more favorable for the growth and development of cultivated species, or to increase tolerance of rainfall deficiency (Piskier, 2006; Janas, 2009; Truba *et al.*, 2012; Szczepanek *et al.*, 2018). In addition, the use of these preparations affects the secretion of plant secondary metabolic compounds that regulate the numbers of numerous pests (Mallikarjuna *et al.*, 2004; Smith and Boyko, 2007; Lamparski, 2016).

Flavonoids are one of the most numerous groups of secondary plant metabolites. In the group of these compounds the most important are flavanones, flavanols and flavones (Czeczot, 2000). In terms of structure and properties they are a diverse group of low molecular weight polyphenolic compounds. Their basic skeleton is 15 carbon atoms forming a C6-C3-C6 moiety. The biochemical activity of flavonoids and their derivatives is determined by the presence and mutual orientation of active groups, mainly the hydroxyl, methyl and glycosidic groups (Małolepsza and Urbanek, 2000). Flavonoids can usually be found in various parts of the plant. They perform many functions, among others as: antioxidants, messenger substances in plant cells, UV filter, inhibitors of many enzymatic reactions, growth regulators, attractants or repellents against various animal organisms, including insects (Simmonds, 2003; Jasiński *et al.*, 2009; Majewska and Czeczot, 2009; Saviranta *et al.*, 2010; Stoclet and Schini-Kerth, 2011; Visioli *et al.*, 2011; Kamiyama and Shibamoto, 2012). They also regulate the behavior of many insects (including aphids) associated with food intake (Bennett and Wallsgrove, 1994; Chrzanowski, 2008; Chrzanowski and Leszczyński, 2008).

The aim of this study was to investigate the response of the bird cherry-oat aphid *Rhopalosiphum padi* L. (Hemiptera: Aphididae) to the treatment of winter wheat with biostimulants based on beneficial microorganisms and to determine the content of flavonoids in these plants.

MATERIAL AND METHODS

The study was carried out in 2014 in the laboratory of the Department of Biology and Plant Protection, UTP University of Sciences and Technology in Bydgoszcz and the laboratory of the Department of Pharmacognosy, Nicolaus Copernicus University in Bydgoszcz.

Winter wheat plants of the cultivar 'Arktis' were sown in pots ($\varnothing = 13$ cm, height 10 cm) filled with soil of a very good rye complex with straw removed. Sowing was conducted under laboratory conditions from 21st to 30th of March and continued in several series until mid-April. The soil was obtained from a field and under laboratory conditions biostimulants and beneficial microorganisms were added: EM Naturalnie Aktywny and/or Asahi SL biostimulant (Table 1). Ten grains were placed in each pot at a depth of 2.5–3.0 cm.

During growth the plants were fed with mineral fertilizers in amounts corresponding to the doses: 140 kg N·ha⁻¹, 30 kg P₂O₅·ha⁻¹ and 60 kg K₂O·ha⁻¹ and watered once a week, in an amount of approx. 100 ml·pot⁻¹. The temperature during plant growth was 21 ± 1°C, with a L16:D8 photoperiod and at 70% humidity.

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Approximately 7 weeks after sowing the plants reached the BBCH 32 stage and constituted the material for proper laboratory tests:

A. Tests for the feeding and development of bird cherry-oat aphids (EPP0, 2007; Lamparski, 2016).

The pots with wheat plants had an oval base made of Styrofoam (fitted to the top edge of the pot), on which Petri dishes (plastic, 10 cm in diameter) were placed. Inside each Petri dish, in the middle, on its underside, a plant leaf was placed and one *R. padi* adults (Müller, 1976) was inserted. Emerging larvae were then counted. The results (based on 2 full generations of the pest) are shown as the number of days from being born to the start of giving birth – the pre-reproductive period, the number of larvae born from 1 female each day – daily fecundity and the

number of days of giving birth by one female – the reproductive period. The experiments were carried out in 5 replications. The effect on plant health of feeding two aphid adults during 5 days was measured (EPPO, 2007). After the end of the research cycle, all aphid larvae found were counted and plant surface damage was assessed organoleptically. The results are presented as (fecundity) the sum of larvae born from 2 females [ind.] and % plant damage [%] after 5 days of insect feeding.

B. Test for determining flavonoids converted to quercetin (Christ-Müller method) (FP VI, 2002).

Plants cut above the soil surface were dried for two days at 32 °C. Two weighed samples were made of each of the raw materials tested. An extract was made from each sample and then a stock solution from which three test solutions and one reference

solution were prepared. Three absorbance measurements were made for each of the solutions using a UV-Vis spectrophotometer (Hitachi U-2900, Japan). The absorbance of the tested solutions was measured at the wavelength $\lambda = 425$ nm. All reagents were purchased at POCH (Gliwice, Poland). The results are presented as mean values taking into account standard deviations. The flavonoid content is given in % dry matter (DM) of the raw material.

The significance of differences between treatment means was estimated using the Tukey test. Relationships between the flavonoid content in winter wheat plants not damaged and damaged by aphids and the tested by us feeding and development of *R. padi* were determined by linear correlation analysis.

Table 1. Treatments, dose, time and method of application

Treatment	Dose (kg·ha ⁻¹)	Date applied	Growth phase	Application method
EM (Effective microorganisms)	40 kg·ha ⁻¹	Mid August 14.08.2013	Before sowing	Applied to soil
2EM (Effective microorganisms)	20 kg·ha ⁻¹	Mid August 14.08.2013	Before sowing	Applied to soil
	20 kg·ha ⁻¹	End of April 22.04.2014	BBCH 21	Applied to leaves
Biostimulant (1 application)	1.0 kg·ha ⁻¹	End of April 24.04.2014	BBCH 21	Applied to leaves
Biostimulant (2 applications)	2 × 0.5 kg·ha ⁻¹	End April 24.04.2014	BBCH 21	Applied to leaves
		Early May 02.05.2014	BBCH 28	

EM Composition: lactic acid bacteria, photosynthetic bacteria, yeast, cane molasses, azotobacter

Biostimulant Composition: sodium para-nitro phenolate – 0.3%, sodium ortho-nitro phenolate b – 0.2%, sodium 5-nitroguajakolan – 0.1%

BBCH 21: Beginning of Tillering, BBCH 28: End of Tillering

RESULTS AND DISCUSSION

Under laboratory test conditions, the bird cherry-oat aphid was feeding without difficulty and developed on wheat plants.

The use of EM Naturalnie Aktywny and Asahi SL preparations significantly differentiated the secretion of flavonoids (expressed as quercetin) by winter wheat not previously damaged by the bird cherry-oat aphid (Fig. 1). It was found that flavonoids were secreted by wheat plants on average in an amount of 0.641% DM. The largest amount was found in the control combination (0.855% DM). Significantly less

was recorded in the 2BA combination, i.e. 0.725% DM. Lamparski *et al.* (2017) noticed that a single plant of winter wheat produced 0.606% DM flavonoids (expressed as an amount of quercetin) regardless of whether effective microorganisms and/or the Asahi SL biostimulant were applied or if plants were damaged or not damaged by cereal leaf beetle. Of the 3 methods of application of EM (1×EM, 2×EM and 0×EM) in wheat plants, the 0×EM (0.653 pp DM) increased the amount of flavonoids. The 2×BA treatment proved most stimulatory for the production of secondary metabolites (0.662 pp DM). Wheat plants undamaged

by cereal leaf beetle and treated with EM and Asahi SL preparations produced 0.013 pp DM more flavonoids than plants exposed to damage of *O. melanopus* for two days and treated with same preparations and there was significant difference (Lamparski *et al.*, 2017). Lamparski (2016) noticed that spring barley that had not been damaged by bird cherry-oat aphid insects contained in dry matter

0.716% flavonoids expressed as quercetin. In the study by Choe *et al.* (2010), they report that the content of flavonoids in barley leaves was, depending on the type of alcohol extract: 94.2 (ethanol) and 58.1 (methanol) mg of kaempferol per g of this plant leaf extract. Sartelet *et al.* (1996) report that flavonoid content reached 500 mg·kg⁻¹ in millet grain.

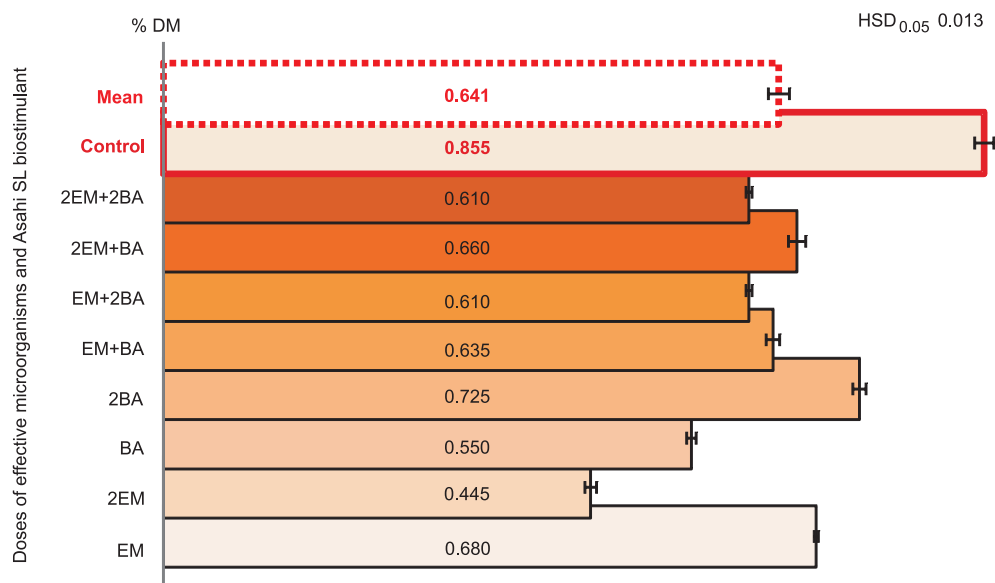


Fig. 1. Flavonoid content expressed as quercetin in the above-ground part of wheat, % DM ± SD

The length of the period from being born to the beginning of giving birth of a bird cherry-oat aphid on wheat plants ranged from 7.8 days (Control) to 8.4 days in treatments with biostimulators: (EM+2BA), (2EM+BA) and (2BA). The average for this series of experiments was 8.2 days (Fig. 2). Similar results have been obtained under controlled conditions, where *R. padi* needed from 7.8 to 8.8 days to undergo nymphal development, and the use of pro-ecological measures slightly extended the pre-reproductive period of the pest compared with the control (Lamparski, 2016). Araya and Foster (1987) report slightly shorter pre-reproductive periods of *R. padi* on wheat and oat plants (5.6–6.2 and 5.4–5.9 days, respectively). Asin and Pons (2001) recorded pre-reproductive periods of *R. padi* on durum wheat plants: 6.5 days at 22°C and

9.3 days at 18°C. Chrzanowski (2013) found that on the seedlings of winter triticale cultivar 'Lamberto', which is relatively resistant to aphid feeding, in comparison with the susceptible cultivar 'Marko', *Sitobion avenae* had a longer pre-reproductive period (8.90 and 6.25 days, respectively).

In the present study, conducted on Petri dishes, an assessment of the number of larvae of the pest born daily found that the population increased by an average of 3.2 larvae per day (Fig. 3). The lowest fecundity of bird cherry-oat aphids on wheat plants (2.6 individuals/day) was noted when the EM Naturalnie Aktywne preparation was introduced into the soil during post-harvest cultivation (in autumn) and Asahi SL was applied twice (EM+2BA).

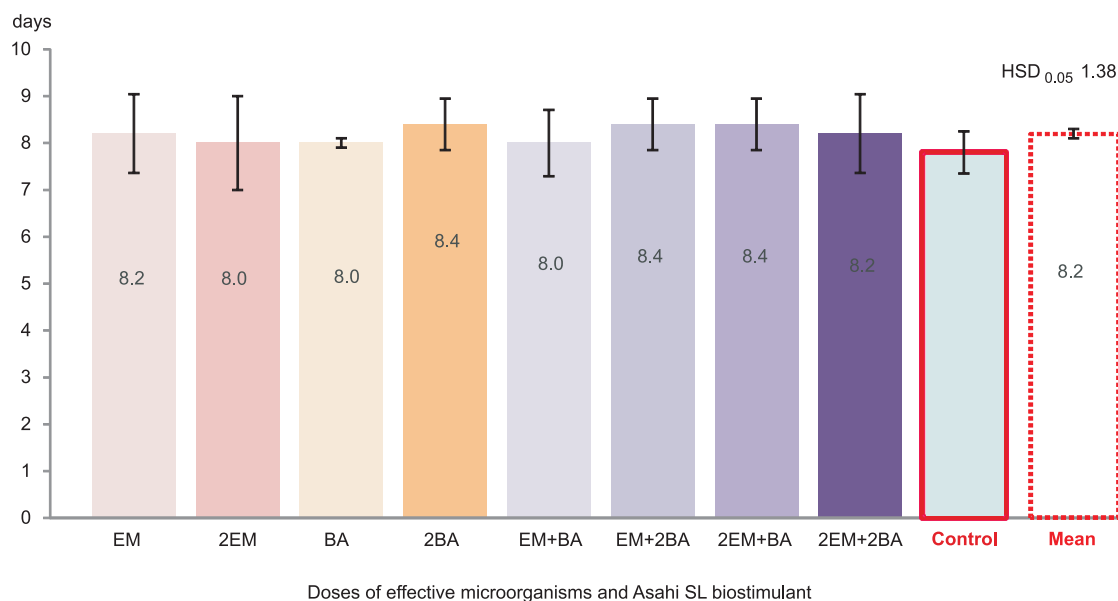


Fig. 2. The length of the pre-reproductive period of bird cherry-oat aphids on wheat plants (Petri dishes), days \pm SD

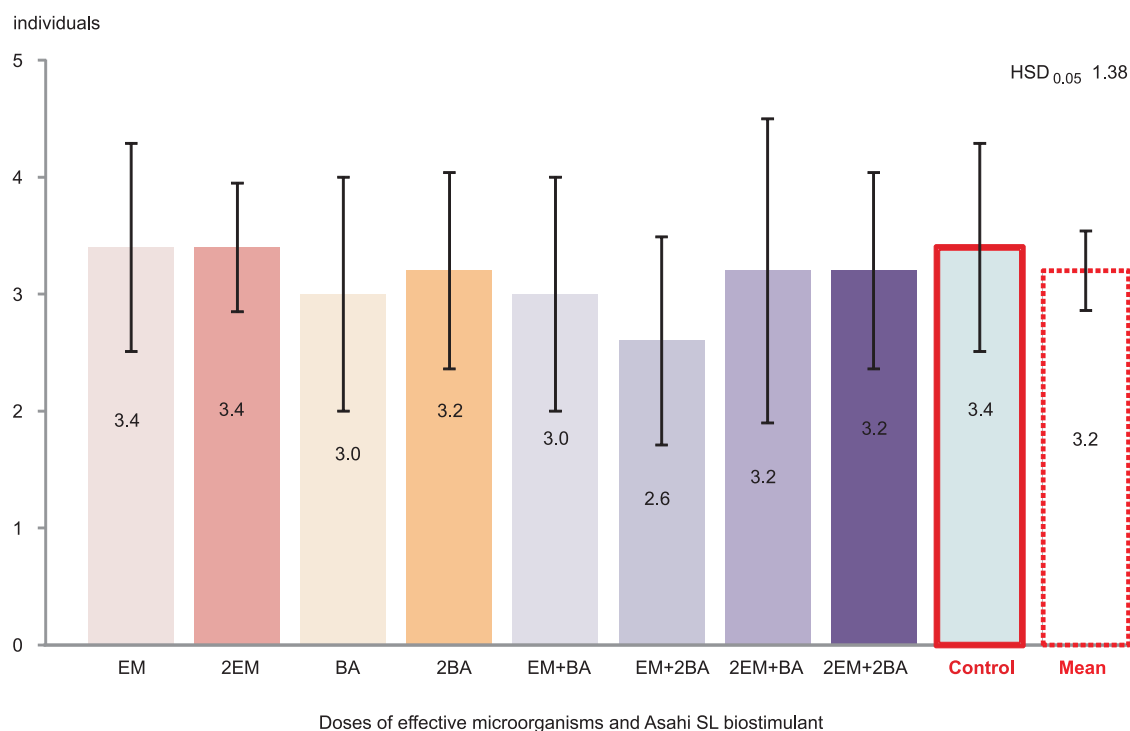


Fig. 3. Daily fecundity of bird cherry-oat aphids on wheat plants (Petri dishes), individuals \pm SD

Comparing these results with the fecundity of aphids obtained in pots it can be concluded that they do not differ significantly for the tested combinations of preparations (Fig. 5). Regardless of the method of application, 25.5 larvae were obtained after 5 days as the offspring of two female pests. According to Lamparski (2016), aphids feeding on spring barley plants with pro-ecological measures applied were characterized by similar fertility (3.5 individuals for the control and 3.0 on average for the tests). Taheri *et al.* (2010) obtained from 2.41 to 2.99 larvae born daily by bird cherry-oat aphids on 6 tested wheat cultivars (from 42.76 to 62.05 larvae, respectively, obtained from one female). Under field conditions on several tested triticale cultivars, the daily fecundity of one female *R. padi* was 1.7 larvae (Wójcicka, 2010). However, Wójcicka (2013) reports that waxy triticale cultivars cause a significant extension of the period needed by these phytophages to reach maturity, and later they reduce the number of individuals appearing from one female. Chrzanowski (2013), found that on seedlings of the winter triticale cultivar 'Lamberto', which is relatively resistant to aphids feeding, compared with the susceptible cultivar 'Marko',

S. avenae had a lower daily fecundity (respectively: 3.39 and 4.13 individuals·day⁻¹).

The effect of the preparations on the period in which the aphids were able to deliver offspring was also compared. It was found that it was similar to what insects needed to pass individual larval stages. The length of the reproductive period of bird cherry-oat aphids on wheat plants ranged from 8 to 9 days. There were no significant differences between the treatments with regard to the reproductive period of aphids. The only thing that merits attention is the fact that the longest reproductive period of aphids was found in the control (Fig. 4). Lamparski (2016) reports that the length of the reproductive period is an important parameter indicating the development possibilities of pests. The same author states that bird cherry-oat aphids on spring barley plants treated with pro-ecological preparations gave birth to larvae for 7.6 to 8.8 days. Asin and Pons (2001) on durum wheat plants recorded the following reproductive periods of *R. padi*: 7.9 days at 22°C and 13.1 days at 18°C. Araya and Foster (1987), however, report slightly longer reproductive periods of *R. padi* on wheat and oat plants (11.4–13.6 and 10.4–11.4 days, respectively).

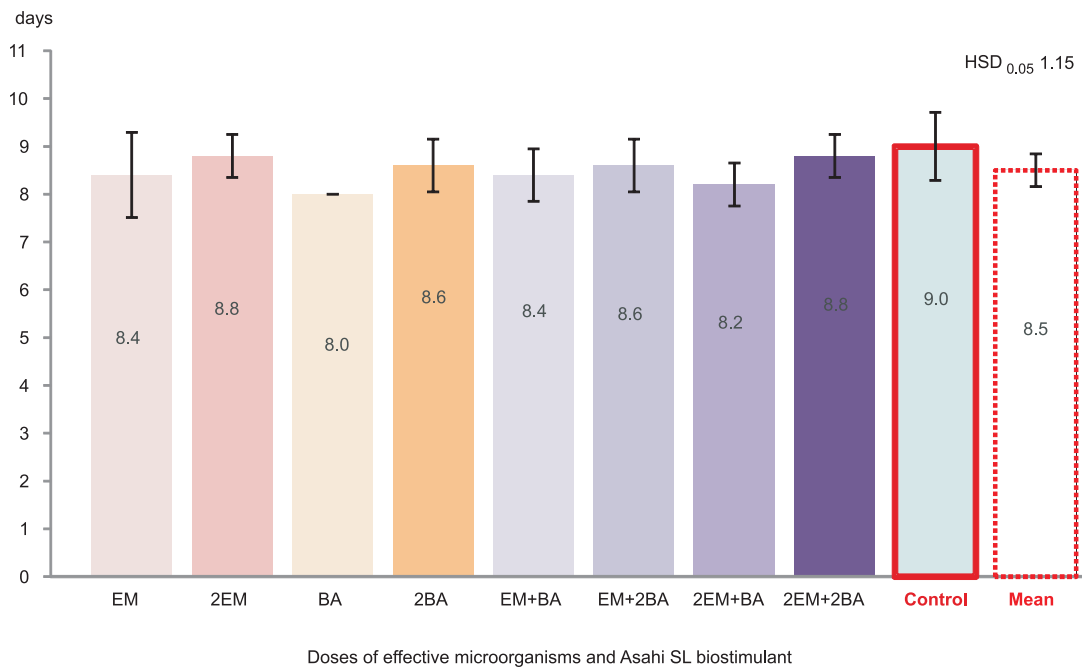


Fig. 4. The length of the reproductive period of bird cherry-oat aphids on wheat plants (Petri dishes), days ± SD

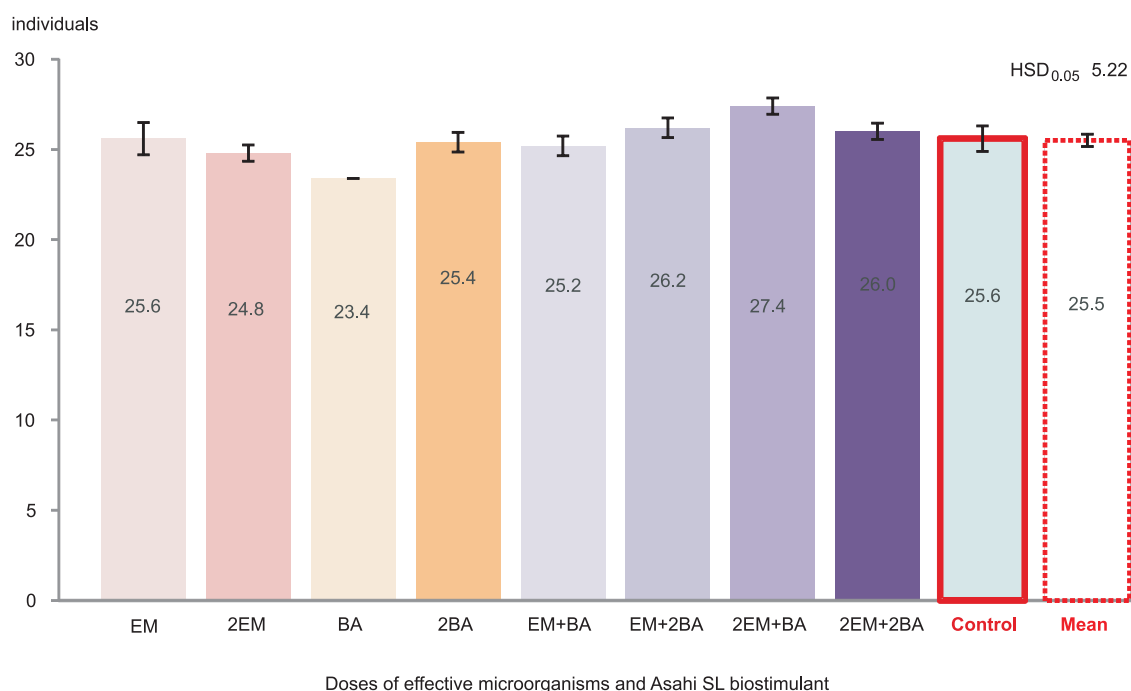


Fig. 5. Fecundity of bird cherry-oat aphids on wheat plants (pots), individuals \pm SD

In the experiment carried out in the pots, on average 14.7 % of plant leaf surface was damaged by the feeding of *R. padi* (Fig. 6). No significant differences between the treatments were found in the percentage of damage caused to the wheat plants. The results obtained range from 11 to 18% of leaf damage caused by two aphid imago forms feeding for 5 days. Compared with these data, slightly smaller damage by *R. padi* was found on spring barley plants (13.2%) (Lamparski, 2016).

The correlation coefficients calculated between the content of flavonoids in winter wheat plants and parameters determining feeding and development of bird cherry-oat aphids were statistically insignificant and usually positive (Table 2). Similarly, in other studies, the calculated correlation coefficients usually showed the absence of strong relationships between the secretion of different groups of phenolic compounds by barley plants and the parameters of

feeding and development of insects on plants on which pro-ecological measures were used (Lamparski, 2016; Lamparski *et al.*, 2018). Research on corn and barley plants showed an inverse correlation between the total phenol content and fecundity of cereal and bird cherry-oat aphids at various stages of cereal development (Eleftherianos *et al.*, 2006). Gomes *et al.* (2005) found that silicon fertilization and a previous infestation with aphids induced wheat plant resistance to the greenbug *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). Leszczyński *et al.* (1985) suggested that preference for the studied winter wheat cultivars was associated with the level of phenolic compounds naturally occurring in the plants. The tested dihydroxy-phenols showed the highest inhibition of the feeding of *Rhopalosiphum padi* (L.) larvae.

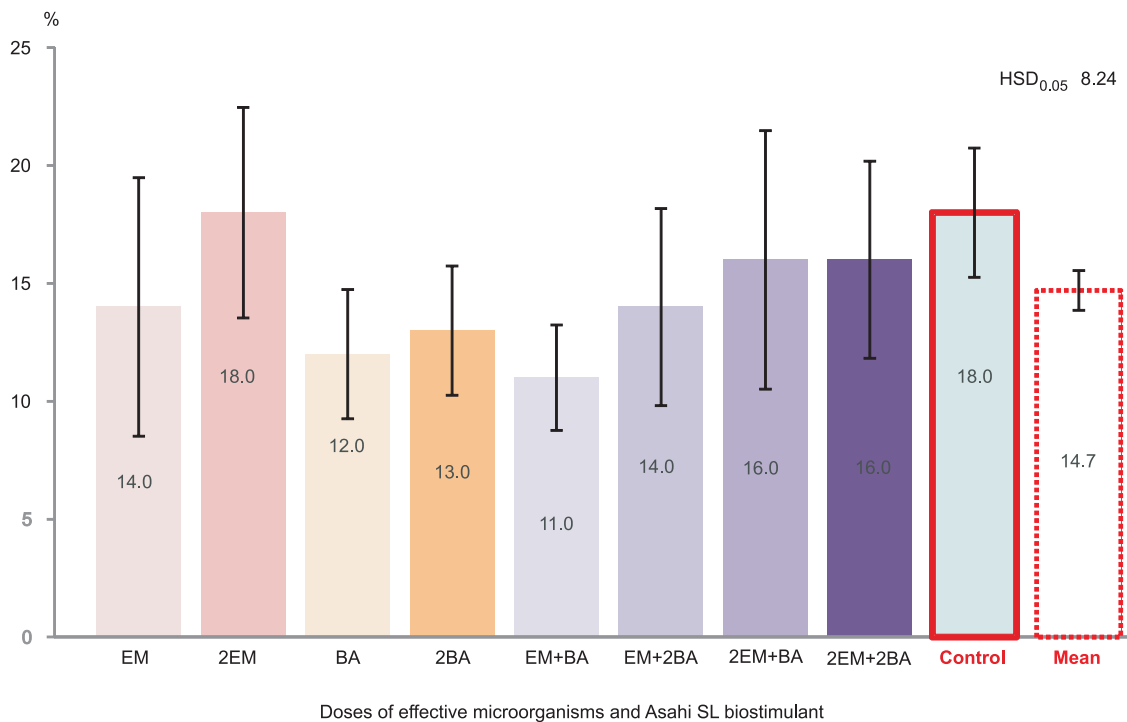


Fig. 6. The plant surface damage caused by bird cherry-oat aphids on wheat plants (pots), % \pm SD

Table 2. Correlation coefficients between the flavonoid content in wheat plants and development parameters and the extent of damage caused by bird cherry-oat aphids

Feature	Development parameters and the extent of damage caused by bird cherry-oat aphids				
	pre-reproductive period	daily fecundity	reproductive period	fecundity	damage
The flavonoid content in wheat plants	-0.063	0.209	0.280	0.351	0.072

*** $r_{crit.}^{0.01} = 0.708$; ** $r_{crit.}^{0.05} = 0.576$; * $r_{crit.}^{0.1} = 0.497$

CONCLUSIONS

Application of beneficial microorganisms (EM Naturalnie Aktywne) and Asahi SL biostimulant in winter wheat cultivation causes a slight reduction in the feeding and development of *R. padi*. The application of the tested preparations did not impact significantly on an extension of the pre-reproductive period of *R. padi*, reduction in the number of aphid larvae born per day, shortening of the reproductive period of *R. padi* or reduction in plant damage caused

by the pest. Wheat plants not previously damaged by *R. padi* and only treated with with biostimulants based on beneficial microorganisms contained less flavonoids.

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REAKCJA *Rhopalosiphum padi* L. (APHIDIDAE, HEMIPTERA) NA ZAWARTOŚĆ FLAWONOIDÓW W PSZENICY OZIMEJ TRAKTOWANEJ BIOSTYMULATOREM I POŻYTECZNYMI MIKROORGANIZMAMI

Streszczenie

Mszyca czeremchowo-zbożowa (*Rhopalosiphum padi* L.) należy do ważniejszych szkodników roślin zbożowych. Ograniczanie jej liczebności poprzez stosowanie proekologicznych metod, jak biostymulanty, nie powoduje zanieczyszczenia środowiska. W laboratorium wysiewano ziarno pszenicy ozimej. Następnie w odpowiedniej fazie rozwojowej rośliny poddano aplikacji szczepionką EM Naturalnie Aktywny, opartej na pożytecznych mikroorganizmach, i/lub biostymulatorem Asahi SL. W fazie BBCH 32 część nadziemną połowy roślin ścięto i ustalono procentową zawartość flawonoidów w przeliczeniu na kwercetynę, a pozostałe posłużyły do badań oceniających poziom żerowania i rozwoju mszyicy czeremchowo-zbożowej. Rośliny pszenicy traktowane szczepionką EM Naturalnie Aktywny i/lub biostymulatorem Asahi SL zawierały średnio 0,641% flawonoidów w suchej masie. Okres przedreprodukcyjny mszyicy czeremchowo-zbożowej wyniósł 8 dni. Jej płodność dzienna wyniosła nieco ponad trzy larwy dziennie. Długość okresu reprodukcyjnego mszyicy czeremchowo-zbożowej na roślinach pszenicy kształtowała się w przedziale od 8 do 9 dni. 2 imago mszyc przez 5 dni żerowania spowodowały 15% uszkodzeń powierzchni roślin. Współczynniki korelacji pomiędzy zawartością flawonoidów w roślinach pszenicy a parametrami rozwoju i żerowania mszyicy czeremchowo-zbożowej były nieistotne. Stosowanie biostymulatora i pożytecznych mikroorganizmów w uprawie pszenicy skutkowało zauważalnym ograniczeniem żerowania i rozwoju *R. padi*. Nie wykazano jednak istotnego statystycznie wpływu tych preparatów na płodność, okres przedreprodukcyjny oraz reprodukcyjny mszyicy.

Słowa kluczowe: flawonoidy, mszyca czeremchowo-zbożowa, zabiegi proekologiczne, żerowanie i rozwój owadów