J. Elem. s. 95–108

DOI: 10.5601/jelem.2014.19.1.605

COEXISTENCE OF *APHIS FABAE* SCOP. PREDATORS ON BROAD BEAN GROWING ON SOIL POLLUTION WITH HEAVY METALS*

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Abstract

The investigations were conducted to determine the effect of soil contamination with heavy metals (cadmium, copper, nickel, lead and zinc) on the level corresponding to 3rd pollution degree in the IUNG classification on interactions between individual aphidophagous groups and their stages of development occurring in *Aphis fabae* Scop. colonies on broad beans. The following were analysed: the occurrence and number of individual stages of predator development (eggs, larvae, pupae and adult specimens). The Agrell's index of coexistence were computed from the achieved results. The values of the Agrell's index for broad bean were low for individual predator groups and their development stages. Among the investigated predator groups, the best mutual tolerance was observed for Syrphidae and Coccinellidae. Co-existence of individual aphidophagous groups seems to be strongly conditioned by food resources, i.e. aphid availability. Heavy metals, by affecting the host plant, modified the degree of its colonization by aphids and their number, hence changes in the incidence of predators. Thus, soil pollution with zinc and nickel contributed to limiting the extent of broad bean colonization by aphids and predators, and consequently to a decrease in the values of their coexistence indices; on the other hand, soil contamination with cadmium and lead favours plant colonization by aphids and predators and therefore the presence of individual aphidophagous groups was noted more frequently on the same plants.

Keywords: heavy metals, broad bean, Ag index, aphidophagous.

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* This work was partly financed by the Polish State Committee for Scientific Research, Project No. N305007 31/0334.

WSPÓŁWYSTĘPOWANIE DRAPIEŻCÓW MSZYCY BURAKOWEJ *APHIS FABAE* SCOP. NA BOBIE W WARUNKACH ZANIECZYSZCZENIA GLEBY METALAMI CIĘŻKIMI

Abstrakt

Celem badań było określenie wpływu skażenia gleby metalami ciężkimi (kadmem, miedzią, niklem, ołowiem i cynkiem) na poziomie odpowiadającym III stopniowi zanieczyszczenia wg klasyfikacji IUNG na wzajemne oddziaływanie między poszczególnymi grupami afidofagów oraz ich stadiami rozwojowymi występującymi w koloniach mszycy burakowej *Aphis fabae* Scop. na bobie. Analizowano występowanie i liczebność poszczególnych stadiów rozwojowych drapieżców (jaj, larw, poczwarek, postaci dorosłych). Na podstawie wyników obliczono wskaźniki współwystępowania Agrella. Wartości wskaźników Agrella na bobie dla poszczególnych grup drapieżców i ich stadiów rozwojowych były niskie. Spośród badanych grup drapieżców największą wzajemną tolerancję obserwowano w przypadku bzygowatych i biedronkowatych. Współwystępowanie poszczególnych grup afidofagów wydaje się być silnie uwarunkowane dostępnością pokarmu – mszyc. Zastosowane metale ciężkie, wpływając na kondycję rośliny żywicielskiej, modyfikowały stopień jej zasiedlenia przez mszyce oraz ich liczebność, a w rezultacie występowanie drapieżców. Skażenie gleby cynkiem oraz niklem przyczyniło się do ograniczenia stopnia opanowania bobu przez mszyce i drapieżców, a tym samym do zmniejszenia wartości wskaźników ich wzajemnego współwystępowania, natomiast skażenie gleby kadmem i ołowiem sprzyjało opanowaniu roślin przez mszyce i drapieżców, w związku z czym częściej notowano obecność poszczególnych grup afidofagów na tych samych roślinach.

Słowa kluczowe: metale ciężkie, bób, wskaźnik Ag, afidofagi.

INTRODUCTION

Heavy metals present in consumed food affect herbivores (e.g. aphid) as well as the subsequent food chain link, i.e. predators. Considerable accumulation of some heavy metals was assessed in bodies of *Aphis fabae* Scop. preying on polluted plants (CRAWFORD et al. 1995, KAFEL et al. 2012). Among aphis predators, Diptera, Syrphidae, which consume big quantities of these pests, are particularly exposed to heavy metals (WINDER et al. 1999). On the other hand, Coleoptera, Coccinellidae, when preying on *Sitobion avenae* aphids and at risk of a higher cadmium level in host plants, showed no signs of biomagnification of this element in their organisms (GREEN et al. 2003), in which they resembled larvae of Neuropetra, Chrysopidae, consuming *Rhopalosiphum padi* aphids (ALONSO et al. 2009). However, cadmium concentrations in the bodies of predators exposed to an elevated Cd content were much higher than in the control specimens. It should be remembered that accumulation of heavy metals in an organism does not equal its toxicity (CORTET et al. 1999). There is great diversification in invertebrates' response to an elevated level of heavy metals in the environment.

Certain antagonisms, which may weigh on the final effect of heavy metals in the environment, appear among individual representatives of a broad range of aphids' natural enemies (ALMOHAMAD et al. 2010). However behaviour strategies of individual aphidophagous groups may be conditioned by several factors, many of which still await explanation.

The aim of the investigations was to determine the influence of soil contamination with heavy metals (cadmium, copper, nickel, lead and zinc) on the level corresponding to the 3rd pollution degree in the IUNG classification on interactions between individual aphidophagous groups and their stages of development occurring in *Aphis fabae* Scop. colonies on broad beans.

MATERIAL AND METHODS

An experiment in pots containing 9.8 kg d.m. of soil was conducted in 2007 and 2008 in Zagaje Stradowskie located in the Świętokrzyskie Province, the commune of Kazimierz. Broad bean (*Vicia faba* L.) was chosen as a model plant because it is a preferred food of aphids (*Aphis fabae* Scop.). Observations were conducted on broad beans of the cultivar White Windsor, grown in the following treatments: unpolluted soil with the natural heavy metal content (C); unpolluted soil with the natural heavy metal content and receiving mineral fertilization (NPK); soil polluted with cadmium, soil polluted with lead, soil polluted with copper, soil polluted with zinc and soil polluted with nickel. The applied doses were: 4 mg Cd kg^{-1}d.m. of soil, 85 mg Cu kg^{-1} d.m., 110 mg Ni kg^{-1} d.m., 530 mg Pb kg^{-1} d.m. and 1000 mg Zn kg^{-1} d.m. of soil, corresponded to the 3rd level of pollution, i.e. medium pollution according to the classification developed by the IUNG in Puławy (Kabata-Pendias et al.1993). Heavy metals were supplied to the soil in spring 2007 as water solutions of the following salts: $3CdSO_4 \cdot 8H_2O$, $NiSO_4 \cdot 7H_2O$, $CuSO_4$, $ZnSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$. The following fertilizer doses were applied: 0.7 g N (as NH_4NO_3); 0.8 g P_2O_5 (as KH_2PO_4); 1.2 g K_2O (as KCl) per pot. The doses were equal in all the treatments except the control (C). In the pots where soil was treated with $Pb(NO_3)_{2,}$ in order to maintain the nitrogen quantity similar to that in the other groups, basic fertilization was diminished accordingly. Each pot contained separately prepared batches of soil. A thin layer of crumbled soil was put on plastic film and sprayed with an appropriate mixture of fertilizer and metal, afterwards it was thoroughly mixed. This procedure was repeated several times. In 2008, the soil contaminated in the previous year was used for the experiment. Prior to it, the soil was loosened and basic fertilization was applied, as in 2007. Five broad bean plants were grown in each pot. The experiment was set up in 8 replications, in a randomized block system. The experimental soil was degraded humus (chernozem) developed from loess. It had acid reaction (pH in 1 mol·dm^{-3} was 5.3 and in water 6.1) and contained 1.13% of organic carbon. The analysis of the texture proved that the soil was a clay silt deposit. The analyses of the soil heavy metal content before the experiment showed that their levels were generally below the threshold limits set for soils unpolluted with these metals. The soil pH was assessed in 1 mol dm^{-3} KCl solution with a potentiometer; organic carbon content was determined with the

Tiurin's method; grain size distribution was checked with the Bouyoucose--Casagrande's method in the Prószyński's modification. The heavy metal content in initial soil was assessed following digestion of a sample in aa mixture of nitric and perchloric acids (2:1). The heavy metal concentrations in the extracts were assessed using atomic absorption spectrometry (AAS).

Aphid fabae Scop. populations were monitored since the first winged migrants were settled on the plants, twice a week, until the end of their presence. All larvae, wingless females and winged migrants were counted. The presence of all pre-imago development stages of predators was observed within the aphid colonies (Diptera, Syrphidae; Coleoptera, Coccinellidae; Neuroptera, Chrysopidae; Heteroptera, Anthocoridae and Aranea). The following were analysed: the occurrence and number of individual stages of predator development (eggs, larvae, pupae and adult specimens) on all plants (not only settled by aphids) because aphidophagous insects could also appear on plants free from aphids. The incidence of so-called tarry black spots, which evidenced the presence of Diptera, Syrphidae larvae and mummies of parasited aphids, was also noted. The values of the Agrell's index of co-existence (GÓRNY, GRÜM 1981) were computed for both individual aphid natural enemies and selected development stages, well as for Formicidae, according to the following formula:

$$Ag = (N_{a,b}/N)\ 100,$$

where: $N_{a,b}$ – number of plants on which units a and b occurred jointly (a and b – comparable pairs of aphid natural enemies, their development stages, and Formicidae);

N – number of all plants.

Also, the percentages of plants on which aphid were observed as well as individual development stages of their natural enemies and Formicidae were computed.

Statistical calculations were made with Statistica 8.0PL software. The significance of differences between the means was assessed by the one-way Anova and means were differentiated using the Duncan's test on the significance level $p<0.05$.

RESULTS AND DISCUSSION

The most numerous aphid colonies were encountered on plants growing on the cadmium polluted soil (Table 1). On the broad bean plants growing on the soil contaminated with either lead or copper, the number of the aphid colonies was the same as in the control, but significantly lower on the plants growing on the soil contaminated either with zinc or nickel. In the previous experiment with the same level of pollution, and in the same location, an average number of aphids on plants growing on zinc contaminated soil con-

Table 1

Occurrence of *Aphis fabae* Scop. aphid, its natural enemies and Formicidae on broad bean plants cultivated on unpolluted soil (control) and soil contaminated with single heavy metals

Specification	*C* *	NPK	Cd	Cu	Pb	Ni	Zn
Mean number of aphids per plant	575.2 *b***	525.1 *b*	656.8 *b*	414.2 *b*	610.0 *b*	14.4 *a*	113.3 *a*
	274.0 *b*	408.3 c	711.4 d	258.6 *b*	359.7 *b* c	5.3 *a*	62.1 *a*
Mean number of *Syrphidae* eggs per plant	0.008 *a*	0.016 *a*	0.016 *a*	0.027 *a*	0.039 *a*	0.000 *a*	0.007 *a*
	0.043 *b*	0.044 *b*	0.019 *ab*	0.010 *a*	0.029 *ab*	0.000 *a*	0.004 *a*
Mean number of *Syrphidae* larvae per plant	0.041 *a*	0.041 *a*	0.024 *a*	0.009 *a*	0.032 *a*	0.000 *a*	0.015 *a*
	0.019 *ab*	0.043 *bc*	0.062 *c*	0.024 *ab*	0.038 *abc*	0.004 *a*	0.017 *ab*
Mean number of *Coccinellidae* eggs per plant	0.008 *a*	0.008 *a*	0.008 *a*	0.018 *a*	0.024 *a*	0.017 *a*	0.007 *a*
	0.010 *a*	0.000 *a*	0.014 *a*	0.000 *a*	0.010 *a*	0.000 *a*	0.008 *a*
Mean number of *Coccinellidae* larvae per plant	0.285 *b*	0.187 *ab*	0.312 *b*	0.186 *ab*	0.333 *b*	0.008 *a*	0.073 *a*
	0.236 *bc*	0.256 *b* c	0.344 c	0.175 *b*	0.196 *b*	0.008 *a*	0.050 *a*
Mean number of *Coccinellidae* pupae per plant	0.081 *abc*	0.098 *bc*	0.088 *bc*	0.071 *abc*	0.143 *c*	0.000 *a*	0.015 *ab*
	0.014 *a*	0.019 *a*	0.019 *a*	0.048 *b*	0.019 *a*	0.000 *a*	0.000 *a*
Mean number of *Coccinellidae* adults per plant	0.130 *bc*	0.081 *abc*	0.096 *abc*	0.142 *c*	0.040 *ab*	0.008 *a*	0.073 *abc*
	0.260 *bc*	0.135 *b*	0.364 *c*	0.228 *b*	0.139 *b*	0.000 *a*	0.017 *a*
Mean number of *Chrysopidae* eggs per plant	0.008 *a*	0.008 *a*	0.000 *a*	0.044 *ab*	0.071 *b*	0.000 *a*	0.007 *a*
	0.000 *a*	0.000 *a*	0.005 *ab*	0.015 *b*	0.005 *ab*	0.000 *a*	0.000 *a*
Mean number of *Formicidae* per plant	0.106 *ab*	0.211 *b*	0.400 *c*	0.106 *ab*	0.238 *b*	0.000 *a*	0.102 *ab*
	0.038 *ab*	0.217 c	0.062 *ab*	0.049 *ab*	0.091 *b*	0.000 *a*	0.059 *ab*
Mean number of *Aranea* per plant	0.049 *abc*	0.073 *c*	0.040 *abc*	0.062 *bc*	0.040 *abc*	0.000 *a*	0.007 *ab*
	0.029 *b*	0.014 *ab*	0.019 *ab*	0.019 *ab*	0.005 *a*	0.000 *a*	0.000 *a*
Mean number of *Anthocoridae* per plant	0.049 *a*	0.057 *a*	0.032 *a*	0.049 *a*	0.055 *a*	0.008 *a*	0.036 *a*
	0.000 *a*	0.000 *a*	0.005 *a*	0.000 *a*	0.010 *a*	0.000 *a*	0.000 *a*
Mean number of mummies of parasitized aphids per plant	0.000 *a*	0.415 *a*	1.232 *a*	0.000 *a*	0.000 *a*	0.000 *a*	0.000 *a*
	0.591 *a*	8.341 *b*	0.019 *a*	0.121 *a*	0.507 *a*	0.025 *a*	0.000 *a*

* *C* – control, NPK – soil with mineral fertilization, Cd – soil contaminated with cadmium, Cu – soil contaminated with copper, Pb – soil contaminated with lead, Ni – soil contaminated with nickel, Zn – soil contaminated with zinc.

** Means marked with the same letter in lines do not differ statistically at $p<0.05$. Upper and lower values relate to experiments from 2007 and 2008, respectively.

taminated in 2002-2005 was about 36 individuals per plant, whereas in the control objects there were 565 specimens per plant (GOSPODAREK 2012*a*).

Syrphiade eggs were absent from plants contaminated with zinc. No marked differences were noted in the number of this predator's eggs in aphid colonies on the control and lead or cadmium containing plants. Significantly fewer Syrphidae eggs were found in 2008 on plants growing on the nickel and copper polluted soil. Their number was below the level established on the control plants. In 2007, no statistically significant differences in the number of Syrphidae larvae were found between the analyzed objects. The larvae were absent from plants contaminated with zinc. In 2008, the most numerous Syrphidae larvae were noticed in aphid colonies on cadmium contaminated plants. They were markedly more numerous than on the control plants without mineral fertilization and on plants growing on soils contaminated with copper, nickel or zinc. Syrphidae larvae were most rarely spotted on plants growing on the zinc polluted soil. No differences were detected between the analyzed treatments in the number of eggs laid by Coccinellidae. Ladybird larvae were several times more numerous than Syrphidae larvae. In 2007, Coccinellidae larvae were most numerous in the aphid colonies on the plants growing in soils contaminated with cadmium and lead. Significantly fewer ladybird larvae were found in aphid colonies on plants growing on soils contaminated with nickel and zinc. In 2008, the most numerous ladybird larvae were found in aphid colonies on cadmium contaminated plants. There were markedly larger numbers of the larvae on these plants than in all the other treatments contaminated with heavy metals. Similarly as in 2007, Coccinellidae larvae were most rarely spotted on plants from soils contaminated with nickel and zinc, where their number was significantly lower than on the control plants. The number of Coccinellidae larvae on broad bean growing on the copper or lead polluted and on the control soil was similar. Adult Coccinellidae were most numerous in 2007 on the treatment where soil was polluted with copper and in 2008 on plants growing on soil contaminated with cadmium, where there were significantly more ladybirds than on the other treatments except for the control without mineral fertilizers. Adult Coccinellidae occurred most rarely on plants polluted with zinc. Markedly fewer adult ladybirds were also found in experimental group with nickel. The number of adult Coccinellidae on plants contaminated with lead remained on the control level.

The other aphid predators, e.g. Chrysopidae and Anthocoridae, occurred occasionally. The number of Anthocoridae did not depend on the analyzed stressors. In 2007, the Chrysopidae eggs were most numerous on plants contaminated with lead and in 2008 – on copper polluted plants. Chrysopidae larvae were not detected. In 2008, Aranea were most numerous on the control non-fertilized plants. Aranea was less common on plants polluted with zinc and nickel, and in 2008 additionally on lead polluted plants. Numerous Formicidae were observed on plants polluted with cadmium (2007) and on the fertilized control plants (2008), which might have helped to lower the number of some predators (e.g. adult ladybirds) in aphid colonies on the plants from this treatments.

Considering the analysed predators, the highest percentage of broad bean plants was settled by predatory Coccinellidae. Larvae of these preda-

Table 2

Mean infestation of broad bean grown on heavy metal contaminated soil with aphids (*Aphis fabae* Scop.), its natural enemies and Formicidae in 2007 and 2008 (infested plants in %)

Specification	*C* *	NPK	Cd	Cu	Pb	Ni	Zn
	2007						
Hover-fly eggs	0.81	1.63	1.60	2.65	1.59	0.72	0.00
Hover-fly larvae	3.25	3.25	2.40	0.88	2.38	1.45	0.00
Hover-fly pupae	0.81	0.81	0.80	0.00	2.38	0.00	0.00
Black spots	0.81	5.69	10.40	2.65	6.35	2.90	0.00
Ladybird eggs	0.81	0.81	0.80	1.77	2.38	0.72	0.84
Ladybird larvae	13.82	10.57	15.20	9.73	19.05	5.80	0.00
Ladybird pupae	7.32	8.13	7.20	7.08	9.52	1.45	0.00
Ladybird adults	10.57	5.69	8.00	7.96	3.97	5.07	0.84
Lacewings eggs	0.81	0.81	0.00	3.54	5.56	0.00	0.00
Ants	8.13	14.63	21.60	10.62	15.87	5.80	0.00
Spiders	4.07	6.50	2.40	5.31	3.97	0.72	0.00
Anthocorids	3.25	4.88	2.40	3.54	3.97	2.90	0.84
Parasitized aphids	0.00	2.44	4.00	0.00	0.00	0.00	0.00
Aphids	55.28	58.54	64.00	51.33	64.29	44.93	12.61
	2008						
Hover-fly eggs	3.85	3.38	1.91	0.97	2.39	0.83	0.00
Hover-fly larvae	1.92	3.38	5.74	1.94	3.35	1.66	0.39
Hover-fly pupae	0.00	0.00	0.48	0.00	0.48	0.00	0.00
Black spots	4.81	3.38	9.09	0.97	3.35	0.41	0.00
Ladybird eggs	0.96	0.00	1.44	0.00	0.96	0.83	0.00
Ladybird larvae	14.90	14.98	22.01	10.68	11.48	3.73	0.78
Ladybird pupae	1.44	1.93	1.91	4.37	1.91	0.00	0.00
Ladybird adults	13.94	10.63	16.75	10.19	10.05	1.66	0.00
Lacewings eggs	0.00	0.00	0.48	1.46	0.48	0.00	0.00
Ants	3.37	12.56	4.78	2.91	4.31	2.90	0.00
Spiders	2.88	0.97	1.91	1.94	0.48	0.00	0.00
Anthocorids	0.00	0.00	0.48	0.00	0.48	0.00	0.00
Parasitized aphids	3.85	26.09	1.44	0.49	5.74	0.83	0.00
Aphids	57.69	65.70	75.12	53.88	56.94	24.90	8.63

* Key: see Table 1

tors were found on about 10-15% of the analyzed plants unpolluted by heavy metals. Also adult forms were quite numerously spotted, i.e. they settled about 6-14% of unpolluted broad bean plants (Table 2). In previous research, the average colonization of aphid colonies on broad bean by these predators was 10-14% (Jaworska, Gospodarek 2003), whereas on *Syringa* plants the presence of adult ladybird beetles was noted in about 8% of analyzed aphid colonies (Gospodarek 2012*b*).

Soil contamination with cadmium in both years and with lead in 2007 contributed to increased plant colonization by Coccinelliade larvae. In all probability, this was the effect of a more extensive colonization of greater broad bean plants by aphids on these plants than on unpolluted plants. On the other hand, about 2.5-4-fold fewer plants with these predators' larvae were identified among broad beans growing on soil contaminated with nickel than among the control plants, although the percentage of plants settled by aphids was respectively twice as low. Also, far fewer plants settled by these predators were observed on soil contaminated with zinc than it might have been suggested by the percentage of plants settled by aphids. In this case, the number of aphids in colonies was also important. The appeal of aphid colonies as a source of food for aphidophagous insects, e.g. Diptera or Coccinelidae depends on the colony size (Wnuk, Starmach 1977, Honěk 1980). Typically, the more numerous the colony, the more numerous predators.

The presence of predatory Syrphidae was much less frequently observed in aphid colonies on broad bean. Larvae of these predators settled only about 2-3% of unpolluted plants. In some previous investigations conducted therein, the occurrence of these predators was recorded on *ca* 4% of control plants (Gospodarek 2012a). As herein, the percentage was much lower on soils contaminated with nickel and zinc (below 1%).

The presence of eggs of such aphidophages as Anthocoridae and Aranae or Neuroptera was rarely noted on broad bean. In 2007, a considerable percentage of aphid colonies was settled by Formicidae (on average about 8-14% of unpolluted plants and between 6 and 22% of plants cultivated in heavy metal polluted soil). Only the plants growing on the soil polluted with zinc were not visited by these insects.

The values of the Agrell's index obtained for individual predator groups and their stages of development were low (Tables 3-5). In 2007 and 2008, no co-occurrence of any predators was noticed on plants growing on zinc polluted soil polluted. On unpolluted soil and not fertilized with mineral salts, the Agrell's index reached the highest value (2.42) for Coccinellidae larvae and adults. It was also high for plants on unpolluted and NPK fertilized soil (Table 3). The co-occurrence of these development stages of predators was observed in almost 4% of plants on cadmium polluted soil (Table 4). On the other hand, on soil contaminated with copper, Coccinellidae larvae and adults occurred together on about 2.2% of plants, whereas on lead polluted soil only 1.19% of plants had these insects (Tables 4, 5). In the latter case, the concurrent presence of Coccinellidae larvae and pupae on the same plants was observed more frequently. Much lower values of this index (0.26%) – Table 5 were registered for plants polluted with nickel. There are reports about limited egg laying by Coccinellidae in colonies settled by other

Table 3

Comparison of Ag index value for individual pairs of aphids' natural enemies, their developmental stages and ants (%) (mean in the years 2007–2008) on broad bean grown on control soil (*C*) – green font and on uncontaminated soil with mineral fertilization (NPK) – black font

Specification	1*	2	3	4	5	6	7	8	9	10	11	12	13
Hover-fly eggs (1)		**0.00**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.00**	**0.00**	**0.30**
Hover-fly larvae (2)	0.30		**0.00**	**0.30**	**0.00**	**0.30**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**
Hover-fly pupae (3)	0.00	0.00		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Black spots (4)	0.30	0.61	0.30		**0.00**	**1.21**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**
Ladybird eggs (5)	0.00	0.00	0.00	0.00		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.00**	**0.00**
Ladybird larvae (6)	0.00	0.61	0.30	2.42	0.00		**0.91**	**2.42**	**0.00**	**0.00**	**0.91**	**0.30**	**0.61**
Ladybird pupae (7)	0.00	0.00	0.00	0.00	0.00	0.61		**0.91**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Ladybird adults (8)	0.00	0.30	0.00	0.30	0.00	3.03	0.91		**0.00**	**0.00**	**0.61**	**0.00**	**0.61**
Lacewings eggs (9)	0.00	0.00	0.00	0.30	0.00	0.30	0.00	0.00		**0.00**	**0.00**	**0.00**	**0.00**
Ants (10)	0.61	0.30	0.00	0.61	0.00	0.91	0.00	0.30	0.30		**0.00**	**0.00**	**0.00**
Spiders (11)	0.00	0.00	0.30	0.91	0.00	1.52	0.30	0.30	0.00	0.30		**0.00**	**0.00**
Anthocorids (12)	0.00	0.30	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.00	0.30		**0.00**
Parasitized aphids (13)	0.61	0.91	0.00	1.52	0.00	5.45	0.00	1.82	0.00	2.42	0.30	0.00	

* numbers (1 – 13) correspond to groups of invertebrates mentioned in the first column

predators (Hemptinne et al. 1992, Doumbia et al. 1998, Oliver et al. 2006). A very rare co-occurrence of Coccinellidae eggs with other predators was also observed in the present research on most of the treatments except the one where soil was contaminated with lead. Clusters of Coccinellidae eggs were spotted on the same plants that were settled by other development forms of these predators, as well as Chrysopidae eggs and Aranea (Table 4). This may be the effect of a high number of plants on which Coccinellidae eggs were found in this experimental variant, particularly in 2007 (Table 1). Soil pollution with lead favoured aphid occurrence on plants, thus the presence of predators, particularly ladybird beetles, was much more frequent.

Table 4

Comparison of Ag index value for individual pairs of aphids natural enemies, their developmental stages and ants (%) (mean in the years 2007–2008) on broad bean grown on cadmium contaminated soil (Cd) – green font, and on copper contaminated soil (Cu) – black font

Specification	1*	2	3	4	5	6	7	8	9	10	11	12	13
Hover-fly eggs (1)		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.60**	**0.00**	**0.00**	**0.00**
Hover-fly larvae (2)	0.00		**0.00**	**2.10**	**0.00**	**2.10**	**0.00**	**0.00**	**0.00**	**0.30**	**0.60**	**0.00**	**0.30**
Hover-fly pupae (3)	0.00	0.00		**0.00**	**0.00**	**0.60**	**0.30**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Black spots (4)	0.00	0.00	0.00		**0.00**	**5.99**	**0.30**	**0.90**	**0.00**	**0.30**	**0.60**	**1.20**	**0.00**
Ladybird eggs (5)	0.00	0.00	0.00	0.31		**0.60**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Ladybird larvae (6)	0.31	0.63	0.00	0.63	0.00		**1.20**	**3.89**	**0.00**	**1.50**	**0.30**	**1.50**	**0.60**
Ladybird pupae (7)	0.00	0.00	0.00	0.00	0.00	0.31		**0.90**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**
Ladybird adults (8)	0.00	0.31	0.00	0.31	0.00	2.19	1.57		**0.30**	**0.60**	**0.90**	**0.30**	**0.00**
Lacewings eggs (9)	0.00	0.00	0.00	0.00	0.00	0.63	0.94	0.31		**0.00**	**0.00**	**0.00**	**0.00**
Ants (10)	0.00	0.00	0.00	0.00	0.00	0.31	0.31	0.63	0.00		**0.00**	**0.30**	**0.30**
Spiders (11)	0.31	0.00	0.00	0.31	0.00	0.63	0.31	0.63	0.31	0.31		**0.00**	**0.00**
Anthocorids (12)	0.00	0.00	0.00	0.00	0.00	0.31	0.31	0.31	0.31	0.00	0.00		**0.00**
Parasitized aphids (13)	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	

* numbers (1 – 13) correspond to groups of invertebrates mentioned in the first column

Predatory Syrphidae revealed the best tolerance to ladybird beetle larvae. Syrphidae larvae were found on the same plants as Coccinellidae larvae in between 0.30 and 2.10% of cases, depending on the treatment. This index reached the highest value on soil contaminated with cadmium, the the same variant, in 2008, was found to comprise the highest number of plants colonized by the above predators. For comparison, on Syringa, both predatory stages were noted jointly in between 0.31 and 0.68% of the analyzed *A. fabae* aphid colonies (Gospodarek 2012b). Usually, tarry black spots evidenced Syrphidae larvae feeding on aphid colonies, and Coccinellidae larvae were found on the same plants (the Ag index ranged from 0.63% for the pot

Table 5

Comparison of Ag index value for individual pairs of aphids natural enemies, their developmental stages and ants (%) (mean in the years 2007–2008) on broad bean grown on lead contaminated soil (Pb) – green font, and on nickel contaminated soil (Ni) – black font

Specification	1*	2	3	4	5	6	7	8	9	10	11	12	13
Hover-fly eggs (1)		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**
Hover-fly larvae (2)	0.00		**0.00**	**0.60**	**0.00**	**0.90**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Hover-fly pupae (3)	0.00	0.00		**0.00**	**0.00**	**0.60**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Black spots (4)	0.00	0.00	0.00		**0.60**	**1.79**	**0.00**	**0.30**	**0.00**	**0.00**	**0.30**	**0.00**	**0.60**
Ladybird eggs (5)	0.00	0.00	0.00	0.00		**0.60**	**0.30**	**0.30**	**0.30**	**0.00**	**0.60**	**0.00**	**0.00**
Ladybird larvae (6)	0.00	0.53	0.00	0.79	0.26		**1.79**	**1.19**	**1.19**	**0.00**	**0.30**	**0.30**	**1.19**
Ladybird pupae (7)	0.00	0.00	0.00	0.26	0.00	0.26		**0.30**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**
Ladybird adults (8)	0.00	0.26	0.00	0.26	0.26	0.26	0.00		**0.30**	**0.30**	**0.00**	**0.00**	**0.60**
Lacewings eggs (9)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		**0.00**	**0.30**	**0.00**	**0.00**
Ants (10)	0.00	0.00	0.00	0.53	0.00	0.53	0.26	0.26	0.00		**0.00**	**0.30**	**0.30**
Spiders (11)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		**0.00**	**0.30**
Anthocorids (12)	0.00	0.00	0.00	0.26	0.00	0.26	0.00	0.26	0.26	0.26	0.00		**0.00**
Parasitized aphids (13)	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

* numbers (1 – 13) correspond to groups of invertebrates mentioned in the first column

with copper contaminated soil to 5.99% in the case of cadmium pollution). On the other hand, Syrphidae eggs were found on the same plants as tarry black spots only on unpolluted plants and only in 0.3% of the cases, despite the report by Alhmedi et al. (2007), noticing the presence of *Episyrphus balteatus* larvae in aphid colonies causing no decrease in the number of eggs laid by Syrphidae. Eggs and larvae of Syrphidae were also found frequently on the same aphid colonies on Syringa (Gospodarek 2012*b*).

Ants in aphid colonies, using honey-dew as food, often become antagonists towards predators such as Coccinellidae (Banks, Macaluay 1967, Styrski, Eubanks 2007, Powell, Silverman 2010), Anthocoridae, Chamaemyiidae

(Winiarska 1997), Chrysopidae (Powell, Silverman 2010). The competitive relationship is especially strong when the number of aphids is low. In their research, Crawford et al. (1995) also noted that *A. fabae* aphids excreted copper together with honey dew, leading to elevated exposure of ants to this element in food. Coexistence of ladybird eggs and ants was not found during the current experiment in any of the treatments, which confirms the results obtained by Sloggett and Majerus (2003), who stated that ladybirds more eagerly lay eggs in aphid colonies free from ants. On the other hand, ladybird larvae were spotted on the same plants as ants in the pots where the soil was contaminated with cadmium, copper and nickel and in the treatment with unpolluted soil fertilized with mineral salts; most frequently on plants growing on soil polluted with cadmium (the Ag index = 1.5%). Plants growing on soil contaminated with cadmium were heavily colonized by aphids, so food availability for both ladybirds and ants was high. Thus, the lack of co-occurrence of ants and ladybird larvae under conditions of soil contaminated with lead and control soil becomes more distinct, despite the considerable colonization of plants by the two insect groups. The index assumed the lowest values (0.26-0.63%) in our analysis of the coexistence of ants and adult ladybirds. In this case, no simultaneous occurrence of ants and adult ladybirds was registered on the control soil (C). On Syringa, the mutual presence of Coccinellidae eggs and Formicidae was noted in the same aphid colonies only in one season of investigations. Adult ladybirds were encountered in the same colonies as ants in just 0.00 to 0.51% of cases (Gospodarek 2012*b*). No co-existence of Syrphidae eggs, larvae and pupae or tarry black spots together with ants was registered on broad bean cultivated on soil contaminated with lead and copper. In the soil contaminated with cadmium, the value of the Ag index was 0.00-0.60%; on nickel polluted soil it varied from 0.00 to 0.53%. It was similar in the NPK pot, but in the unfertilized control, ants were spotted only with Syrphidae eggs. On Syringa, ants and Syrphidae eggs were noted in the same colonies quite frequently, but Syrphidae larvae occurred together with ants in the same colonies in just 0.00-0.52% of cases (Gospodarek 2012*b*). This is consistent with previous observations conducted by Winiarska (1997) on burdock and pigweed and by Gospodarek (2012*a*) on broad bean.

Neuroptera eggs and other predators were very rarely spotted on the same plants. Co-occurrence of these forms with various development stages of ladybirds was observed only on soil contaminated with copper and lead, which is undoubtedly connected with their more numerous occurrence on plants in these treatments.

The Ag index asumed low values also in the analysis of the coexistence of Aranea, Anthocoridae and aphids parasitized together with other predators in the majority of the experimental variants. It was only on the control soil fertilized with mineral salts that parasitized aphid mummies and predators were often observed on the same plants.

CONCLUSIONS

1. The values of the Agrell's index were low for individual predator groups and their development stages on broad bean, which demonstrates that predators may avoid competition when much food is available. Among the investigated predator groups, the best mutual tolerance was observed for Syrphidae and Coccinellidae.

2. Co-existence of individual aphidophagous groups seems to be strongly conditioned by food, i.e. aphid availability. The applied heavy metals, by influencing the host plant, modified the degree of its colonization by aphids and their number, consequently causing changed in the occurrence of predators. Thus, soil pollution with zinc and nickel contributed to limiting the extent of broad bean colonization by aphids and predators, hence decreasing the values of their coexistence indices, whereas soil contamination with cadmium and lead favoured plant colonization by aphids and predators and therefore the presence of individual aphidophagous groups was noted more frequently on the same plants.

REFERENCES

ALMOHAMAD R., VERHEGGEN F.J., FRANCIS F., LOGNAY G., HAUBRUGE E. 2010. A*ssessment of oviposition site quality by aphidophagous hoverflies: reaction to conspecific larvae.* Animal Behav., 79(3): 589-594. DOI:10.1016/j.anbehav.2009.11.032

ALONSO E., GONZALEZ-NUNEZ M., CARBONELL G., FERNANDEZ C., TARAZONA J.V. 2009. *Bioaccumulation assessment via an adapted multi-species soil system (MS.3) and its application using cadmium.* Ecotoxicol. Environ. Saf., 72: 1038-1044. DOI:10.1016/j.ecoenv.2008.08.004

ALHMEDI A., FRANCIS F., BODSON B., HAUBRUGE E. 2007. *Intraguild interactions of aphidophagous predators in fields: effect of Coccinella septempunctata and Episyrphus balteatus occurrence on aphid infested plants.* Comm. Appl. Biol. Sci., Ghent Univ., 72(3): 381-390.

BANKS C.J., MACAULAY E. D. M. 1967. *Effects of Aphis fabae Scop. and of its attendant ants and insect predators on yields of field beans (Vicia faba* L.*).* Ann. Appl. Biol., 60: 445-453.

CORTET J.G., GOMOT-DE VAUFLERY A., POINSOT-BALAGUERA N., GOMOT L., TEXIER C., CLUZEAU D. 1999. *The use of invertebrate soil fauna in monitoring pollutant effects*. Eur. J. Soil Biol., 35(3): 115-134.

CRAWFORD L.A., HODKINSON I.D., LEPP N.W. 1995. *The effects of elevated host-plant copper and cadmium on the performance of the aphid Aphis fabae (Homoptera: Aphididae).* J. Appl. Ecol., 32: 528-535.

DOUMBIA M., HEMPTINNE J.L., DIXON A.F.G. 1998. *Assessment of patch quality by ladybirds: role of larval tracks*. Oecologia, 113: 197-202.

GÓRNY M., GRÜM L. 1981. *Methods used in soil zoology*. PWN, Warszawa, pp. 483 (in Polish)

GOSPODAREK J. 2012*a*. *Occurrence of black bean aphid (Aphis fabae Scop.) and its natural predators on broad bean under the conditions of soil pollution with heavy metals.* Zesz. Nauk. UR w Krakowie, 480 (357), ser. Rozprawy, 207 pp. (in Polish)

GOSPODAREK J. 2012*b*. *Co-occurrence of predators of black bean aphid* (*Aphis fabae* Scop.) on *Philadelphus coronaries*. Prog. Plant Prot./Post. Ochr. Roślin, 52 (1): 20-25. (in Polish)

GREEN I.D., MERRINGTON G., TIBBETT M. 2003. *Transfer of cadmium and zinc from sewage* *sludge amended soil through a plant–aphid system to newly emerged adult ladybirds (Coccinella septempunctata).* Agric. Ecosyst. Environ., 99: 171-178. DOI: 10.1016/S0167-8809(03)00147-6

Hemptinne J.L., Dixon A.F.G., Coffin J. 1992. *Attack strategy of ladybird beetles (Coccinellidae): factors shaping their numerical response.* Oecologia, 90: 238-245.

Honěk A. 1980. *Population density of aphids at the time of settling and ovariole maturation in Cocinella septempunctata (Col.: Coccinellidae).* Entomophaga, 25: 427-430.

Jaworska M., Gospodarek J. 2003. *The effect of liming and fertilization with magnesium on the aphis fabae natural enemies in conditions of soil contaminated with heavy metals.* Prog. Plant Prot./Post. Ochr. Roślin, 43(1): 157-163. (in Polish)

Kabata-Pendias A., Motowicka-Terelak, Piotrowska M., Terelak H., Witek T. 1993. *Assessment of the degree of soil and plants pollution with heavy metals and sulphur.* Wyd. IUNG Puławy, ser. P, 53 pp. (in Polish)

Kafel A., Gospodarek J., Nadgórska-Socha A., Babczyńska A., Zawisza-Raszka A., Wilczek G., Świergosz-Kowalewska R. 2012. *Anti-oxidant responses of Aphis fabae aphids fed on Vicia faba plants grown on soil contaminated with heavy metals.* Physiol. Entomol. (in press).

Oliver T.H., Timms J.E.L., Taylor A., Leather S.R. 2006. *Oviposition responses to patch quality in the larch ladybird Aphidecta obliterata (Coleoptera: Coccinellidae): effects of aphid density, and con- and heterospecific tracks.* Bull. Entomol. Res., 96: 25-34. DOI: 10.1079/BER2005395

Powell B.E., Silverman J. 2010. *Impact of Linepithema humile and Tapinoma sessile (Hymenoptera: Formicidae) on three natural enemies of Aphis gossypii (Hemiptera: Aphididae.* Biol. Control, 54(3): 285-291. DOI: 10.1016/j.biocontrol.2010.05.013

Sloggett J.J., Majerus M.E.N. 2003. *Adaptations of Coccinella magnifica, a myrmecophilous coccinellid, to aggression by wood ants (Formica rufa group).* II. *Larval behaviour, and ladybird oviposition location.* Eur. J. Entomol., 100: 337-344.

Styrsky J.D., Eubanks M.D. 2007. *Ecological consequences of interactions between ants and honeydew-producing insects.* Proc. Royal Soc., B, 274: 151-164.

Winder L., Merrington G., Green I. 1999. *The tri-trophic transfer of Zn from the agricultural use of sewage sludge.* Sci. Tot. Environ., 229: 73-81. DOI: http://dx.doi.org/10.1016/S0048-9697(99)00070-4

Winiarska W. 1997. *Natural enemies of the aphid Aphis fabae Scop complex. (Aphididae) on selected weed species in urban green areas of Lublin.* Rozpr. hab. 208, Wyd. AR Lublin, 80 pp.

Wnuk A., Starmach M. 1977. *The effect of aphids colony size on Diptera, Syrphidae* oviposition. Zesz. Nauk. AR Krak., 125: 199-207. (in Polish)