

Biodiversity of Epigeic Spiders in Genetically Modified (Bt) and Conventional (non-Bt) Potato Fields in Bulgaria

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Abstract: Investigations on epigeic spiders in potato fields were conducted in Bulgaria in 2000, 2001 and 2004. Pitfall traps were used to compare biodiversity of spiders in *Bacillus thuringiensis* (Bt), and conventional (non-Bt) potato cultivars. A total of 7128 individuals from 85 species of spiders were collected. The species *Titanoeca veteranica*, *Haplodrassus aenus*, *Chalcoscirtus nigrinus* and *Zodarion epirense* are new to the Bulgarian fauna. The species *Pardosa agrestis*, *Oedothorax apicatus*, *Trichoncoides piscator*, *Araeoncus humilis*, *Erigone dentipalpis* and *Meioneta rurestris* are present in all years, at all fields of investigations. *Pardosa agrestis* represented 79 – 84% of all spider species in 2000 and 2004. No negative effect of Bt potatoes on spiders could be detected. The insecticidal treatments in non-Bt cultivars also had no direct effect on epigeic spiders. The analyses of data confirmed the hypothesis of similarity of the epigeic spider's fauna in Bt and non-Bt potato fields.

Key-words: spiders, Bt potatoes, non-Bt potatoes, insecticides

Introduction

In some regions of Bulgaria uncontrolled Colorado potato beetle (CPB) populations can defoliate an entire field in mid-season. Traditional interventions include treatment with a range of broad-spectrum insecticides. The reliance on conventional insecticides has resulted in multiple resistances in CPB and a variety of untoward effects on non-target organisms and environment (CASAGRANDE 1987, HARE 1990).

Advances in plant molecular biology and biochemistry in the past two decades have allowed the development of modern genetic engineering technology that offers the potential to improve agronomic traits of crop cultivars. Several species of crop have been modified with genetic engineering methods to express genes from various subspecies of *Bacillus thuringiensis* (BERLINER) that encode Crystalline

(Cry) proteins. These Cry proteins confer effective protection to the crop plants from damage by certain phytophagous insect pests. Bt Cry toxins are generally considered to have fewer adverse impacts on environment than many broad-spectrum and persistent chemical insecticides (SCHULER 2000). Although the intrinsic insecticidal activity of Bt protein toxins is not altered in the transgenic crops, the continuous expression of Bt Cry proteins in large portions of the plant throughout most of the growth seasons has raised some environmental concerns. One such concern is the possible impact of this novel pest control technology on various groups of non-target organisms of ecological and economic value through crop plant-based food chains.

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The aim of the present work is to evaluate the effects of Bt (carrying Cry3A protein) and non-Bt potatoes on the epigeic spiders biodiversity under field conditions.

Material and Methods

In 2000 the investigated fields were situated near Samokov at 900 m a.s.l. (W Bulgaria). The potatoes were planted at the end of April (Bt – a monoculture field of area 1.6 ha) and in the middle of May (conventional - 4 ha) and harvested in the beginning of September. Bt potatoes (Superior Newleaf®) containing Cry 3Aa Bt-toxin were planted in a monoculture field of area 1.6 ha. One hundred meters from this field separated by a bare land there was a non-Bt field with conventional cultivar (Santana® 4 ha). Both fields were free of weeds. Non-Bt field was sprayed twice in the season by pyrethroid alfa-cypermethrin (Vaztak® - 10 EC, 100 ml/ha). There was no rain during the season and potatoes were irrigated every two weeks. Samples were taken from ten pairs of pitfall traps (GREENSLADE 1964) in each field, six times in the season. The trap pairs were 15 m apart in three rows, each 15 m apart, situated in the centre of each field.

In 2001 the investigated fields were situated at 600 m a.s.l. near Ihtiman (W Bulgaria). The potatoes were planted in late April and harvested at the end of August. Experiments were carried out in 40 ha field divided into halves with a 5 m wide road. In the middle of the field, at both sides of the road, there were experimental plots – 1.5 ha Bt potatoes (Superior Newleaf®) of one side and 1.5 ha control field (standard cultivar Arinda®). The non-transgenic cultivars (Santana®, Arinda®, Sante®) also surrounded both fields. The non-Bt field and all other plantations of the conventional potatoes were sprayed twice in the season with fipronil (Regent® - 800 WG, 20 g/ha). Weeds covered virtually all space between the rows of potatoes for much of the season. Samples were taken from ten pairs of pitfall traps eight times in the season from middle of May to the end of August. Pitfall trap pairs were 15 m apart in a single row, situated in the centre of each field.

In 2004 the investigated fields were situated in the same region as in 2000. Three Bulgarian Bt potato cultivars (Bor®, Kalina®, Koral®) were studied. There were three Bt plots (4 x 30 m) and three conventional plots 4 x 10 m separated from Bt plots by other conventional potatoes. Potatoes were planted at the beginning of June and harvested in the beginning of September. Other nontransgenic cultivars surrounded both fields. No insecticides were used in conventional fields and all other surrounding plantations. The fields were free of weeds and it was raining often. Samples were taken from nine pitfall traps (three in every Bt and non-Bt plot) five times in the season from the end of June to the end of August.

Pitfall traps were with an opening of 8 cm diameter and 0.5 l volume. In 2000 as a preserving solution we used ethyleneglycol : water (1:1) and in 2001 and 2004 formaldehyde : water (1:8).

The potatoes were planted with 0.7 m spacing between rows and 0.25 m spacing between plants within a row.

The spiders community structure from Bt and non-Bt plots was compared by calculating Sørensen Similarity Index according to the formula $I_s = (2c/a+b)*100$, where: 'Is' is the Sørensen Index, 'c' is the number of common species, 'a' is the number of species from one of the fields, and 'b' is the number of species from the other field. 100 % indicates that there is no difference between the two faunas compared and 1 % that they are completely different.

Dynamic density per 100 trap-days was calculated according to the formula

$Dd = (Ni/Tr * dn)*100$, where: 'Dd' is Dynamic density, 'Ni' is the number of specimens of the respective species, 'Tr' is the number of the traps and 'dn' is the number of the trap-days.

Results and Discussion

Till now, the spiders were not investigated in both regions. A total 7128 individuals from 85 species were recorded. Four species: *Titanoeca veteranica*, *Haplodrassus aenus*, *Chalcoscirtus nigrinus*,

Zodarion epirense are new to the Bulgarean fauna. The first three species were collected in Bt potato fields, while *Z. epirense* in a non-Bt field. The species *P. agrestis*, *O. apicatus*, *T. piscator*, *A. humilis*, *E. dentipalpis*, *M. rurestris* are present in all fields, in all years of investigations (Table 1).

In 2000, 1392 individuals from 42 species of spiders were collected in the Bt field and 1311 individuals from 28 species in the conventional field. The analysis of spider's community revealed enormous differences between the dominating and the rare species (Table 1). Two species dominated in Bt field (*P. agrestis* and *P. prativaga*) and two species in non-Bt field (*P. agrestis* and *O. apicatus*). *P. agrestis* represented 79 – 81 % of all spider species. Only three other species go beyond 1% in Bt field (*O. apicatus*, *P. palustris* and *W. vigilax*) and in non-Bt field (*W. vigilax*, *P. prativaga* and *T. piscator*). In Bt field we collected 15 species more than in a conventional field. These species were represented from single specimens and probably the differences are not due the presence of Bt toxin.

In 2001, there were 1201 individuals from 41 species of spiders collected in the Bt field and 1107 individuals from 39 species were caught in the conventional field. Again *P. agrestis* dominated in both plots representing 53% of all individuals in Bt plot and 61% in non-Bt plot. The second most common species in 2001 was *M. rurestris*, and it made up 15-25% of the total number of spiders. The third species representing more than 5% was *T. piscator*. Four other species go beyond 1% (*O. apicatus*, *P. vegans*, *H. dalmatensis*, *Z. gracilis*).

In 2004, a total of 1037 individuals from 23 species were recorded in the Bt plots and 1080 individuals from 24 species were caught in the conventional plots. All species collected in this year were present in potato fields in 2000. *P. agrestis* dominated with 82-84 % of all spider species, followed by *O. apicatus* and *P. prativaga* with about 4%. The dominance of only a few species of spiders is typical of agroecosystems (HÄNGGI *et al.* 1995, SAMMU, SZINETAR 2002, SEHNAL *et al.* 2004, VOLKMAR *et al.* 2004, LUDY, LANG 2004, MEISSELE, LANG 2005).

Seven species had dynamic density > 1.0 per 100 trap-days (Table 1). *P. prativaga* and *P. palustris* had higher dynamic density in Bt plot near Samokov in 2000 while *P. vegans* had higher dynamic density in non-Bt plot near Ihtiman. Nevertheless there was no statistically significant difference between the number of observed spiders in Bt and non-Bt fields in all years (NEDVED *et al.* 2006).

Similarity more than 50% was observed between Bt and non-Bt potato fields every year, and between fields near Samokov region (2000 and 2004). Low similarity was found between the fauna near Ihtiman and Samokov (Table 2).

Formaldehyde solution used in 2001 and 2004 instead of ethyleneglycol in 2000 fixed the tissues of all trapped animals, including small mammals. The tissues did not decay and attract carnivorous and necrophagous insects by the smell. On the epigeic spiders both preserving solutions have the same effect.

In Bulgaria *Pardosa agrestis*, *Pardosa prativaga*, *Meioneta rurestris*, *Oedothorax apicatus* colonize agroecosystems and *Pardosa palustris*, *Walckenaeria vigilax*, *Trichoncoides piscator*, *Prinerigone vagans*, *Haplodrassus dalmatensis*, and *Zelotes gracilis* are typical inhabitants of open habitats, cultivated fields included. The differences in spider fauna between 2000 and 2004 (Samokov), on one hand and 2001 (Ihtiman), on the other, are due the probably to the different study site (different altitude, respectively climate conditions).

According to our results, no negative effect of Bt potatoes on the biodiversity of epigeic spiders could be detected. The insecticidal treatments in non-Bt cultivars also had no direct effect on spider's biodiversity. According to PEKAR (1999) the density of understorey plants and herbicide applications on weeds had a greater influence on the abundance of epigeic arachnids than the different insecticides. By the way, Bt potatoes, Bt cotton and Bt maize had no negative effect on spiders, while insecticides reduced plant-dwelling spider populations (FITT *et al.* 1994, PILCHER *et al.* 1997, REED *et al.* 2001, LUDY, LANG 2004, DUAN *et al.* 2004, MEISSELE, LANG 2005). In all

Table 1. Araneae in Bt and non-Bt potato fields during the seasons 2000, 2001 and 2004.
(Bt – Bt potato field; C – non-Bt potato field; Dd – dynamic density per 100 trap-days; * - new species to the Bulgarian spider fauna).

Species	Samokov 2000			Ihtiman 2001			Samokov 2004		
	Bt	Dd	C	Bt	Dd	C	Bt	Dd	C
<i>Harpactea rubicunda</i> (C.L.KOCH, 1838)				1	0.04				
<i>Enoplognatha thoracica</i> (HAHN, 1833)			1			3			
<i>Robertus arundineti</i> (O.P.-CAMBRIDGE, 1871)	1	0.06	2	1	0.04		5	0.31	1
<i>Steatoda albomaculata</i> (DE GEER, 1778)	6	0.34	2	13	0.56	3			2
<i>Steatoda phalerata</i> (PANZER, 1801)			2	2	0.9	6			1
<i>Theridion impressum</i> L. KOCH, 1881						3			
<i>Acartauchenius scurrilis</i> (O.P.-CAMBRIDGE, 1872)				3	0.13				
<i>Araeoncus humilis</i> (BLACKWALL, 1841)	1	0.06	8	1	0.04	16	2	0.12	4
<i>Dicymbium tibiale</i> (BLACKWALL, 1836)			1						
<i>Erigone dentipalpis</i> (WIDER, 1834)	3	0.17	11	2	0.09	5	9	0.55	7
<i>Lepthyphantes istrianus</i> KULCZYNSKI, 1914				1	0.04				
<i>Meioneta fuscipalpis</i> (C.L. KOCH, 1836)				3	0.13				
<i>Meioneta rurestris</i> (C.L. KOCH, 1836)	1	0.06	7	306	13.03	166	3	0.18	1
<i>Microlinyphia pusilla</i> (SUNDEVALL, 1830)				1	0.04	2			
<i>Oedothorax apicatus</i> BLACKWALL, 1850)	54	3.03	99	21	0.91	22	46	2.84	41
<i>Oedothorax retusus</i> (WESTRING, 1851)	2	0.11	6				3	0.18	4
<i>Pelecopsis parallela</i> (WIDER, 1834)						1		0.04	
<i>Pocadicnemis juncea</i> LOCKET & MILLIDGE, 1953	5	0.28	3				7	0.43	4
<i>Porrhomma convexum</i> (WESTRING, 1851)			1						1
<i>Prinerigone vegans</i> (AUDOUIN, 1826)				12	0.52	47		2.04	
<i>Temiphantes tenuis</i> (BLACKWALL, 1852)						1		0.04	
<i>Trichonoides piscator</i> (SIMON, 1884)	7	0.39	15	94	4.08	57	8	0.49	11
<i>Walckenaeria capito</i> (WESTRING 1861)						1		0.04	

Table 1. Continued.

Species	Samokov 2000			Ihtiman 2001			Samokov 2004		
	Bt	Dd	C	Bt	Dd	C	Bt	Dd	C
<i>Walckenaeria vigilax</i> (BLACKWALL, 1853)	18	1.01	36				21	1.30	12
<i>Pachygnatha degeeri</i> SUNDEVALL, 1830	2	0.11		2	0.09	2	2	0.12	
<i>Hypsosinga pygmaea</i> (SUNDEVALL 1831)				1	0.04				
<i>Alopecosa barbipes</i> (SUNDEVALL, 1833)	1	0.06							
<i>Alopecosa cursor</i> (HAHN, 1831)						1			
<i>Aulonia albimana</i> (WALCKENAER, 1805)	1	0.06		1	0.04				
<i>Hogna radiata</i> (LATREILLE, 1817)	4	1.80		7	0.30	2	4	0.25	9
<i>Pardosa agrestis</i> (WESTRING, 1861)	1095	61.52	1058	642	27.91	677	846	52.22	910
<i>Pardosa bifasciata</i> (C.L. KOCH, 1834)	11	0.62	1		0.06		6	0.37	8
<i>Pardosa hortensis</i> (THORELL, 1872)									
<i>Pardosa italica</i> TONGIORGI, 1966	1	0.06							
<i>Pardosa palustris</i> (LINNAEUS, 1758)	35	1.97	12		0.67		12	0.74	19
<i>Pardosa prativaga</i> (L. KOCH, 1870)	95	5.34	25	1	1.40		42	2.59	33
<i>Pardosa proxima</i> (C.L. KOCH, 1847)	1	0.06							
<i>Pardosa roscai</i> (ROEWER, 1951)	2	0.11	9		0.51		4	0.25	2
<i>Pardosa vittata</i> (KEYSERLING, 1863)	3	0.17		2	0.09	2	2	0.12	2
<i>Pirata latitans</i> (BLACKWALL, 1841)	1	0.06							
<i>Pirata piscatorius</i> (CLERCK, 1757)						1		0.04	
<i>Trochosa ruricola</i> (De Geer, 1778)	10	1.56	3		0.17		7	0.43	4
<i>Xerolycosa miniata</i> (C.L. KOCH, 1834)	11	0.62	2		0.11				
<i>Xerolycosa nemoralis</i> (WESTRING, 1861)				2	0.09	1		0.04	
<i>Titanoea tristis</i> L. KOCH, 1872			1		0.06				
* <i>Titanoea veteranica</i> HERMAN, 1879				2	0.09				

Table 1. Continued.

Species	Samokov 2000			Ihtiman 2001			Samokov 2004		
	Bt	Dd	C	Bt	Dd	C	Bt	Dd	C
<i>Phrurolithus festivus</i> (C.L. KOCH, 1835)						1		0.04	
<i>Phrurolithus pullatus</i> KULCZYŃSKI, 1897				1	0.04				
* <i>Zodartion epirense</i> BRIGNOLI, 1984						1		0.04	
<i>Drassodes lapidosus</i> (WALCKENAER, 1802)						1		0.04	
<i>Drassodes pubescens</i> (THORELL I, 1856)	1	0.06							
<i>Drassyllus praeficus</i> (L. KOCH, 1866)	1	0.06		1	0.04	3		0.13	
<i>Drassyllus pusillus</i> (C.L. KOCH, 1833)	1	0.06							
<i>Gnaphosa lucifuga</i> (WALCKENAER 1802)				6	0.26	5		0.22	
* <i>Haplodrassus aenus</i> THALER, 1984	1	0.06							
<i>Haplodrassus dalmatensis</i> (L. KOCH, 1866)	4	0.22		20	0.87	15		0.65	
<i>Haplodrassus signifer</i> (C.L. KOCH, 1839)						4		0.17	
<i>Micaria albimana</i> O.P.-CAMBRIDGE, 1972				1	0.04				
<i>Micaria fulgens</i> (WALCKENAER, 1802)						1		0.04	
<i>Micaria pulicaria</i> (SUNDEVALL 1832)	1	0.06							
<i>Micaria albovittata</i> (LUCAS, 1846)	1	0.06	1		0.06			0.04	0.06
<i>Micaria rossica</i> THORELL 1875				2	0.09	5		0.22	
<i>Phaeoedus braccatus</i> (L. KOCH, 1866)						1		0.04	
<i>Zelotes caucasicus</i> (L. KOCH, 1866)				3	0.13				
<i>Zelotes erebius</i> (L. KOCH, 1866)						1		0.04	
<i>Zelotes exiguus</i> (MÜLLER & SCHENKEL, 1895)			1		0.06				1
<i>Zelotes gracilis</i> (CANESTRINI, 1868)				22	0.96	30		1.30	
<i>Zelotes hermani</i> (CHYZER, 1897)	1	0.06		2	0.09				
<i>Zelotes latreillei</i> (SIMON, 1878)				1	0.04				
<i>Zora spinimana</i> (SUNDEVALL, 1833)	1	0.06							

Table 1. Continued.

Species	Samokov 2000			Ihtiman 2001			Samokov 2004		
	Bt	Dd	C	Bt	Dd	C	Bt	Dd	C
<i>Thanatus vulgaris</i> SIMON, 1870	1	0.06							
<i>Tibellus oblongus</i> (WALCKENAE 1802)	1	0.06	1				2	0.12	1
<i>Ozyptila trux</i> (BLACKWALL, 1846)	1	0.06							
<i>Xysticus kochi</i> THORELL, 1872	1	0.06		2	0.09	6			
<i>Xysticus ninnii</i> THORELL, 1872	1	0.06	1						1
<i>Chalcoscirtus infimus</i> SIMON, 1868)				10	0.43	1		0.04	
* <i>Chalcoscirtus nigrinus</i> (THORELL, 1875)				1	0.04				
<i>Bianor auroinctus</i> (Ohlert, 1865)	1	0.06					1	0.06	
<i>Evarcha laetabunda</i> (C.L. KOCH, 1846)	1	0.06	1				2	0.12	
<i>Euophrys herbigrada</i> (SIMON 1871)				1	0.04				
<i>Heliophanus auratus</i> C.L. KOCH, 1835			1						1
<i>Heliophanus kochi</i> SIMON 1868				1	0.04				
<i>Pellenes nigrociliatus</i> (SIMON, 1875)				2	0.09				
<i>Sitticus distinguendus</i> (SIMON, 1868)				3	0.13				
<i>Talavera aequipes</i> (O.P.-CAMBRIDGE, 1871)	2	0.11		1	0.04		2	0.12	
Total number of species	42		28	41		39	23		24
Total number of specimens	1392		1311	1201		1107	1037		1080

Table 2. Similarity of spider's fauna from the fields cultivated with Bt and non-Bt potato (Sörensen Index).

//////	Samokov Bt ₂₀₀₀	Samokov C ₂₀₀₀	Ihtiman Bt ₂₀₀₁	Ihtiman C ₂₀₀₁	Samokov Bt ₂₀₀₄	Samokov C ₂₀₀₄
Samokov Bt ₂₀₀₀	//////	60.00	43.90	39.51	64.61	60.61
Samokov C ₂₀₀₀	60.00	//////	28.98	35.82	58.06	69.84
Ihtiman Bt ₂₀₀₁	43.90	28.98	//////	52.50	37.50	36.92
Ihtiman C ₂₀₀₁	39.51	35.82	52.50	//////	35.48	38.09
Samokov Bt ₂₀₀₄	64.61	58.06	37.50	35.48	//////	76.60
Samokov C ₂₀₀₄	60.61	69.84	36.92	38.09	76.60	//////

probability insecticides had effect on plant-dwelling arthropods, while this effect on epigeic fauna is lower.

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Биоразнообразие на епигейните паяци в генномодифицирани (Vt) и конвенционални (не-Vt) картофени полета в България

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(Резюме)

През 2000, 2001 и 2004 г. бяха изследвани епигейните паяци от полета, засадени с Vt и стандартни (не-Vt) сортове картофи в България. Земни капани бяха използвани, за да се сравни биоразнообразието на паяците. Общо бяха уловени 7128 екземпляра от 85 вида. Видовете *Titanoeca veteranica*, *Haplodrassus aenus*, *Chalcoscirtus nigrinus* и *Zodarion epirense* са нови за българската фауна. През годините на изследване във всичките полета с картофи присъстваха видовете *Pardosa agrestis*, *Oedothorax apicatus*, *Trichoncoides piscator*, *Araeoncus humilis*, *Erigone dentipalpis* и *Meioneta rurestris*. *Pardosa agrestis* представлява 79-84% от всички паяци, събрани през 2000 и 2004 г. Не бе установено негативно влияние на Vt посева върху епигейните паяци. Инсектицидите, използвани в конвенционалните посеви, също не оказваха съществено въздействие върху тях. Анализът на данните показва сходство във фауната на епигейните паяци в изследваните полета.