

CAN MIXTURE OF FLOWERING PLANTS WITHIN INTENSIVE AGRICULTURAL LANDSCAPE POSITIVELY AFFECT GROUND-DWELLING SPIDER ASSEMBLAGES?

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ABSTRACT

Spiders are dominant predators in agroecosystems. Terrestrial semi-natural habitats, such as different field margins, can enhance the abundance and diversity of spiders in adjoining fields. We compared the effect of a mixture of flowering plants and an adjacent maize crop for ground-dwelling spiders (Arachnida: Araneae). It was assumed that occurrence of these beneficial arthropods will benefit from the adjacent strip of mixed plants. They were collected by using pitfall traps in a two-year study carried out in Poland during whole growing seasons. The species similarity between the two studied treatments was low, which was indicated with the redundancy analysis (RDA). Furthermore, the species richness and overall abundance of spiders were significantly higher in the mixture of flowering plants than in the adjacent maize field, therefore we assumed that this kind of habitat diversification might increase their role in biological pest control.

Keywords: Araneae, predators, biological control, non-cropped areas, arable field.

INTRODUCTION

The simplification of environmental structures within agroecosystems creates unfavorable conditions for beneficial organisms. Intensive management of agricultural areas can negatively affect their abundance, diversity and efficiency (Kovács-Hostyánszki et al., 2013). Different non-crop ecological structures such as forest margins, shrubs or field boundaries have an important role in supporting biodiversity (Bianchi et al., 2006; Drapela et al., 2006). Non-cropped areas within agricultural landscapes provide an important contribution to ecosystem services through the conservation of native wildlife, habitat connectivity through ecological corridors, and for beneficial organisms which utilize this environment (Molina et al., 2014; Mkenda et al., 2019). Food and Agricultural Organization of the United Nations promotes landscape practices like hedges or vegetative buffer strips as effective methods for increasing soil organic matter content and to reduce the impact of extreme weather phenomena associated mainly with climate

change (Food and Agricultural Organization of United Nations, 2007).

One of the ways to enhance populations of natural enemies of pests is to enrich the field vicinity with flowering plants (Haaland et al., 2011). In both natural and managed ecosystems, arthropod species diversity is often positively correlated with the diversity of plant species and with plant density (Midega et al., 2008). This habitat can act as alternative food sources, e.g. flowers providing nectar and pollen; alternative prey or hosts can improve the microclimate and overwintering conditions (Mkenda et al., 2019). Such a diverse habitat attracts many groups of beneficial insects: coccinellids, syrphids, chrysopids, parasitoids, bees, and ground-dwelling arthropods including carabid beetles, rove beetles and also spiders (Hurej et al., 2014; Twardowski et al., 2020).

Spiders are one of the dominant groups of arthropods within agricultural ecosystems, with some species able to significantly reduce pest populations, including on maize fields (Samu et al., 1999; Nyffeler and Sunderland, 2003). A wide range of predatory ground-dwelling Araneae occur in arable fields, of

which Lycosidae and Linyphiidae are the most abundant (Campbell et al., 2020). Among these, only a few dominant species represent 60-90% of the whole community (Samu and Szinetar, 2002). They are also often used as bioindicators of environmental changes, including agroecosystems (Kovács-Hostyánszki et al., 2013). Nowadays, it is proposed to enhance the level of agricultural biodiversity to increase the impact of beneficial organisms on herbivores and consequently minimize the use of insecticides (Tschumi et al., 2015). One of the possibilities is to use natural structures present within the agricultural landscape (Pluess et al., 2010). It is also possible to create new areas providing similar functions, for example strips of flowering plants, natural weedy strips, or even intercrops (Hurej and Twardowski, 2006; Twardowski et al., 2020; Hummel et al., 2012; Schmidt-Entling and Döbeli, 2009; Kosewska and Nietupski, 2015; Kosewska et al., 2016). It is assumed that non-crop habitats and enhanced levels of landscape heterogeneity will increase spider populations (Bertrand et al., 2016). From these floristically diverse sites spiders probably will migrate to neighboring cultivated fields (Kajak and Oleszczuk, 2004). This was correctly noted in the work of Pfister et al. (2015) in the case of aerial web-building spiders in cereal fields. They achieved clear results merely in the case of chosen species and some positive consequences, which indicate that these species should be studied. To our knowledge there have been no such studies on ground-dwelling spiders identified to species level and their relocation from an artificially established mixture of flowering plants to arable fields. Therefore, it is still an open question as to how strong the effects of non-crop areas are on the arable field situation. In this preliminary study, we analyzed the effect of flowering plants on the abundance of epigeic spiders in an adjacent arable field. We hypothesized that dispersal of spider populations will benefit from the adjacent strip of mixed plants. The abundance and species diversity of spiders in the adjacent arable field will also increase during the season.

MATERIAL AND METHODS

Study site and experimental design

The study was carried out at the Experimental Station in Pağów, Poland (51°8'34.54"N, 17°38'13.27"E) in 2013-2014. Fields belonging to BASF, the Chemical Company, were located in an intensively used agricultural landscape, on sandy loam soil (in the terms of granulometry). A mixture of annual flowering plants was sown in a 1 ha area, at the beginning of May each year, comprising the seeds of 19 species. The seed mixture was provided by BASF and based on local climatic conditions. The diversified, non-crop area was a rectangular shape which was directly adjacent to the cultivated maize field for a distance of 500 meters. The width of the strip of the mixture of flowering plants was 20 meters. For each treatment (maize and plant mixture), four traps were placed separately as four replications, in the straight line within 20 meters of each other. The distance between each pair of traps set up in the flower mixture and maize fields was 40 m.

Maize LG 32.58 variety (FAO 250) was sown each year at the end of April, at a rate of 85,000 seeds per hectare. Weed control was done once with a mixture of two herbicides, Stellar 210 SL (dicamba + topramezone) and Zeagran 340 SE (bromoxynil + terbuthylazine) at a rate of 1.0 and 1.5 l/ha, respectively, when the maize was at BBCH stages 14-15. Fungicide Retengo Plus 183 SE (pyraclostrobin + epoxiconazole) was applied at a rate of 1.0 l/ha was at BBCH stages 37-39 to control maize diseases. The fields under both treatments were ploughed after the season and prepared for sowing in the same way for the following year. The agrotechnical conditions for spider development were the same in both habitats. The mean daily temperature and relative humidity were measured using a highly accurate temperature and humidity USB data logger during the growing season in both years of the study (Figure 1).

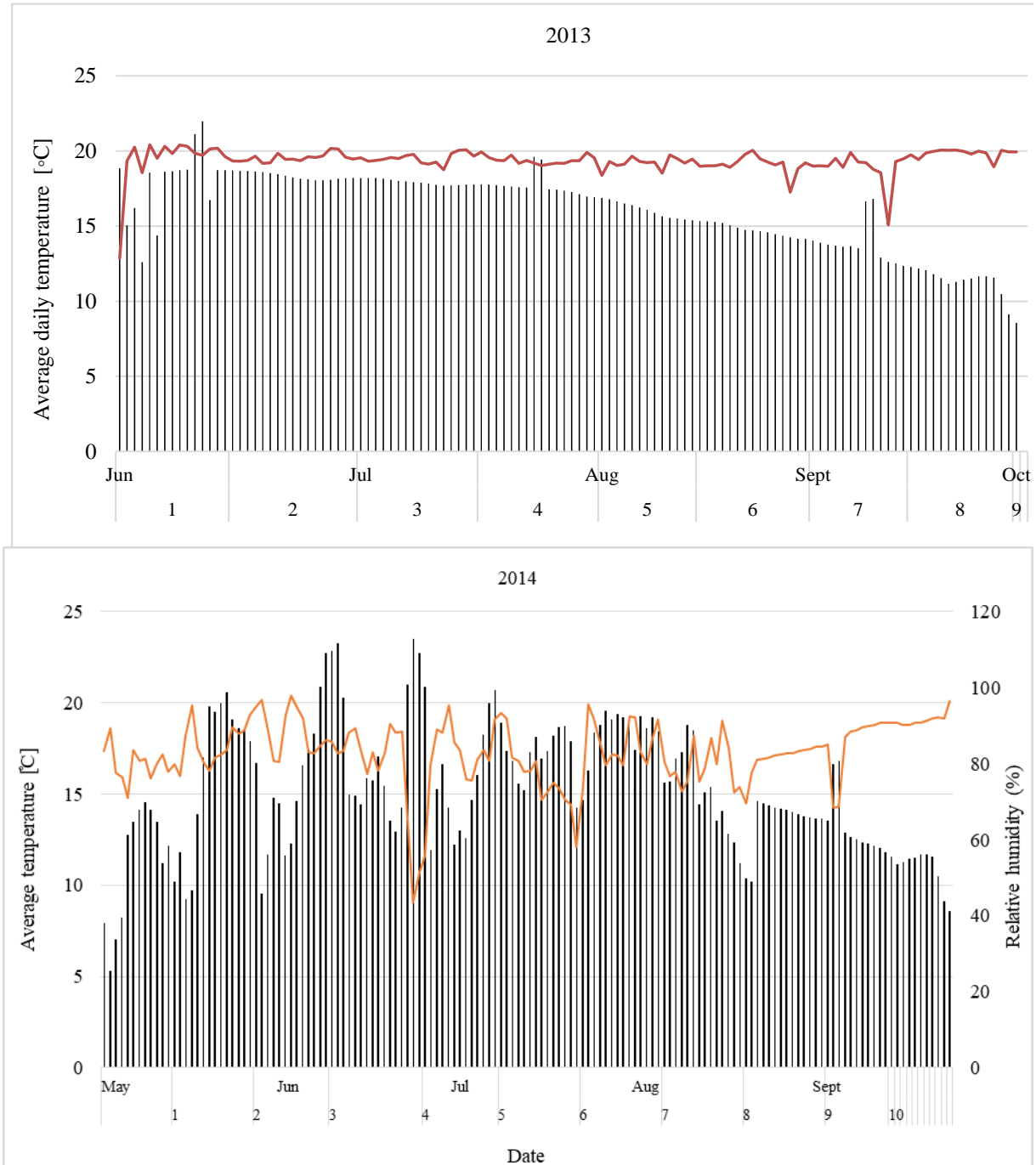


Figure 1. Mean daily temperature (°C) and relative humidity (%) during the experiment in 2013 and 2014

Vegetation assessment of the plant mixture

The number of plants and their species structure in the mixture were calculated for one-metre-square sample areas, taken randomly four times in the experimental flowering plant mixture strip in each of the growing seasons. The composition of plant species was determined three times for each year of the study; after their emergence, one month later, and two weeks after that. The species abundance in the mixture was

determined according to the Braun-Blanquet cover-abundance scale (Braun-Blanquet, 1965).

Spider sampling and data analysis

Active ground-dwelling spiders were sampled using Barber's pitfall traps (Barber, 1931). Eight circular (four in maize and four in plant mixture), transparent plastic traps (9 cm in diameter, 14 cm in height) were used throughout the whole vegetation period. The traps were dug into the soil with

the opening at the soil surface and filled with 50:50 water with ethylene glycol, used as a preservative. To prevent rainwater from filling the cup a cover made of a transparent plastic square was installed. In both of the study years, the traps were emptied every second week from the end of May (beginning of flowering of the plants in the mixture) until the end of September (decaying plants). As a result, arthropods were sampled on nine dates in 2013 and ten dates in 2014.

In the laboratory the samples were sorted, and specimens were counted and identified. Spiders were identified to species level by Dr. Robert Rozwałka from the Department of Zoology, Maria Curie-Skłodowska University in Lublin, Poland. He used the key of Heimer and Nentwig (1991) for spider identification, and the nomenclature follows Platnick (2020). The individuals from each species were sorted into males, females and juveniles. Only adults (males and females) were considered in the analysis. The juveniles caught in the pitfall traps reached low densities (less than 5% of spiders in the single sample). Sørensen's and Simpson's indexes were calculated for each sample.

The Sørensen's index was calculated as $SS = 2a/(b + c)$, where a = number of species common to both quadrats, b = number of species unique to the first treatment, and c = number of species unique to the second treatment. Simpson's index was calculated as:

$$D = \sum \left(\frac{n_i(n_i-1)}{N(N-1)} \right)$$

where n_i = the total number of organisms of a particular species and N = the total number of organisms of all species.

Simpson's index represents the probability that two randomly chosen individuals belong to the same species (Morris et al., 2014). It includes not only the number of species, but also the relative abundance of each species. Simpson's index is in the range 0-1, where 0 is the highest value, with 1 the lowest. The

normality of the data was checked using Shapiro-Wilk test. Data analysis was performed separately for each of the years. The spider abundance in maize and plant mixture were compared using repeated analysis of variance (proc mixed) in software of SAS University Edition. Date was the repeated factor in the analysis. The spider community response to experimental treatments was analyzed using redundancy analysis (RDA). For the analysis the abundance data of the species were used. The data were log-transformed during the analysis. The significance of the first RDA axis was calculated using Monte-Carlo test. Ordinations were calculated with CANOCO 4.5. The ordination plots were performed separately for each of the years of the study.

RESULTS AND DISCUSSION

Botanical analysis of the mixture of plants

In the conducted trials, the plant species composition and their abundance in the crop mixture were very similar both in 2013 and 2014 and because of this only the results of 2014 are presented (Figure 2). On the first sampling date of 2014 (after plant germination), four species were recorded, which together covered 75% of the soil surface: *Phacelia tanacetifolia*, *Chrysanthemum segetum*, *Trifolium pratense* and *Anethum graveolens*. On the second sampling date (a month later), 77% of the soil surface was covered by *Ph. tanacetifolia*, *Ch. segetum*, *A. graveolens*, *Camelina savita* and *Calendula officinalis*. On the third sampling date (two weeks after the previous observation), three species covered more than 60% of the soil surface: *Ph. tanacetifolia*, *Ch. segetum*, and *Fagopyrum esculentum*. The degree of surface cover of *Ph. tanacetifolia* increased from the first to the third sampling date, while *Ch. segetum*, *T. pratense* and *A. graveolens* decreased over time. In total, the botanical analysis of the flower mixture revealed the presence of 17 plant species of the 19 sown together.

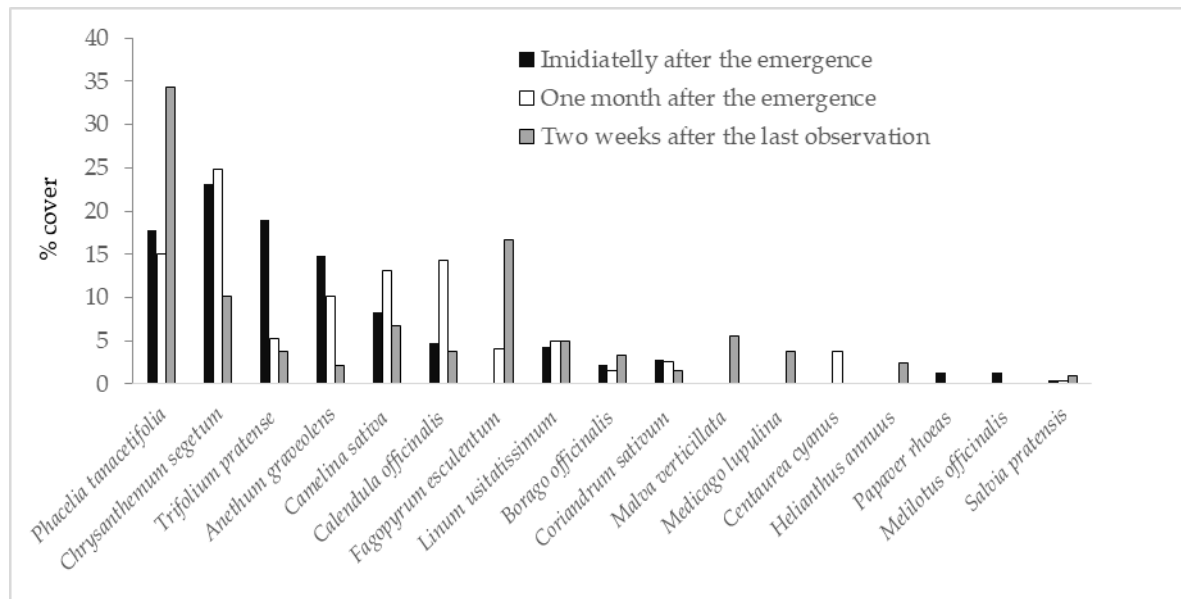


Figure 2. Mean degree of surface cover by plants of the mixture on three dates in 2014 (in %)

Abundance and species composition of ground-dwelling spiders

The mean number of epigeic spiders was significantly higher in the plant mixture in comparison to the maize crop in both years of the study (Table 1). In more detail, in 2013, total abundance of studied organisms was more than 3 times higher ($F=94.94$, $p<0.001$), and in 2014 more than 2.5 times higher ($F=20.36$, $p<0.001$) within the more diverse habitat compared to the maize monoculture.

The species number of identified spiders differed significantly between treatments in both years of the study. In 2013, 14 species were found in the maize and 23 species in the

flower mixture ($F=27.31$, $p<0.001$), whereas in 2014 it was 11 and 19, respectively ($F=13.65$, $p<0.0005$). In both years of the study, a significant effect of the date was observed (2013: $F=2.26$, $p=0.04$; 2014: $F=3.72$, $p<0.0015$). The Simpson's index, which responds to species diversity, was distinctly higher in the plant mixture in comparison to maize, but only in 2013 ($F=7.20$, $p<0.0091$). The species similarity between experimental treatments was calculated using Sørensen's index. Its value was 0.28 in 2013 and 0.30 in 2014, what indicates a small species similarity between the flower mixture and the maize crop.

Table 1. The abundance and species diversity of spiders per pitfall trap caught in the maize crop and the plant mixture in 2013 and 2014

	Parameter	Maize	Mixture	DF*	F	p
2013	Mean abundance	5.55	16.80	1	94.94	.0001
	Total abundance	200	605			
	Mean species number	1.86	3.77	1	27.31	.0001
	Total species number	14	23			
	Simpson's index	0.47	0.34	1	7.20	0.0091
Sørensen's index		0.28				
2014	Mean abundance	6.72	16.60	1	20.36	.0001
	Total abundance	282	722			
	Mean species number	2.55	5.45	1	13.65	.0005
	Total species number	11	19			
	Simpson's index	0.13	0.10	1	0.33	0.56
Sørensen's index		0.30				

*DF, F, p - results of repeated analysis of variance.

Seasonal abundance of spiders

In 2013 (Figure 3), the individual number increased drastically in the plant mixture between the 25th of May and 25th of June. In the maize, the mean abundance of spiders was more stable. On the 13th of August, the mean number of spiders was equal in both habitats, and thereafter by an increase in the plant mixture and a decrease in the maize. In 2014, the abundance of spiders increased at a similar rate until the 9th of July. The maximum of abundance of spiders was observed on the 6th of August in the plant mixture. In the maize, spider abundance was more stable with much smaller numbers.

The seasonal dynamics of the species number in the flower plant mixture and maize in 2013 were similar (Figure 4). The higher abundance and declines of the species

number were observed at the same time, e.g. on the 13 and 25th of June or 12th of September. In the maize crop, the lowest number of species was 0.5 per sample, the highest 3.5 species. In the plant mixture, an average from 3.5 to 5.5 species was found. In 2014, the number of species per sample was considerably higher. In the maize crop it was from 0 to 5 species, while in the plant mixture from 2 to 10 species per sample were found (Figure 4). In both cases, the species number slightly increased from the beginning of the season up to the maximum on the 23rd of July (plant mixture) and 20th of August (in maize). After this, the species number considerably decreased in both treatments. In 2014 there is also no clear relation between the species number and the weather conditions.

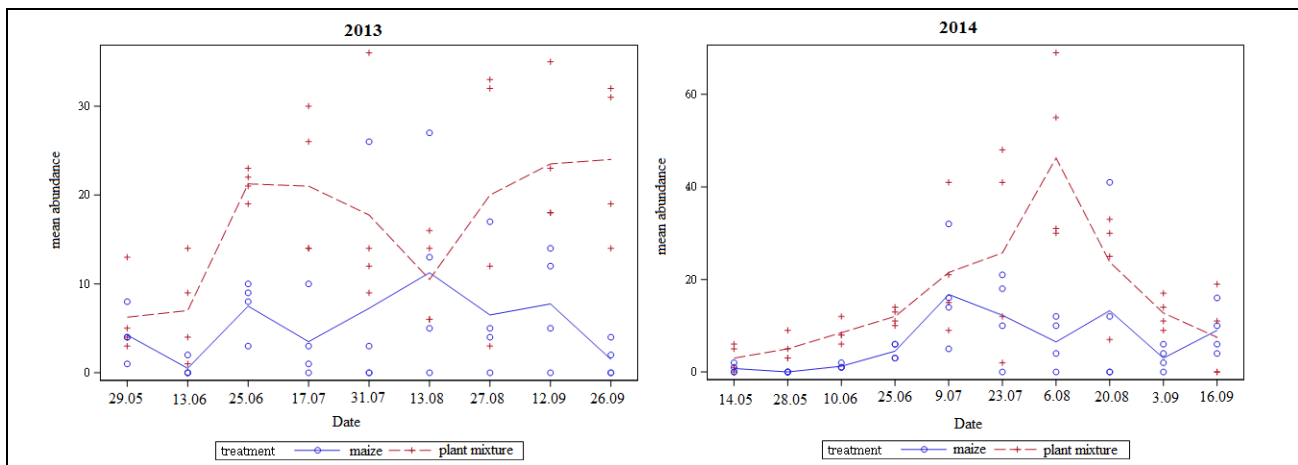


Figure 3. Seasonal dynamic of individual number of spiders in maize and plant mixture in two years study

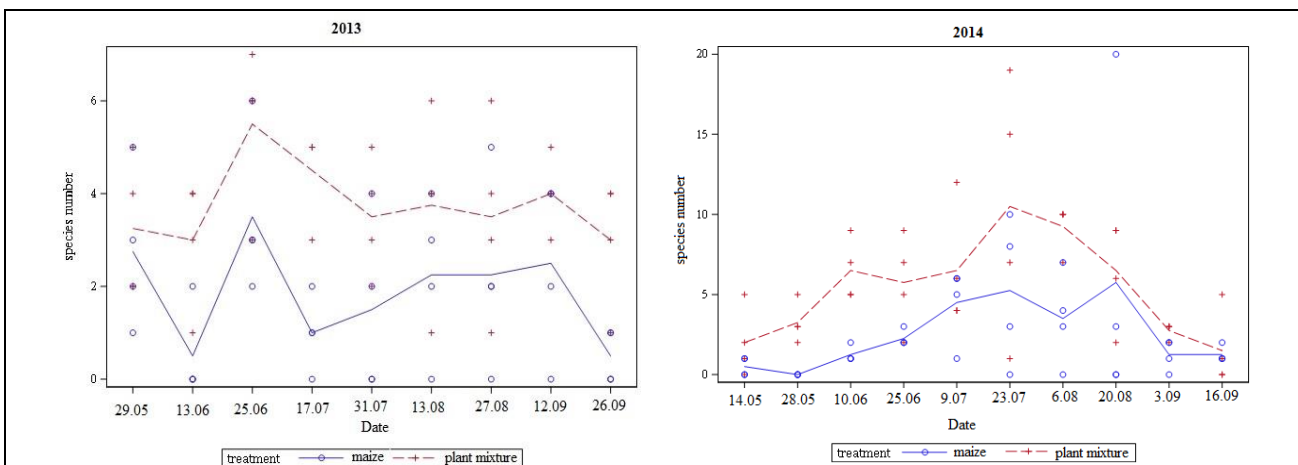


Figure 4. Seasonal dynamic of species number of spiders in maize and plant mixture in two years study

Redundancy analysis of species diversity

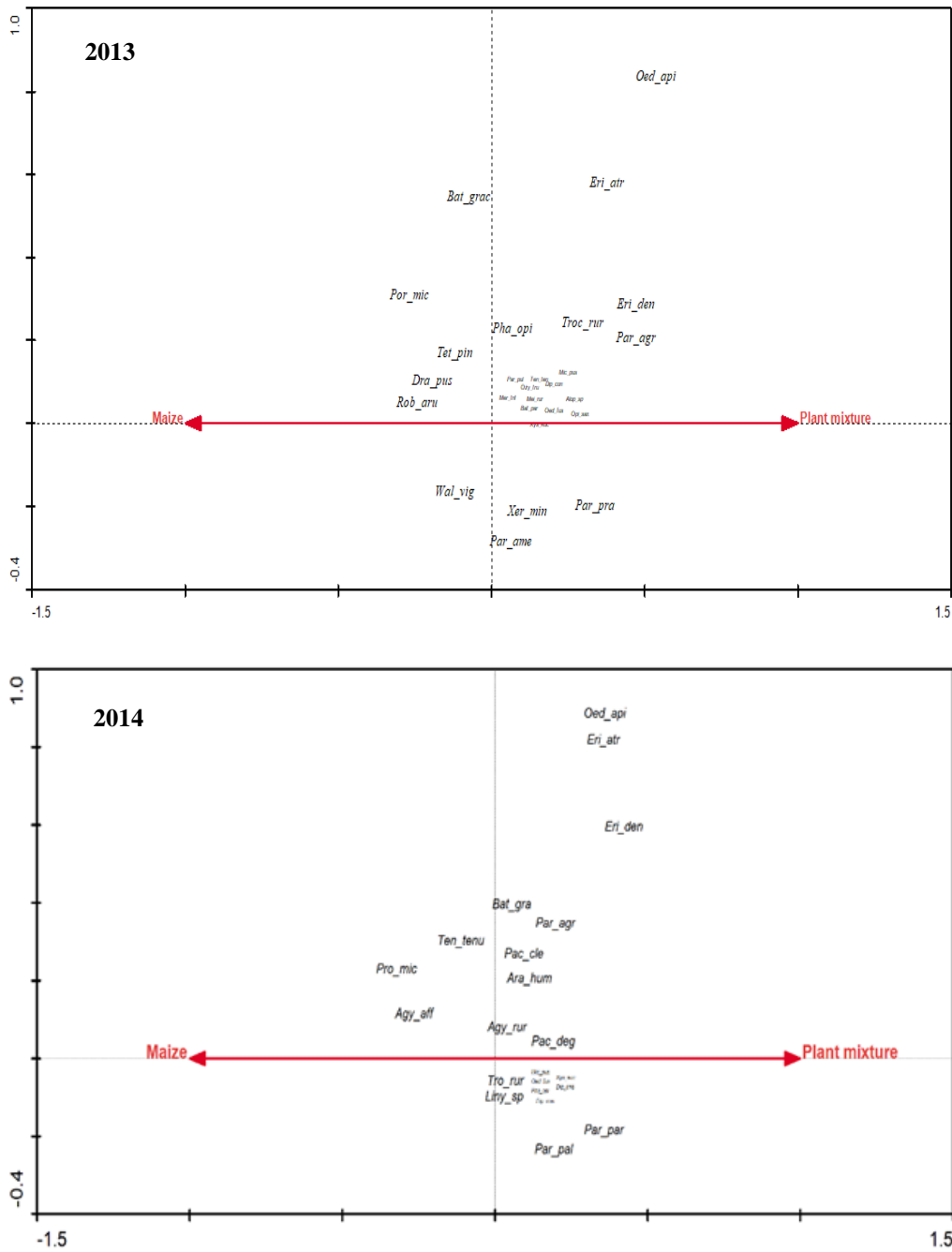
The most abundant species in both years of the study were *Oedothorax apicatus* and *Erigone atra* (Table 2). In 2013, the sum of eigenvalues from two first RDA axes was 0.551 (Table 3, Figure 5). There was the group of 12 species related to plant mixture (e.g. *Pardosa pullata*, *Mermessus trilobatus*, *Oedothorax fuscus*). Species, which occurred more frequently in maize were: *Tetragnatha pinicola*, *Drassyllus pusillus* and *Robertus arundineti*. The remaining species were rare

or affected by other factors, not included in the analysis.

In 2014, the sum of eigenvalues of RDA 1 and RDA 2 was 0.44. All RDA axes were significant (Table 3, Figure 5). Similarly to 2013, there was a group of 7 species clearly related to plant mixture (e.g. *Xysticus kochi*, *Diplocephalus cristatus* or *Microlinyphia pusilla*). The species with distinct preferences to maize was *Agyneta affinis*, while species which occurred in both treatments were *Agyneta rurestris* and *Trochosa ruricola*.

Table 2. Species composition of all spiders collected to pitfall traps in two years of the study

Species	Abbreviation in RDA	2013		2014		Total
		Mixture	Maize	Mixture	Maize	
<i>Oedothorax apicatus</i> (Blackwall, 1850)	Oed_api	424	131	482	178	1215
<i>Erigone atra</i> (Blackwall, 1833)	Eri_atr	64	19	119	43	245
<i>Erigone dentipalpis</i> (Wider, 1834)	Eri_den	27	5	65	14	111
<i>Bathyphantes gracilis</i> (Blackwall, 1841)	Bat_gra	12	18	18	13	61
<i>Pardosa agrestis</i> (Westring, 1861)	Par_agr	28	3	3		34
<i>Porrhomma microphthalmum</i> (O. P. - Cambridge, 1871)	Por_mic	1	8	2	15	26
<i>Trochosa ruricola</i> (De Geer, 1778)	Tro_rur	15	4	2	2	23
<i>Pardosa prativaga</i> (L. Koch 1870)	Par_pra	10	1	6		17
<i>Agyneta rurestris</i> (C.L. Koch, 1836)	Agy_rur			8	8	16
<i>Meioneta rurestris</i> (C.L.Koch, 1836)	Mei_rur	8	5			13
Linyphiidae	Lyny_sp.			4	2	6
<i>Pardosa palustris</i> (Linnaeus, 1758)	Par_pal			4		4
<i>Diplostyla concolor</i> (Wider, 1834)	Dip_con	2		1		3
<i>Phalangium opilio</i> (Linnaeus, 1758)	Pha_opi	1	1	1		3
<i>Tenuiphantes tenuis</i> (Blackwall, 1852)	Ten_ten	1			2	3
<i>Xysticus kochi</i> (Thorell, 1872)	Xys_koc	1		1	1	3
<i>Agyneta affinis</i> (Kulczynski, 1898)	Agy_aff				3	3
<i>Oedothorax fuscus</i> (Blackwall, 1834)	Oed_fus	1		1		2
<i>Ozyptila trux</i> (Blackwall, 1846)	Ozy_tru	2				2
<i>Robertus arundineti</i> (O. P. - Cambridge, 1871)	Rob_aru		2			2
<i>Bathyphantes parvulus</i> (Westring, 1851)	Bat_par	1				1
<i>Drassyllus pusillus</i> (C.L. Koch, 1833)	Dra_pus		1			1
<i>Mermessus trilobatus</i> (Emerton, 1882)	Mer_tri	1				1
<i>Microlinyphia pusilla</i> (Sundevall, 1830)	Mic_pus	1				1
<i>Opilio saxatilis</i> (C.L. Koch, 1839)	Opi_sax	1				1
<i>Pardosa amentata</i> (Clerck, 1757)	Par_ame	1				1
<i>Pardosa pullata</i> (Clerck, 1757)	Par_pul	1				1
<i>Tetragnatha pinicola</i> (L. Koch, 1870)	Tet_pin		1			1
<i>Walckenaeria vigilax</i> (Blackwall, 1853)	Wal_vig		1			1
<i>Xerolycosa miniata</i> (C.L. Koch, 1834)	Xer_min	1				1
<i>Araeoncus humilis</i> (Blackwall, 1841)	Ara_hum			1		1
<i>Diplocephalus cristatus</i> (Blackwall, 1833)	Dip_cri			1		1
<i>Microlinyphia pusilla</i> (Sundevall, 1830)	Mic_pus			1		1
<i>Pachygnatha clercki</i> (Sundevall, 1823)	Pac_cle			1		1
<i>Pachygnatha degeeri</i> (Sundevall, 1830)	Pac_deg			1		1
<i>Alopecosa</i> sp. (Simon, 1885)		1				1
<i>Phylloneta</i> sp. (Archer, 1950)					1	1
Total		605	200	722	282	1809
Number of species		23	14	19	11	37



*Species marked with small font in 2013: Par_pul, Mer_tri, Oed_fus, Mei_rur, Dip_con, Alop_sp, Mic_pus, Bat_par, Opi_sax, Xys_koc, Ozy_tru, Ten_ten.

Species marked with small font in 2014: Xys_koc, Dip_cris, Mic_pus, Oed_fus, Pha_opi, Dip_con.

The complete species names and its abundance are presented in Table 2.

Figure 5. RDA biplots of Araneae species in relation to the treatment in two years study

Table 3. Results of RDA analysis

Parameter		RDA 1	RDA 2	RDA 3	RDA 4
Eigenvalues	2013	0.181	0.370	0.128	0.071
	2014	0.112	0.532	0.137	0.06
Significance of all RDA axes	2013	F-ratio = 15.474, P-value = 0.0010			
	2014	F-ratio = 9.865, P-value = 0.0020			

One approach to enhance the populations of natural enemies within agroecosystems, such as ground-dwelling spiders, is to modify the habitat to favor their mobility. We propose to enhance the significance of these arthropods within the intensive agricultural landscape by establishing and sustaining a mixture of flowering plants near to the maize crop. The sown flower mixture can effectively enhance the natural enemies of crop pests (Haaland et al., 2011; Twardowski et al., 2020). Another authors, Oleszczuk et al. (2010) observed the greatest diversity and the highest total density of Araneae close to the forest edge, but an opposite relation was found for orb webs, built by spiders from the Araneidae and Tetragnathidae families, whose abundance was the lowest in close proximity to the forest. It is known that only some arthropod groups migrate effectively to a considerable distance from their semi-natural habitats. For example, the distance from a non-crop area was studied in the case of pollinators (Bailey et al., 2014) and aphidophagous hoverflies (Bowie, 2010; Bortolotto et al., 2016). In all the cases the abundance of beneficial organisms in the field was higher closer to the edge (forest of flowering plants). Spider web research was done by Oleszczuk et al. (2010), who tested the effect of distance from the forest edge on the distribution and diversity in an adjacent maize field.

In our experiment it was hypothesized that dispersal of the spider population will benefit from the adjacent strip of mixed plants. For instance, Denys and Denys and Tscharrntke (2002) observed the dispersal of natural enemies from red clover into cereal crops. Considering our study, the assumption was not confirmed. The maximum of the species similarity index was 0.4, which indicates a low number of common species between both treatments. The RDA biplots also show differences in species composition between the analyzed treatments. To compare, the mean species similarity of epigeic rove beetles between the plant mixture and the

adjacent field was 0.6 (Twardowski et al., 2020). In this case, periodic migration between two vegetation stands was observed.

During both seasons we observed relatively large variability in the seasonal dynamics of the abundance, species richness and similarity of spiders. The main factors which impact the spider activity densities are prey densities, season or climatic factors (Haddad et al., 2016).

In our trials, significantly more spiders were trapped in pitfall traps within the more diverse habitat of the flower mixture than in the adjacent arable field. Also, with respect to the number of Araneae species, the habitat in the mixture was clearly richer than in the arable field. The greater number of plants and the higher diversity of the vegetation probably had a positive effect on spiders' assemblages. The important factors which affect the arthropod communities are plant communities for food and as habitat, and also the changes in plant species richness, plant biomass or plant functional composition (Siemann et al., 1998; Hertzog et al., 2017). Schmidt-Entling and Döbeli (2009) found that the abundance of wolf and ground spiders increased towards field edges. Lemke and Poehling (2002), who observed the dispersal of Linyphiidae species *O. apicatus* and *E. atra*, found distinctly more spiders in fields with weed strips or in the weed strips themselves in comparison to fields without a weed strip.

Our results do not support the hypothesis that the abundance and species diversity of spiders will increase in the adjacent field during the growing season. The seasonal dynamics of spiders were variable between sampling dates and not linked with the weather conditions. We also found that the more diverse habitat (flower mixture) increased the spiders' abundance and species richness. In future studies we plan to observe the population of natural enemies in the fields adjacent to mixtures of flowering plants in correlation with their prey.

CONCLUSIONS

Spiders were significantly more abundant in the plant mixture in comparison to the maize crop. The species similarity was low, therefore the assumption about the migration of spiders from strips to crop was not confirmed. Ecological structures like a mixture of flowering plants may be considered as a hunting ground for most of the spider species that utilize agri-environmental schemes. However, it would be favorable if the beneficial arthropods attracted significantly reduced pest populations in nearby crops.

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