BIODIVERSITY OF GROUND BEETLES (*COLEOPTERA: CARABIDAE*) IN GENETICALLY MODIFIED (BT) AND CONVENTIONAL (NON-BT) POTATO FIELDS IN BULGARIA

P. Kalushkov[1], B. Gueorguiev[2], L. Spitzer[3], O. Nedved[3,4]
Institute of Zoology, Bulgarian Academy of Sciences, Sofia, Bulgaria[1]
National Museum of Natural History, Sofia, Bulgaria[2]
University of South Bohemia, Faculty of Biological Sciences, České Budějovice, Czech Republic[3]
Institute of Entomology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic[4]
Correspondence to: Plamen Kalushkov
E-mail: plamenkalushkov@yahoo.com

ABSTRACT

Investigations on ground beetles in potato fields were conducted in Bulgaria in 2000, 2001 and 2004. Pitfall traps were used to compare the biodiversity of carabid beetles in Bacillus thuringiensis (Bt) and conventional (non-Bt) potato cultivars. A total of 8663 individuals from 59 species of carabids were collected. The species Poecilus cupreus, Poecilus versicolor and Harpalus rufipes dominated in the Samokov region in 2000 and 2004, and Zabrus spinipes, Harpalus distinguendus and H. rufipes dominated in the region of Ihtiman in 2001. No negative effect of Bt potatoes on ground beetles could be detected. The insecticidal treatments in non-Bt cultivars also had no direct effect on the carabids fauna. Similarity more than 75% was observed between Bt and non-Bt potato fields every year.

Keywords: ground beetles, Bt potatoes, non-Bt potatoes, insecticides

Introduction

Colorado potato beetle *Leptinotarsa decemlineata* (Say) is the major insect pests of potatoes on the Balkan Peninsula (7). Reliance on conventional insecticides has resulted in multiple resistances in this pest and variety of untoward effects on non-target organisms and environment. *Bacillus thuringiensis* (Berliner) is the most successful microbial agent in the control of insect pests during last decades. Several species of crops have been modified with genetic engineering methods to express genes from various subspecies of this bacterium that encode Crystalline (Cry) proteins. The toxin Cry3A, which was introduced into the potato genome (Bt potatoes), provides excellent protection against the Colorado potato beetle (4, 6).

Ground beetles were used for evaluating effects of pesticides treatments or effects of transgenic crops on non-target species. Duan et al. (1) studied ground dwelling arthropods in Bt and non-Bt potato fields, treated with diverse insecticides or untreated and found that species composition of ground beetles did not differ significantly among treatments. The effect of transgenic potatoes resistant to CPB on the abundance of predators was compared to non-transgenic fields in the USA by Riddick et al. (17). None of the predators (coccinellids, carabids, ants, bugs) were affected, but spiders were more abundant in the transgenic fields.

The aim of the present work was to evaluate the effects of Bt and non-Bt potatoes on the ground beetles biodiversity in Bulgaria.

Materials and Methods

In 2000 the investigated fields were situated near Samokov at 900 m a.s.l. Western Bulgaria. The potatoes were planted in the end of April (Bt) and middle of May (conventional) and harvested in the beginning of September. Bt transgenic potatoes (Superior Newleaf®) containing Cry 3A Bt-toxin were planted in a monoculture field of 1.6 ha area. One hundred meters from this field separated by a bare land there was a non-Bt, 4 ha field with conventional cultivar (Santana®). Both fields were free of weeds. Non-Bt field was sprayed twice in the season (8th and 26th July) with the pyrethroid alfa-cypermethrin (Vaztak® - 10 EC, 100 ml/ha). There was no raining during the season and potatoes were irrigated every two weeks. Samples were taken from ten pairs of pitfall traps in each field, six times in the season (June 15 and 28, July 11 and 21, August 10 and 28). Each pitfall trap pair consisted of two 0.5 l plastic cups. The trap pairs were 15 m apart in three rows, each also 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, in Western Bulgaria. The potatoes were planted in the late April and harvested in the end of August. Experiments were carried out in 40 ha field divided in 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 (30 m x 500 m) Bt potatoes (Superior Newleaf®) of one side and 1.5 ha (30 m x 500 m) control field (standard cultivar Arinda®). The nontransgenic 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 (23rd June and 9th July) with fipronil (Regent® - 800 WG, 20 g/ha). Weeds covered virtually all space between the

TABLE 1

Carabid beetles collected in Bt and non-Bt potato fields in 2000 and 2004 near Samokov and in 2001 near Ihtiman

Species	2000				2001				2004			
	Bt	%	C	%	Bt	%	C	%	Bt	%	C	%
Nebriini												
Leistus ferrugineus (Linnaeus)	1	0.04	1	0.08								
Carabini												
Calosoma auropunctatum (Herbst)					5	0.45	2	0.19				
Carabus coriaceus Linnaeus					1	0.09						
C. scabriusculus Olivier					1	0.09						
Brachinini												
Brachinus crepitans (Linnaeus)	1	0.04			11	0.99	16	1.48				
B. explodens Duftschmid					4	0.36	1	0.09				
Clivinini												
Clivina fossor (Linnaeus)	4	0.15										
Bembidiini												
Bembidion femoratum Sturm	3	0.11	2	0.15							2	0.15
B. lampros (Herbst)	73	2.84	7	0.52					32	2.72	45	3.55
B. properans (Stephens)	16	0.64	3	0.23					10	0.85	10	0.74
B. quadrimaculatum (Linnaeus)	5	0.19	5	0.37					8	0.68	2	0.15
B. subcostatum (Motschulsky)	1	0.04										
Tachyini												
Tachys bistriatus (Duftschmid)							1	0.09				
Trechini												
Blemus discus (Fabricius)			1	0.08								
Pterostichini												
Poecilus cupreus (Linnaeus)	1422	52.89	718	55.86	2	0.18	11	1.11	714	60.86	758	56.44
P. versicolor (Sturm)	281	11.12	36	2.72					177	15.09	185	13.77
Pterostichus macer (Marsham)					3	0.27	1	0.09				
P. melanarius (Illiger)	14	0.57			1	0.09			2	0.17	12	0.89
Xenion ignitum (Kraatz)									1	0.08		
Zabrini												
Amara aenea (Degeer)			3	0.23	2	0.19	1	0.09	5	0.44		
A. apricaria (Paykull)	25	1.02	6	0.45	32	2.88	13	1.20	13	1.11	15	1.12
A. arenaria (Putzeys)					10	0.90	1	0.09				
A. aulica (Panzer)	5	0.19	4	0.30	6	0.54	1	0.09	1	0.08		
A. consularis (Duftschmid)							1	0.09				
A. chaudoiri Schaum					3	0.27	6	0.56				
A. equestris (Duftschmid)											4	0.29
A. eurynota (Panzer)	1	0.04	4	0.30								
A. ingenua (Duftschmid)	5	0.19	5	0.37								
A. lucida (Duftschmid)					1	0.09						
A. majuscula (Chaudoir)	16	0.64	3	0.23					4	0.34	10	0.74
A. plebeja (Gyllenhal)	4	0.15							2	0.17	5	0.37
A. similata (Gyllenhal)	2	0.08	2	0.15			3	0.28				
Zabrus spinipes (Fabricius)					163	14.77	140	13.53				
Z. balcanicus Heyden					3	0.27						
Z. *tenebrioides* (Goeze)					46	4.23	56	5.93				
Harpalini												
Acupalpus meridianus (Linnaeus)					2	0.18						
Anisodactylus binotatus (Fabricius)	10	0.38										
A. signatus (Panzer)	36	1.40	28	1.73					5	0.44	16	1.19
Harpalus affinis (Schrank)	7	0.26	4	0.30	3	0.275	5	0.46	8	0.68	4	0.29
H. albanicus Reitter					2	0.18						
H. autumnalis (Duftschmid)	5	0.19	3	0.23					1	0.08		
H. caspius (Steven)	2	0.08			3	0.27	11	1.02	1	0.08		
H. distinguendus (Duftschmid)	65	2.50	118	9.04	279	25.14	282	25.58	31	2.64	38	2.83

H. pygmaeus Dejean							1	0.09				
H. rubripes (Duftschmid)	2	0.08									2	0.15
H. rufipes (Degeer)	532	19.96	325	24.20	397	35.41	435	39.39	143	12.19	203	15.12
H. serripes (Quensel)	2	0.08										
H. smaragdinus (Duftschmid)	8	0.30	2	0.15	23	2.16	11	1.02	9	0.76	2	0.15
H. signaticornis (Duftschmid)					4	0.36	3	0.28				
Ophonus azureus (Fabricius)					2	0.18	1	0.09				
O. cribricollis Dejean							2	0.19				
Sphodrini												
Calathus fuscipes (Goeze)	5	0.19									2	0.15
C. melanocephalus (Linnaeus)	21	0.83	5	0.37							5	0.37
Dolichus halensis (Schaller)	11	0.45	4	0.30	3	0.27	27	2.41	1	0.08	4	0.29
Laemostenus punctatus (Dejean)									1	0.08		
Platynini												
Agonum muelleri (Herbst)	1	0.04										
Anchomenus dorsalis (Pontoppidan)					1	0.09	1	0.09				
Lebiini												
Microlestes maurus (Sturm)	46	1.85	19	1.36	23	2.16	18	1.76	4	0.34	18	1.34
M. minutulus (Goeze)	13	0.49	4	0.30	74	6.67	28	2.69	1	0.08	1	0.07
Total number of species	**34**		**25**		**30**		**28**		**23**		**22**	
Total number of specimens	**2645**		**1313**		**1110**		**1079**		**1173**		**1343**	

rows of potatoes for much of the season. Samples were taken from ten pairs of pitfall traps eight times in the season (May 15, June 1, 15 and 29, July 14 and 28, August 9 and 24). 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 non-transgenic cultivars surrounded both fields. No insecticides were used in the 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 (June 25, July 9 and 23, August 6 and 20).

In 2000 ethyleneglycol/water (1:1) was used as the preserving solution and in 2001 and 2004 the preserving solution was formaldehyde/water (1:8) (14). The potatoes were planted with 0.7 m spacing between the rows and 0.25 m spacing between the plants within a row.

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

Results and Discussion

A total of 59 species from 13 tribes were recorded (**Table 1**). In 2000, 2645 individuals from 34 species and 10 tribes of ground beetles were collected in the Bt field and 1313 individuals from 25 species and 8 tribes in the conventional field. The analysis of the carabid community revealed the big differences between the dominating and the rare species (**Table 1**). Three species dominated in the Bt field (*P. cupreus, P. rufipes, P. versicolor*) and three species in the non-Bt field (*P. cupreus, H. rufipes*, *H. distinguendus*). *Poecilus cupreus* represented 53% and 56% of all carabid species in the Bt and non-Bt fields, respectively. Only five other species were beyond 1% in the Bt field (*B. lampros, A. signatus, H. distingiendus, M. maurus* and *A. apricaria*) and three in the non-Bt field (*A. signatus, M. maurus* and *P. versicolor*). In the Bt field we collected 9 species more than in the conventional field. These species were represented from single specimens and probably the differences are not due the presence of the Bt toxin. Interestingly, the numbers of two more abundant species showed significant increase in the Bt field compared with the non-Bt field: *B. lampros* (more than 10 times) and *P. versicolor* (more than 7.8 times).

In 2001, there were 1110 individuals from 30 species and 8 tribes of carabid beetles collected in the Bt field and 1079 individuals from 28 species and 9 tribes caught in the conventional field. Four species dominated in both fields. *Harpalus rufipes* represented 35 - 39% of all carabid beetles in the Bt and non-Bt fields, followed by: *H. distingiendus* (25%), *Z. tenebrioides* (15%) and *M. minutulus* (7%) in the Bt field, and *H. distingiendus* (26%), *Z. tenebrioides* (14%) and *Z. spinipes* (6%) in the non-Bt field. Four other species in the Bt field and eight in the non-Bt field were represented beyond 1%.

In 2004, a total of 1173 individuals from 23 species and 6 tribes were recorded in the Bt plots and 1343 individuals from 22 species and 6 tribes were caught in the conventional plots. Except *X. ignitum* and *L. punctatus,* all other species collected during this year were present in the potato fields in 2000. *P. cupreus* dominated in both plots with 56 - 61% followed by

TABLE 2

Similarity of carabid'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}	///////////////	78.0	37.5	41.9	71.4	75.0
Samokov C_{2000}	78.0	///////////////	40.0	45.3	76.6	76.6
Ihtiman Bt_{2001}	37.5	40.0	///////////////	79.3	50.0	38.5
Ihtiman C_{2001}	41.9	45.3	79.3	///////////////	48.0	36.0
Samokov Bt_{2004}	71.4	76.6	50.0	48.0	///////////////	77.3
Samokov C_{2004}	75.0	76.6	38.5	36.0	77.3	///////////////

P. versicolor (14 - 15%) and *H. rufipes* (12 - 15%). Three other species in the Bt field and five in the non-Bt field were documented beyond 1%.

Similarity more than 75% was observed between the Bt and non-Bt potato fields every year (**Table 2**). Low similarity was found between the fauna near Ihtiman and Samokov.

A few very abundant arthropod species dominate the agricultural habitats (9). This is also clearly seen in our investigations. In Bulgaria *L. ferrugineus, C. coriaceus, C. scabriusculus*, *B. subcostatum, T. bistriatus, B. discus, A. consularis, A. lucida* and *H. pygmaeus* are rare species and *L. ferrugineus* are not typical for the agroecosystems.

In 2000 the control field was generally poorer in individuals and species than the Bt field. In 2001 and 2004 both the control and Bt fields were similar in terms of number of trapped individuals and species. We suppose that the experimental design in 2000 and 2004 affected the results. When the experimental plots were separated as in 2000, the differences were clearer. Carabids are highly mobile and when the experimental plots are small, one to another as in 2004, they can easy migrate from one to another plot.

Representation of carabid fauna was different in 2001 (Ihtiman) in comparison to 2000 and 2004 (Samokov). In Ihtiman region most numerous were omnivorous (Harpalini) and phytophagous (Zabrini) carabids, according to the food classification of the carabid beetles (8). In Samokov region most numerous were carnivorous beetles (Pterostichini, Bembidiini, Sphodrini, Lebiini) and phytophagous were rare.

Pterostichus melanarius and *P. cupreus* were the dominating carabid species in the agricultural fields in Czech Republic (18, 19). The same species, altogether with *P. rufipes* dominated in southern Poland and a region of Moscow in Russia (13, 20). Luka et al. (11) examined the seasonal population dynamics of the carabid species in Switzerland and concluded that *P. cupreus* is most common in spring, being replaced by *P. melanarius* at the beginning of summer. In Germany, the domination of *P. melanarius* and *P. cupreus* was associated with high abundance of *P. versicolor* and *Anchomenus dorsalis* (missing in our pitfall catches) (3). *P. melanarius* was rare species in our collections. In 2000, the numbers of two more abundant species (*B. lampros, P. versicolor*) showed significant increase in the Bt field compared with the non-Bt field and in our opinion, this fact may be due to indirect causes (such as food preferences).

It should be concluded that ground beetle predators are an important component of the agricultural ecosystems. Their prey includes insects feeding on both the aerial and the subterranean parts of the potato plants and potentially transmitting the Cry 3A toxin. In spite of this expected exposure to the toxin, we found no difference in terms of biodiversity between the carabid beetles in the Bt and the non-Bt potato plots. Other studies also reported negligible or no effect of Bt potatoes on various non-target insects, both phytophagous and carnivores (1, 16, 17). Bt potatoes, Bt cotton and Bt maize had no negative effect on epigeic spiders, while insecticides reduced plant-dwelling spider populations (2, 10, 12, 15).

In our experiments insecticidal treatments in non-Bt cultivars also had no direct effect on ground beetle biodiversity but they reduced the aphidophagous coccinellids (5). In all, probability insecticides had effect on plant-dwelling arthropods, while this effect on epigeic fauna (ground beetles, spiders) was lower.

Conclusions

Bt potatoes providing excellent protection against the Colorado potato beetle and the ground beetles are an important component of the agricultural ecosystems. Field experiments indicated that Bt potatoes had no negative effect on the biodiversity of ground beetles, but insecticides also had slight effect on the epigeic fauna including the carabid communities.

Acknowledgments

These investigations were supported by the grant B-1105 and the preparation of the paper by the grant B-1508 of Bulgarian National Research Fund. The study has been undertaken as a part of official cooperation program between the Institute of Zoology, Bulgarian Academy of Sciences and Institute of Entomology, Academy of Sciences of the Czech Republic.

REFERENCES

1. **Duan J.J., Head G., Jensen A., Reed G.** (2004) Environ. Entomol., **33**, 275-281.
2. **Fitt G.P., Mares C.L., Llewellyn D.J.** (1994) Biocontrol Science and Technology, **4**, 535-548.
3. **Imler U.** (2003) Agric. Ecosyst. Environ., **98**, 141-151.
4. **Kalushkov P., Batchvarova R.** (2005) Biotechnol. & Biotechnol. Eq., **19**, 28-34.
5. **Kalushkov P., Hodek I.** (2005) Eur. J. Entomol., **102**, 449-452.
6. **Kamenova I., Batchvarova R., Flasinski S., Dimitrova L., Christova P., Slavov S., Atanassov A., Kalushkov P., Kaniewski W.** (2008) Agron. Sustain. Dev., **28**, 481-488.
7. **Kostic M., Drazic S., Popovic Z., Stankovic S., Sivcev I., Zivznovic T.** (2007) Biotech. & Biotech. Equipment, **21**, 426-430.
8. **Larochelle A.** (1990) The food of carabid beetles (Coleoptera: Carabidae, Including Cicindelidae). Fabreries, Suplement, **5**, 1-132.
9. **Luczak J.** (1979) Spider communities in the crop fields. Polish Ecol. Studies, **5**, 151-200.
10. **Ludy C., Lang A.** (2004) J. Appl. Entomol., **128**, 501-509.
11. **Luka H., Pfiffner L., Niggli U.** (2000) Mittl. Entomol. Geselsch. Basel, **50**, 18-38.
12. **Meissle M., Lang A.** (2005) Agriculture Ecosystems & Environment, **107**, 359-370.
13. **Olbrycht T., Czerniakowski Z.W.** (2002) Progr. Plant Protect., **42**, 415-416.
14. **Pekar S.** (1999) J. Appl. Entomol., **123**, 115-120.
15. **Pilcher C.D., Obrycki J.J., Rice M.E., Lewis L.C.** (1997) Environ. Entomol., **26**, 446-454.
16. **Reed G.L., Jensen A.S., Riebe J., Head G., Duan J.J.** (2001) Entomol. Exp. Appl., **100**, 89-100.
17. **Riddick E.W., Dively G., Barbosa P.** (2000) J. Anim. Ecol., **33**, 301-310.
18. **Sehnal F., Habustova O., Spitzer L., Hussein H., Ruzicka V.** (2004) IOBC wprs Bulletin, **27**(3), 147-160.
19. **Stastna P., Bezdek J.** (2002) Acta Univ. Agricult. Silvicult. Mendelianae Brunensis, **50**, 103-109.
20. **Timraleev Z.A., Aryukov V.A., Bardin O.D.** (2002) Zool. J., **81**, 1517-1522 (in Russian).