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Genetically modified potatoes expressing Cry 3A protein do not affect aphidophagous coccinellids

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Abstract: Field investigations showed that genetically modified *Bacillus thuringiensis* (Bt) potato plants ('New Leaf' expressing Cry 3Aa toxic protein) were not damaged by the Colorado potato beetle (CPB) *Leptinotarsa decemlineata* and contained diverse populations of the aphidophagous coccinellids *Coccinella septempunctata*, *Coccinula quatuordecimpustulata*, *Hippodamia variegata* and *Propylea quatuordecimpunctata*. The insecticides, alpha-cypermethrin and fipronil, were used for control of CPB in the fields with the non-transgenic standard potatoes 'Santana' and 'Arinda'. Both insecticides caused significant decrease in the abundance of aphidophagous coccinellids. Laboratory experiments revealed that Bt potatoes expressing Cry 3Aa had no effect on the aphid *Myzus persicae* and that the aphids fed on Bt potatoes had no effect on the larval development and mortality of *C. septempunctata*.

Key words: *Bacillus thuringiensis*, Coccinellidae, GMO, insecticides, ladybeetles, non-target organism, predators

1 Introduction

Several crop species were genetically modified to incorporate the *Bacillus thuringiensis* (Bt) toxin. Obrycki et al. (2004) reviewed various laboratory experiments of direct and indirect toxicity of these plants to the natural enemies, as well as results of field surveys in transgenic insecticidal fields. Coccinellids seem to be important predators of non-target or secondary pests. Aphids, which are considered major prey of aphidophagous coccinellids – besides pollen, do not ingest the toxin when feeding on the Bt plants in which the Bt toxin is not transported in the phloem (Head et al., 2001; Dutton et al., 2002; Raps et al., 2001).

A possible adverse effect on coccinellids by transgenic plants expressing protease inhibitors was also investigated. The larvae of *Harmonia axyridis* (Pallas) consumed prey (the moth *Plutella xylostella* L.) reared on oilseed rape expressing the cysteine protease inhibitor oryzacystatin-1. No significant effects upon survival or overall development or reproductive fitness of adults were observed. A shortening of the development of the second instar larvae was accompanied by an increase in weight (Ferry et al., 2003).

A complicated situation was found when plants expressing snowdrop lectin (GNA) were studied. When adult *Adalia bipunctata* (L.) were fed for 12 days on *Myzus persicae* (Sulzer) colonizing transgenic potatoes expressing GNA in leaves, coccinellid fecundity, egg viability and longevity significantly decreased over the following 2–3 weeks (Birch et al., 1999). However, no significant effects on development and survival of coccinellid larvae fed on those aphids were observed, and there were also no effects on subsequent longevity in this second study (Down et al., 2003). Preying on aphids fed on artificial diet containing GNA even improved the fecundity.

Reactions of the coccinellid, *Coleomegilla maculata* DeGeer, to several insecticides used against the Colorado potato beetle (CPB) were recently studied by Lucas et al. (2004b). Cyromazine (insect growth regulator) and *Bacillus thuringiensis* Berliner (Bt), var. *tenebrionis* (microbial insecticide), had no lethal effects on larvae. Cryolite (inorganic insecticide) caused very low predator mortality. However, imidacloprid (systemic organic insecticide) was highly toxic to adult and larval *C. maculata*. Toxicity of pyrethroids (alphamethrin, cyhalothrin) to the coccinellid *Cycloneda sanguinea limbifer* Casey was demonstrated by Nedvěd and Kindlmann (1991).

We report the results of a real-situation field study conducted near Sofia, Bulgaria to evaluate the potential non-target impact of a transgenic Bt potato (*Solanum tuberosum* L.) cultivar and sprayed insecticides on predatory coccinellids dwelling on potato plants. The major arthropod pest of potatoes in Bulgaria is the CPB, *Leptinotarsa decemlineata* (Say) (Chrysomelidae). In some regions there are additional pests such as aphids and click beetles (Elateridae) but they are of less importance.

2 Materials and Methods

2.1 Experimental plots

The investigated fields were situated at about 900 m above sea level (a.s.l.) near Samokov in 2000 and at about 600 m a.s.l. near Ihtiman in 2001 in western Bulgaria. In both the years, the experiments were conducted in a real-culture situation, not on artificial experimental plots. There were only one Bt and one control field available for our study each year, no real plot replications were included, and the control cultivars were not isogenic to the transgenic one.

Transgenic potatoes Newleaf Superior® (Monsanto, St. Louis, Missouri, USA) containing Cry 3Aa Bt-toxin were planted in one monoculture field of 1.6 ha in 2000. One hundred metres from this field, separated by a strip of bare land, was a control field (4 ha) with a classic cultivar (Santana®) which was sprayed twice in the season (8 and 26 July) by pyrethroid alpha cypermethrin (Vaztak® 10 EC (BASF, Lufthafen, Germany), 100 ml/ha). Bt potatoes were planted in late April and the Santana potatoes 2 weeks later. Both fields were harvested in the beginning of September. Susceptible cultivars were planted all around, which functioned as a refuge decreasing the risk of development of resistance in CPB.

In 2001, experiments were carried out in a 40-ha field divided in halves with a 5-m path. Experimental plot of 1.5 ha was picketed in the centre of each half. One plot was planted with the Bt potatoes Newleaf Superior® and the other with the classic cultivar Arinda®. The remainder of the field was planted with the Santana® and Arinda® cultivars. The non-transgenic potatoes were sprayed twice in the season (23 June and 9 July) with fipronil (Regent® (BASF, Lufthafen, Germany) 800 WG, 20 g/ha). The potatoes were planted in late April and harvested in late August. These fields were strongly infested by weeds.

2.2 Field sampling

Coccinellids were sampled by beating 25 randomly selected plants into an entomological net. Larvae and adults were identified (see the pictorial field key to larvae by Klausnitzer and Kovář, 1973) in the field and released. In 2000, we collected material four times (7, 11, 21 July and 10 August) and in 2001 seven times (1, 16, 29 June, 14 and 28 July, 9 and 23 August). Numbers of larvae and adults were pooled, as there was no obvious difference in the seasonality and field preference between the two stages. Egg masses found by visual inspection of the potato plants could not be identified to species level. Pupae were probably overlooked.

Four coccinellid species found in the fields were all aphidophagous: *Coccinella septempunctata* Linnaeus, 1758, *Coccinula quatuordecimpustulata* (Linnaeus, 1758), *Hippodamia variegata* (Goeze, 1777), and *Propylea quatuordecimpunctata* (Linnaeus, 1758). Aphids sampled by the shaking of the 25 plants were counted, but their number was relatively low and stable, and hence not included in subsequent analyses. Other arthropods (Carabidae, Staphylinidae, Aranea, Opiliones) were sampled at the same dates in the same fields using pitfall traps (Spitzer L., Gueorgniev B., Kalushkov P., Nedvěd O., unpubl. data). No significant correlation of abundance and species composition between coccinellids and those of other taxonomic groups was found, and was not further analysed.

2.3 Laboratory experiment

The polyphagous aphid, *M. persicae*, was reared either on the Bt or on the standard potato plants. Potatoes were cultivated in 4-l pots inside nylon isolators (cube of half metre side) at 20–25°C temperature, 53–75% relative humidity and 16 : 8 h light : dark (L : D) photoperiod conditions. Individual aphids were fed on potato leaves in Petri dishes. Developmental time (from neonate to adult female), longevity and life-span fecundity of aphids were recorded.

Adult *C. septempunctata* were collected in alfalfa fields near Sofia, transferred to the laboratory and reared on the aphid *Acyrthosiphon pisum* (Harris), which was grown on the broad been, *Vicia faba* L. The larvae hatched from eggs laid by these coccinellids were used (30 individuals per treatment) in the experiments. Newly hatched larvae were reared individually in 9-cm Petri dishes, fed by the adult wingless females (virginogenies) of aphid *M. persicae* from either Bt or non-Bt potatoes.

The experiments were performed in laboratory conditions (23 ± 2°C temperature, 55–76% relative humidity and 16 : 8 h L : D photoperiod). Developmental time of the fourth instar of *C. septempunctata* and larval mortality were recorded.

2.4 Data analysis

Direct unimodal gradient analysis [canonical correspondence analysis (CCA)] was chosen because of the multivariate nature of the predictors and response variables (Lepš and Šmilauer, 2003). With our data, a conventional anova could suffer from inflation of type-I error. CANOCO for Windows version 4.5 (Ter Braak and Šmilauer, 1998) was run for the evaluation of the role of three environmental variables – date, Bt, and spray – on the coccinellid community composition, separately for the 2 years of study. Time (D for days) was coded as the number of days from 1 June to the date of sampling. Bt was coded as +1 for the samples on transgenic cultivar, and as −1 for those on classical, sprayed cultivar. Spray was calculated as a reciprocal time (1/number of days – estimation of decaying of residues) after the day of spraying, or coded always zero for the Bt field. No transformation of abundance data was needed. Monte Carlo test (the advantage of which is that it is distribution-independent) with 999 permutations was run to get a probability value (P).

Mann–Whitney *U*-test for two independent samples was used to compare abundance between two plots or dates. We used individual plants (25 per sample) as observed units, which may be considered a pseudoreplication, as there were only one Bt and one control field available for our study each year. Abundance was generally low and skewed, thus calculating a variance within sampling dates and using a parametric test would be incorrect. The *U*-test was also employed to evaluate life-table characteristics of aphids and coccinellids in the laboratory experiments.

3 Results

3.1 Species abundance

In both seasons, 2000 and 2001, there were the same four coccinellid species in both fields, irrespective of differences in the agrotechnical history (mainly the high infestation by weeds in 2001). Total numbers of coccinellids were rather small in both 2000 and 2001: *C. septempunctata* 90 and 139, *C. quatuordecimpustulata* 67 and 23, *H. variegata* 17 and 59, and *P. quatuordecimpunctata* 40 and 21 respectively.

In both 2000 and 2001, abundance of coccinellids was slightly but non-significantly (Mann–Whitney test 2000: $Z = 0.5$, $P = 0.6$; 2001: $Z = 1.8$, $P = 0.07$) higher in the non-transgenic cultivars before the first insecticide treatment than in the Bt plot (fig. 1). The abundance of aphids was very low (about 10 aphids per stem), and did not peak or crash during the season.

The abundance of coccinellids strongly decreased after the first spraying in 2000 (Mann–Whitney test: $Z = 3.8$, $P = 10^{-4}$) and conspicuously but non-significantly decreased in 2001 ($Z = 1.5$, $P = 0.12$; fig. 1), whereas in the Bt plots, it remained more or less constant throughout the season. After spraying, the abundance decreased below the level found on the Bt plots and remained low for most of the remaining season. The second spray did not have such a conspicuously damaging effect as the first one. Coccinellid larvae were present in the Bt fields from the beginning of June to early August, but were absent in the control fields for several weeks after the spraying.

3.2 Community structure

Multivariate analysis (CCA) of the abundance data of individual species in 2000 revealed only slight seasonal changes in species composition, or in other words, different timing of individual species occurrence (table 1, row D: 27% explained variability, but low test significance: $P = 0.1$; and fig. 2). In 2001, when sampling covered most of the season, the sample date had a strong influence on the species composition (table 2, row D: 61% explained variability, significance: $P = 0.001$; and fig. 3).

Coccinellid species composition was affected neither by the Bt toxin inside plants nor by the sprayed insecticides that temporarily damped the overall abundance (fig. 1). In 2000, the environmental variable spray appeared opposite to the date variable, not to Bt (fig. 2). The most common *C. septempunctata* was abundant early in the season and readily re-colonized plots deprived of coccinellids by the insecticides after spraying most easily. None of the coccinellid species showed a preference or avoidance of one of the two cultivars (Bt and control) or of spraying in 2001. The environmental variables Bt and spray were opposite to each other on the ordination diagram (fig. 3), and perpendicular to the date. The long arrows of the two former environmental variables indicate that they had strong effect on particular samples. However, the position of all species points close to the x-axis means that no species was selectively affected by Bt toxin or insecticide spraying during entire season. All four species showed strong seasonal tendency: *C. septempunctata* and *H. variegata* were early season species, while *C. quatuordecimpustulata* and *P. quatuordecimpunctata* were abundant late in the summer.

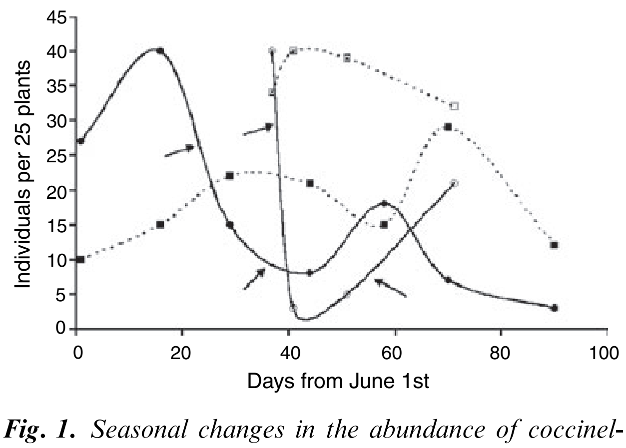

Fig. 1. *Seasonal changes in the abundance of coccinellids (four species pooled) in the potato fields. White marks – 2000, black marks – 2001; squares and dashed lines – Bt, circles and solid lines – sprayed control. Arrows show the spraying dates*

Table 1. *CCA analysis of environmental impact on the abundance of coccinellids in potato fields in Samokov, 2000*

ENV	COV	P	F	Eign. (0.224)	(%)
Bt	–	0.396	1.10	0.035	15.5
D	–	0.101	2.22	0.061	27.0
S	–	0.432	0.69	0.023	10.3
D + S	–	0.189	1.61	0.088	39.2
Bt + S	–	0.573	0.73	0.050	22.5
Bt + D	–	0.140	1.80	0.094	41.9
Bt	S	0.526	0.78	0.027	13.6
Bt	D	0.298	1.28	0.033	20.4

Bt, Bt-cultivar; D, date of sampling; S, insecticide treatment (spraying); ENV, environmental variables; COV, used covariables; P, permutation test probability; F, statistical test value; Eign., eigenvalue; (%), explained variability.

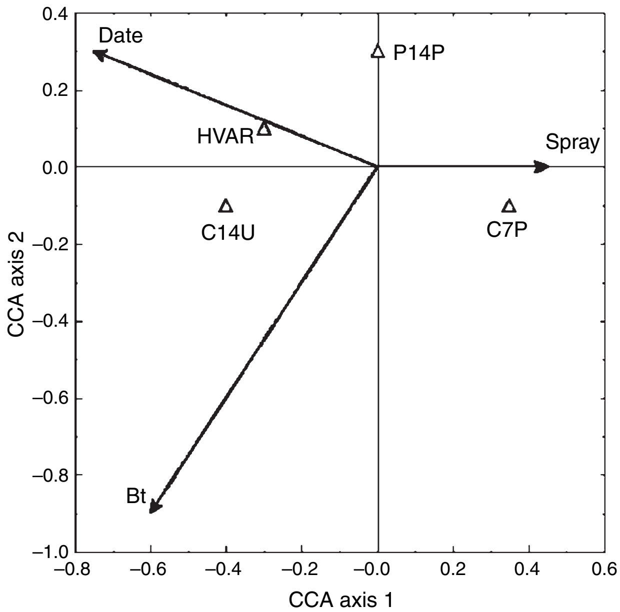

Fig. 2. *Ordination diagram of coccinellid community in two potato fields in 2000. Arrows – environmental variables; Triangles – species scores; C7P –* Coccinella septempunctata*; HVAR –* Hippodamia variegata*; C14U –* Coccinula quatuordecimpustulata*; P14P –* Propylea quatuordecimpunctata

Table 2. CCA analysis of environmental impact on the abundance of coccinellids in potato fields in Ihtiman, 2001

ENV	COV	P	F	Eign. (0.594)	EXP(%)
Bt	–	0.553	0.48	0.023	3.9
D	–	0.001	18.86	0.363	61.1
S	–	0.878	0.11	0.005	0.9
D + S	–	0.001	9.39	0.375	63.1
Bt + S	–	0.882	0.26	0.026	4.4
Bt + D	–	0.001	9.98	0.383	64.5
Bt	S	0.605	0.41	0.021	3.6
Bt	D	0.350	1.04	0.020	8.6

Bt, Bt-cultivar; D, date of sampling; S, insecticide treatment (spraying); ENV, environmental variables; COV, used covariables; P, permutation test probability; F, statistical test value; Eign., eigenvalue; (%), explained variability.

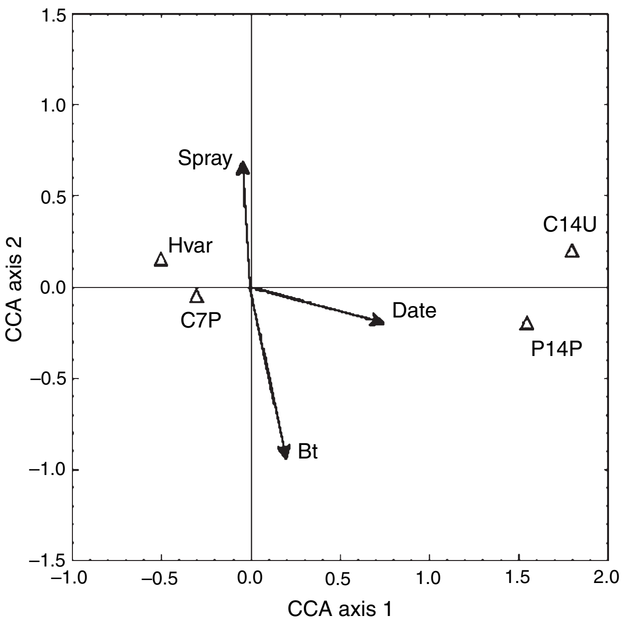

Fig. 3. *Ordination diagram of coccinellid community in two potato fields in 2001. Arrows – explaining environmental variables. Triangles – species scores. C7P –* Coccinella septempunctata*; HVAR –* Hippodamia variegata*; C14U –* Coccinula quatuordecimpustulata*; P14P –* Propylea quatuordecimpunctata

3.3 Larval development

Laboratory experiments showed no effect of Bt transgene on the performance of the aphid *M. persicae* on potatoes. Feeding *M. persicae* on the Bt potatoes did not affect larval development and mortality of *C. septempunctata* grown exclusively on this aphid.

None of the three studied parameters – length of larval development, longevity of virginogenies, and average fecundity – differed (Mann–Whitney tests) between aphids grown on the control and those reared on the Bt potatoes (table 3). Similarly, larvae of *C. septempunctata* offered aphids from the two types of potatoes did not differ with respect to the developmental time (Mann–Whitney test), and the mortality rate (*G*-test; table 4).

Table 3. Effect of Bt and non-Bt potatoes on selected life parameters of Myzus persicae

	n	Larval development (in days)	Longevity of virginogenies (in days)	Fecundity per female
Bt	28	8.4 ± 0.5	16.2 ± 2.7	41.1 ± 5.5
Non-Bt	28	8.6 ± 0.6	15.7 ± 1.5	38.9 ± 4.0

No pair of values (mean ± SD) was significantly different (Mann–Whitney tests).

Table 4. Effect of Myzus persicae *reared on Bt and non-Bt potatoes on mean larval development time and mortality of* Coccinella septempunctata

	n	Larval development (days)	Mortality [% (individuals)]
Bt	28	9.2 ± 1.0	7 (2/30)
Non-Bt	27	9.6 ± 1.2	10 (3/30)

Neither developmental time (mean ± SD; Mann–Whitney test), nor mortality (*G*-test) differed on 5% level.

4 Discussion

Field investigations showed that Bt potatoes Newleaf Superior® (carrying Cry3Aa protein) are very effective against the CPB *L. decemlineata* and preserve aphidophagous coccinellids. By contrast, both tested insecticides proved very toxic to the coccinellids, causing statistically significant decrease in the abundance of adults and larvae. Multivariate analysis confirmed seasonality of individual coccinellid species, and no affinity to either Bt transgenic or standard cultivars.

The presented results are based on comparisons of two treatments, i.e. a standard (classical) cultivar of potatoes protected by the insecticide sprays, and a transgenic cultivar without insecticidal treatment. It is not possible to cultivate classical potatoes without insecticides in Bulgaria. The potato plants of non-sprayed plots were totally destroyed at the end of July. In the middle July 2002 we found on average 16 (min. 9, max. 42, $n = 25$), and in 2003 19 (min. 10, max. 48, $n = 25$) larvae of the CPB per plant.

Our laboratory experiments showed that Bt potatoes had no effect on the aphid *M. persicae* and subsequently, *M. persicae* from the Bt potatoes had no effect on the larval development and mortality of the most common coccinellid *C. septempunctata*. Dutton et al. (2002) reared aphids *Rhopalosiphum padi* (L.) on Bt maize and found that aphids did not ingest the Bt-toxin and therefore had no effect on survival, development and weight of *Chrysoperla carnea* (Stephen). We verified this observation for another tritrophic system (*S. tuberosum*–*M. persicae*–*C. septempunctata*). We hypothesize that the other three coccinellid species found in the potato fields during our study have similar ability to develop on aphids fed on both potato cultivars but we did not have the capacity to repeat all experiments.

All four coccinellid species observed are primarily aphidophagous but they may by chance prey on other organisms, including eggs of the CPB. However, these eggs are not expected to contain Bt toxin. Riddick and Barbosa (1998) fed *Coleomegilla maculata* – a ladybird with a broad food spectrum – with neonate larvae of *L. decemlineata* fed on turn on either Cry3A-transgenic or nontransgenic foliage of potato. After differences in prey weight were considered, no significant difference was found between the body mass, mortality and fecundity of ladybirds. *L. decemlineata* larvae fed on Bt potatoes was found a suitable prey also for other generalist predators like *Lebia grandis* (Col., Carabidae) (Riddick and Barbosa, 2000). *L. grandis* probably rapidly dispersed from seed-mixed and pure transgenic potato fields because of the low densities of prey – *L. decemlineata* – in these fields. However, *C. maculata* was abundant in fields containing transgenic potato, thanks to alternative prey and plant pollen (Riddick et al., 1998).

In both 2000 and 2001, we found the same four ladybird species in both localities and fields, irrespective of different agrotechnical histories and the high field infestation by weeds in 2001. This contrasts with differences in the species composition of carabid beetles sampled in a parallel study using pitfall traps (Spitzer L., Gueorgniev B., Kalushkov P., Nedvěd O., unpubl. data). The weeds might harbour an alternative aphid prey for the coccinellids. However, this potential prey was not important, since *M. persicae* on the Bt potatoes provided a suitable, non-toxic prey. Possible effects of coccinellids on the populations of aphids could not be detected due to the consistently low aphid abundance.

While the numbers of coccinellids remained moderate and almost constant in the Bt fields throughout the seasons, the abundance of coccinellids decreased markedly after spraying in the control field. Larvae were almost absent for several weeks, while flying adults later restored the population.

Similar to our observation, Delrio et al. (2003) found no significant differences between the Bt and non-Bt (but non-sprayed) corn in the infestation by aphids, mainly *R. padi*. The abundance and species composition of coccinellids were also similar, except for the number of *P. quatuordecimpunctata* captured with the aid of yellow traps that was significantly lower in the Bt plots.

Several arthropod taxa were studied in Bt and non-Bt corn for three years in Hungary (Szentkirályi et al., 2003). Abundance, dominance structure, species composition, and the species diversity profile did not differ between the cultivars for several non-target groups of insects including coccinellids. However, there were great differences between years.

Generalist predators like *Nabis* spp. feed on two common insect herbivores co-occuring on potato, *M. persicae* and *L. decemlineata*. In a greenhouse microcosm experiment, the bugs ate more aphids when beetle eggs were included, compared to microcosms with no alternative prey present (Koss et al., 2004). Similar increase of voracity was found in a specialist predator *C. septempunctata* – the total number of prey killed and the total biomass consumed were higher when both prey – *Aphis pomi* DeGeer and *Choristoneura rosaceana* (Harris) (Lep., Tortricidae) were present than in single-prey treatments (Lucas et al., 2004a). Thus, a lower performance of coccinellids in fields with decreased prey diversity may be suggested. Anyway, both our Bt-field and sprayed non-Bt field were probably similarly impoverished.

The cultivars used, Newleaf Superior®, and either Santana® or Arinda® are similar but not isogenic, so that possible differences in insect community, if they had occurred, could be attributed not only to the Bt toxin but also to other possible differences between plants. However, no such differences were found. The relatively low number of species and also relatively low overall abundance of ladybirds in this study might be responsible for no detection of possible weak effect of Bt plants. On the contrary, strong effects like the decrease of abundance after spraying and seasonality in species composition were proven even using our poor samples.

In Oregon, Bt potatoes or weekly sprays of a microbial Bt-based formulation did not significantly impact the beneficial predators, however, bi-weekly applications of permethrin significantly reduced the abundance of several major generalist predators (Reed et al., 2001). In the case of our potato fields, casual differences were also mostly because of the insecticide spray. However, the effect was not significant if the entire season was taken into account.

A significant trend was found in Minnesota (USA) for a coccinellid *C. maculata* that was more abundant in the non-Bt than in the Bt sweet corn (Wold et al., 2001), possibly due to high ingestion of pollen. High concentrations of a microbial insecticide of *B. thuringiensis* were found to reduce predation rates by adult *C. maculata*, and prolonged larval development when applied to pollen fed to the larvae (Giroux et al., 1994a,b). Feeding on pollen is infrequent in our four coccinellid species, and rarely occurs on potatoes.

While it was already found several times that Bt transgenic crop cultivar had no adverse effect on predatory coccinellid in a field experiment, this study proves such conclusion also in a real-situation agricultural system in Europe, despite the above discussed weaknesses in the methodology. As the effects of synthetic insecticides and of the season were statistically significant in our study, it is unlikely that any effect of Bt cultivar alone would ever contribute strongly to the predator–prey dynamics, pest control, and biodiversity in agroecosystems.

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References

Birch, A. N. E.; Geoghegan, I. E.; Majerus, M. E. N.; McNicol, J. W.; Hackett, C. A.; Gatehouse, A. M. R.; Gatehouse, J. A., 1999: Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. Mol. Breeding **5,** 75–83.

Delrio, G.; Verdinelli, M.; Serra, G., 2003: Monitoring of pest and beneficial insect populations in a summer sown cultivation of Bt corn. In: Book of Abstracts, IOBC/WPRS Working Group Meeting of the Ecological Impact of Genetically Modified Organisms. České Budějovice: Institute of Entomology AS ČR, 19.

Down, R. E.; Ford, L.; Woodhouse, S. D.; Davison, G. M.; Majerus, M. E. N.; Gatehouse, J. A.; Gatehouse, A. M. R., 2003: Tritrophic interactions between transgenic potato expressing snowdrop lectin (GNA), an aphid pest (peach-potato aphid; *Myzus persicae* (Sulz.) and a beneficial predator (2-spot ladybird; *Adalia bipunctata* L.). Transgenic Res. **12,** 229–241.

Dutton, A.; Klein, H.; Romeis, J.; Bigler, F., 2002: Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecol. Entomol. **27,** 441–447.

Ferry, N.; Raemaekers, R. J. M.; Majerus, M. E. N.; Jouanin, L.; Port, G.; Gatehouse, J. A.; Gatehouse, A. M. R., 2003: Impact of oilseed rape expressing the insecticidal cysteine protease inhibitor oryzacystatin on the beneficial predator *Harmonia axyridis* (multicoloured Asian ladybeetle). Mol. Ecol. **12,** 493–504.

Giroux, S.; Coderre, D.; Vincent, C.; Cote, Jc., 1994a: Effects of *Bacillus thuringiensis* var San-Diego on predation effectiveness, development and mortality of *Coleomegilla maculata lengi* (Col, Coccinellidae) larvae. Entomophaga **39,** 61–69.

Giroux, S.; Cote, Jc.; Vincent, C.; Martel, P.; Coderre, D., 1994b: Bacteriological insecticide M-One effects on predation efficiency and mortality of adult *Coleomegilla maculata lengi* (Coleoptera, Coccinellidae). J. Econ. Entomol. **87,** 39–43.

Head, G.; Brown, C. R.; Groth, M. E., 2001: Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Appl. **99,** 37–45.

Klausnitzer, B.; Kovář, I., 1973: A simple key for field use. In: Biology of Coccinelidae. Ed. by Hodek, I. Praha: Academia.

Koss, A. M.; Chang, G. C.; Snyder, W. E., 2004: Predation of green peach aphids by generalist predators in the presence of alternative, Colorado potato beetle egg prey. Biol. Control **31,** 237–244.

Lepš, J.; Šmilauer, P., 2003: Multivariate analysis of ecological data using CANOCO. Cambridge: Cambridge University Press.

Lucas, E.; Demougeot, S.; Vincent, C.; Coderre, D., 2004a: Predation upon the oblique-banded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae), by two aphidophagous coccinellids (Coleoptera: Coccinellidae) in the presence and absence of aphids. Eur. J. Entomol. **101,** 37–41.

Lucas, E.; Giroux, S.; Demougeot, S.; Duchesne, R. M.; Coderre, D., 2004b: Compatibility of a natural enemy, *Coleomegilla maculata lengi* (Col., Coccinellidae) and four insecticides used against the Colorado potato beetle (Col., Chrysomelidae). J. Appl. Entomol. **128,** 233–239.

Nedvěd, O.; Kindlmann, P., 1991: Poisoning of ladybirds by pyrethroids. In: Behaviour and impact of aphidophaga. Ed. by Polgár, L.; Chambers, R. J.; Dixon, A. F. G.; Hodek, I.. The Hague: SPB Academic Publishing, 319–322.

Obrycki, J. J.; Ruberson, J. R.; Losey, J. E., 2004: Interactions between natural enemies and transgenic insecticidal crops. In: Genetics, Evolution and Biological Control. Ed. by Ehler, L. E.; Sforza, R.; Mateille, T.. Wallingford: CABI Publishing, 183–206.

Raps, A.; Keher, J.; Gugerli, P.; Moar, W. J.; Bigler, F.; Hilbeck, A., 2001: Detection of Cry 1Ab in phloem sap of *Bacillus thuringiensis* corn and in the selected herbivores *Rhopalosiphum padi* (Homoptera: Aphididae) and *Spodoptera littoralis* (Lepidoptera: Noctuidae). Mol. Ecol. **10,** 525–533.

Reed, G. L.; Jensen, A. S.; Riebe, J.; Head, G.; Duan, J. J., 2001: Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts. Ent. Exp. Appl. **100,** 89–100.

Riddick, E. W.; Barbosa, F., 1998: Impact of Cry3A-intoxicated *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and pollen on consumption, development, and fecundity of *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. **91,** 303–307.

Riddick, E. W.; Barbosa, F., 2000: Cry3A-intoxicated *Leptinotarsa decemlineata* (Say) are palatable prey for *Lebia grandis* Hentz. J. Entomol. Sci. **35,** 342–346.

Riddick, E. W.; Dively, G.; Barbosa, P., 1998: Effect of a seed-mix deployment of Cry3A-transgenic and nontransgenic potato on the abundance of *Lebia grandis* (Coleoptera: Carabidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. **91,** 647–653.

Szentkirályi, F.; Kádár, F.; Szekeres, D.; Kiss, J.; Szénási, Á.; Tóth, F., 2003: Comparative studies on predatory insect assemblages in experimental Bt- and non-Bt maize fields in Hungary. In: Book of Abstracts, IOBC/wprs working group meeting of the Egological Impact of Genetically Modified Organisms. České Budějovice: Institute of Entomology AS ČR, 50.

Ter Braak, C. J. F.; Šmilauer, P., 1998: CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Ithaca, NY: Microcomputer Power.

Wold, S. J.; Burkness, E. C.; Hutchison, W. D.; Venette, R. C., 2001: In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin. J. Entomol. Sci. **36,** 177–187.

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