Tritrophic Interactions of Transgenic *Bacillus thuringiensis* Corn, *Anaphothrips obscurus* (Thysanoptera: Thripidae), and the Predator *Orius majusculus* (Heteroptera: Anthocoridae)

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ABSTRACT Laboratory feeding experiments using transgenic *Bacillus thuringiensis* variety *kurstaki* (Berliner) corn plants were carried out to study the effects of *B. thuringiensis*-fed herbivorous prey on the predator *Orius majusculus* (Reuter). Host plants were a transgenic *B. thuringiensis*-expressing (Cry1Ab) corn hybrid and the corresponding untransformed isogenic *B. thuringiensis*-free corn hybrid. The herbivorous prey species used in the experiment was *Anaphothrips obscurus* (Müller), a thysanopteran pest of corn, not sensitive to Cry1Ab toxin. The objectives were to quantify the effects of *B. thuringiensis*-fed prey on the development and mortality of immature *O. majusculus*. There was no significant difference in total mean mortality from hatch to adult eclosion between *O. majusculus* nymphs reared on *B. thuringiensis*-fed or *B. thuringiensis*-free prey. Similarly, no significant differences in total developmental time of *O. majusculus* was detected when reared on the two different prey types. Overall mortality was low, confirming that the methodology used was appropriate. We propose this approach as an efficient standardized preregistrational testing for side effects of transgenic plants on small predators such as *Orius* spp.

KEY WORDS *Bacillus thuringiensis*, *Orius majusculus*, transgenic plants, natural enemies, nontarget organisms, standardized testing

ADOPTION OF TRANSGENIC plants in agriculture is proceeding very rapidly. It is predicted that transgenic seed sales will grow at ≈30% per year worldwide, driven by further adoption, new genetic traits, and enhanced crop outputs (Thayer 1999). In 1998, transgenic insect-resistant plants expressing a truncated, modified gene (cry1Ab) from *Bacillus thuringiensis* variety *kurstaki* (Berliner) HD-1 that encodes for the expression of an insecticidal δ-endotoxin were commercially grown on ≈8 million hectares worldwide, almost doubling the production area since 1997 (James 1998). Because transgenic plants are resistant to primary insect pest species, a substantial reduction of pesticide use is expected.

A tremendous influx of *B. thuringiensis*-protein into the agroecosystem is accompanying this large-scale commercial production of transgenic *B. thuringiensis*-plants (cotton, corn, and potato). The *B. thuringiensis*-genes used in the *B. thuringiensis*-crop plants currently commercially available are primarily Cry1Ab and Cry1Ac, two similar proteins that differ somewhat in efficacy towards the target pest insects, *Heliothis virescens* (F.) (tobacco budworm), *Helicoverpa zea* (Boddie) (cotton bollworm), and *Ostrinia nubilalis* (Hübner) (European corn borer) (Höfte and Whiteley 1989, MacIntosh et al. 1990, Van Frankenhuyzen 1993, Gould and Tabashnik 1998). Transgenic plants express the *B. thuringiensis*-proteins in high doses and in most of their tissues throughout the season. This insecticidal toxin will become available to natural enemies in a new and modified form via nonsusceptible or sublethally affected nontarget herbivorous prey feeding on these plants. Herbivores that feed on plant cell contents will most likely ingest *B. thuringiensis* proteins and pass them on to piercing-sucking natural enemies such as hemipteran predators. Therefore, *B. thuringiensis* proteins will become more widely spread in the food web than previously used *B. thuringiensis* insecticides. Because preservation of the predatory fauna associated with crop pests is one of the most important tactics of modern pest management, risk assessment is advisable given the modified form of release of the insecticidal gene product and the extended duration of exposure (compared with *B. thuringiensis* as a conventional insecticide) (Jepson et al. 1994).

Previous studies that examined plant-produced *B. thuringiensis* proteins or their microbially produced equivalents for side-effects on nontarget organisms yielded conflicting results. Some of these studies reported no effects on nontarget organisms (Ahl Goy et al. 1995, Dogan et al. 1996, Pilcher et al. 1997, Riddick and Barbosa 1998), whereas others demonstrated effects on beneficial insects, for example, the predator

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Chrysoperla carnea (Stephens) (Salama et al. 1982; Croft 1990; Hilbeck et al. 1998a, 1998b, 1999). However, the differences in results are probably caused by different methods used in these studies, including varying duration of the experiments, which demonstrates that more research is necessary regarding the use and development of appropriate methods (see Hilbeck et al. 1998b).

The polyphagous predator *Orius majusculus* (Reuter) is a common, native insect species in Europe. Its immatures and adults are natural enemies of thrips, spider mites, white fly larvae, aphids, lepidopteran eggs, and other soft-bodied arthropods (Chambers and Long 1992). *O. majusculus* also feeds on pollen (Bühl and Bässler 1992). Because of its importance in biological control, mainly for thrips control, it has been commercially used for several years. *Anaphothrips obscurus* (Müller) is a pest of corn and other Poaceae (Brohmer et al. 1966). Like other Thysanoptera, they feed on the contents of plant cells. Thysanoptera are of increasing economic importance, and some pest species like *Frankliniella occidentalis* (Pergande), *Thrips tabaci* (Lindeman), or *Taeniothrips inconsequens* (Uzel) are now known from many parts of the world (Moritz 1994). In this study, extended tritrophic feeding experiments were made to assess and quantify the mortality and development of immature *O. majusculus* when reared on *A. obscurus* that were fed transgenic *B. thuringiensis* corn and to develop an efficient method for testing *O. majusculus*—herbivore—plant interactions.

Materials and Methods

Plants. Two corn hybrids were used in the experiments. One was genetically modified corn from Northrup King (Bt11) (Golden Valley, MN) containing a truncated, synthetic version of a gene from *Bacillus thuringiensis* variety *kurstaki* coding for the expression of the insecticidal *B. thuringiensis*-δ-endotoxin Cry1Ab. The second corn hybrid was identical to the previously described one, except it was not genetically modified. The plants were cultivated in plastic pots in greenhouses at temperatures between 20 and 25°C. The leaves of the plants were used in the 7–10 leaf stage. The concentration of Cry1Ab toxin in leaves was ≈3.3 μg/g fresh weight (EPA 1997).

Insect Species. *Herbivores.* The nontarget herbivore *A. obscurus* was used as prey. The thrips used in the experiments occurred naturally on maize plants in the greenhouses of the Federal Research Station. After collecting the herbivores, they were reared in cages on the two corn hybrids in the greenhouse at temperatures between 20 and 25°C.

Predators. Eggs of *O. majusculus* were obtained from Andermatt Biocontrol AG, Grossdietwil, Switzerland and Bioconsulting, Tappernøje, Denmark. The nymphs were allowed to hatch in the same environment chamber as the experiments were carried out.

Tritrophic Feeding Experiments. The experiments were carried out in a controlled environment chamber at fluctuating temperatures (25°C for 10 h during photophase and 20°C for the remaining 14 h) averaging 22°C per day, 70% RH, and a photoperiod of 16:8 (L:D) h.

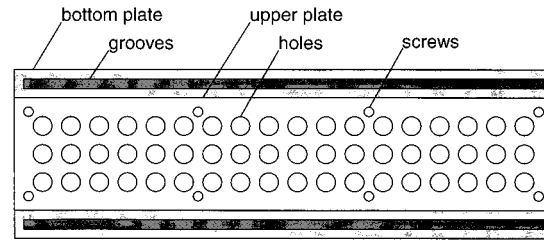

Fig. 1. Experimental cage unit: bottom plate (light gray) with grooves (dark gray), upper plate containing 54 holes (i.e., 54 cages).

Units of special experimental cages (Fig. 1) were manufactured by Isoplex AG, Regensdorf, Switzerland. Each unit consisted of two Plexiglas plates placed on top of each other. The bottom plate (52.6 by 13.9 by 1.9 cm) contained two small grooves running alongside of the plate lengths that were filled with fresh water. The upper plate (52.6 by 10.1 by 1.9 cm) contained 54 holes (1.8 cm diameter) arranged in a 3×18 grid representing 54 small cages (Fig. 1). A thin layer of foam cloth was placed between the two plates to seal the space between the two plates and prevent escape of the insects inside the holes. The tops of the small cages were closed with plastic caps that contained a small hole covered with fine mesh netting to allow for air circulation. Eighteen pieces of transgenic *B. thuringiensis*-maize leaves or nontransgenic maize leaves, respectively (i.e., two treatments), were placed across a three-hole row. The cut leaf ends were placed into the waterfilled grooves of the bottom plate, thereby keeping the leaves fairly fresh for a period of 3 d. Neonate *O. majusculus* nymphs were placed individually into each hole, resulting in 54 individuals per replication and treatment. Number and size of the thrips prey provided was adjusted to the size of the predator. For the *B. thuringiensis*-treatment, *A. obscurus* reared on *B. thuringiensis*-maize were used, and *A. obscurus* reared on *B. thuringiensis*-free maize were supplied in the *B. thuringiensis*-free control treatment. A small piece of rubber tube (0.8 cm diameter by 0.5 cm long) was added into each hole to serve as a shelter for the small *O. majusculus* nymphs. Mortality and immature stage of *O. majusculus* was noted twice a week. After 12 d, the developmental stage of the predators was recorded daily to determine the date of adult eclosion.

The experiment was repeated three times. For the first two runs the number of neonate nymphs was 54 for each treatment (Bt+/Bt−), for the third run the number of neonate nymphs was 28 on *B. thuringiensis*-fed prey and 30 on *B. thuringiensis*-free prey.

Herbivore Bioassays. To determine whether the Cry1Ab toxin was expressed in the transgenic corn leaves used in our experiments, bioassays with *Ostrinia nubilalis* (Hübner), the European corn borer, were conducted. *O. nubilalis* is a target herbivore species and highly susceptible to the Cry1Ab toxin expressed

in transgenic *B. thuringiensis* corn. For each replication and treatment, four neonate larvae were placed into each of 10 vials (1.2 cm diameter by 7.5 cm high). Small pieces of maize leaves (5 cm long) were added. The vials were closed with perforated plastic lids to allow for air circulation. A total of 240 *O. nubilalis* was examined. The bioassays were carried out in the same environment chamber as the tritrophic feeding experiments. After 4 d, the numbers of dead larvae were recorded.

Data Analysis. *Tritrophic Feeding Experiments.* For the statistical analyses of stage-specific and total mortality from egg hatch to adult eclosion, a logistic regression was carried out using the GENMOD procedure of the SAS statistical package including a DSCALE and Type 3 statement producing the appropriate *F*-statistics (SAS Institute 1996). This method accounts for the binomial probability distribution of mortality data. A model was used that tested for significant replication and treatment main effects. In addition, mean mortality and standard errors were determined. Means were compared by carrying out the MEANS procedure and a least significant difference (LSD) test, respectively (SAS Institute 1996).

As an indicator of *O. majusculus* developmental time, the proportion of individuals that had molted to the third, fourth, fifth nymphal stage and to adult on a given check date (day 5, 8, 12, 15 after hatch) was calculated. For the statistical analyses, a one-way analysis of variance (ANOVA) was carried out. A model was used that tested for replication and treatment main effects. Analyses were performed using the general linear models (GLM) procedure of the SAS statistical package (SAS Institute 1996). Also, mean developmental stage and standard errors were determined for both treatments, and means were compared by carrying out the MEANS procedure and a LSD test of the SAS statistical package (SAS Institute 1996).

In addition, total developmental time (days from egg hatch to adult) was analyzed for differences between the two treatments. A regular one-way ANOVA was carried out using the GLM procedure of the SAS statistical package (SAS Institute 1996). Also, mean total developmental time and standard errors were determined for both treatments. Means were compared by carrying out the MEANS procedure and a LSD test of the SAS statistical package (SAS Institute 1996).

Herbivore Bioassays. For the analysis of the herbivore bioassays, a one-way ANOVA was carried out on the arcsine-transformed mortality data of *O. nubilalis.* Because four *O. nubilalis* larvae per vial were used in these assays, the observations were not independent. Therefore, a logistic regression was not appropriate. A model was used that tested for significant replication and treatment main effects. Analyses were performed using the GLM procedure of the SAS statistical package (SAS Institute 1996). In addition, mean mortality and their standard errors were determined. Means were compared by carrying out the MEANS procedure and an LSD test of the SAS statistical package, respectively (SAS Institute 1996).

Results

Mortality. There was no significant difference in mortality between *O. majusculus* nymphs reared on *B. thuringiensis*-fed prey and *O. majusculus* nymphs reared on *B. thuringiensis*-free prey, except during the fourth nymphal stage (N4). Significantly more *O. majusculus* fourth immature stage died when fed with *B. thuringiensis*-free prey compared with those fed with *B. thuringiensis*-fed prey (Fig. 2d) ($F_{\mathrm{Tmt}} = 5.17$; df = 1, 222; $P = 0.024$). Mortality during the first immature stage was very low, increased somewhat during second immature stage, and was low again during third, fourth, and fifth immature stages (Fig. 2a–e).

For the entire immature development from hatch to adult (N1-A), 14.3% of *O. majusculus* nymphs raised on *B. thuringiensis*-fed prey and 16% of *O. majusculus* nymphs raised on *B. thuringiensis*-free prey died (Fig. 2f). This difference was not significant.

Overall levels of mortality varied significantly between replications but remained always quite low, never exceeding 25% (Fig. 2). (N1: $F_{\mathrm{Rep}} = 33.82$; df = 2, 266; $P = 0.0001$; N2: $F_{\mathrm{Rep}} = 9.30$; df = 2, 256; $P = 0.0001$; N3: $F_{\mathrm{Rep}} = 9.51$; df = 2, 235; $P = 0{,}0001$; N4: $F_{\mathrm{Rep}} = 11.33$; df = 2, 222; $P = 0.0001$; N5: $F_{\mathrm{Rep}} = 12.27$; df = 2, 214; $P = 0.0001$; N1-A: $F_{\mathrm{Rep}} = 4.31$; df = 2, 254; $P = 0.0144$).

Development. Generally, *O. majusculus* nymphs reared on *B. thuringiensis*-fed prey developed to the third, fourth, and fifth nymphal stage at similar rates as *O. majusculus* nymphs reared on *B. thuringiensis*-free prey (Table 1). There were significant replication effects at days 12 and 15 (day 12: $F_{\mathrm{Rep}} = 211.00$; df = 2, 5; $P = 0.0047$; day 15: $F_{\mathrm{Rep}} = 26.75$; df = 2, 5; $P = 0.036$).

Similarly, total immature developmental time was not significantly different. *O. majusculus* nymphs reached the adult stage 14.07 ± 0.14 d after egg hatch when reared on *B. thuringiensis*-fed prey and 13.94 ± 0.14 d after hatch when reared on *B. thuringiensis*-free prey. There was a significant replication effect ($F_{\mathrm{Rep}} = 86.96$; df = 2, 212; $P = 0.0001$).

Herbivore Bioassays. Mortality of *O. nubilalis* fed transgenic *B. thuringiensis* corn leaves was significantly higher (96.67 ± 1.58%) than in the control (3.33 ± 1.58%) when *O. nubilalis* were fed nontransgenic maize leaves ($F_{\mathrm{Tmt}} = 946.09$; df = 1, 59; $P = 0.0001$).

Discussion

No lethal or sublethal effects were observed when *O. majusculus* nymphs were reared on *B. thuringiensis*-fed prey compared with the *B. thuringiensis*-free control. Mortality of *O. majusculus* was generally low in both treatments. The significantly higher mortality of the nymphs during fourth nymphal stage when fed with *B. thuringiensis*-free thrips appeared to be negligible because the difference in mortality was small

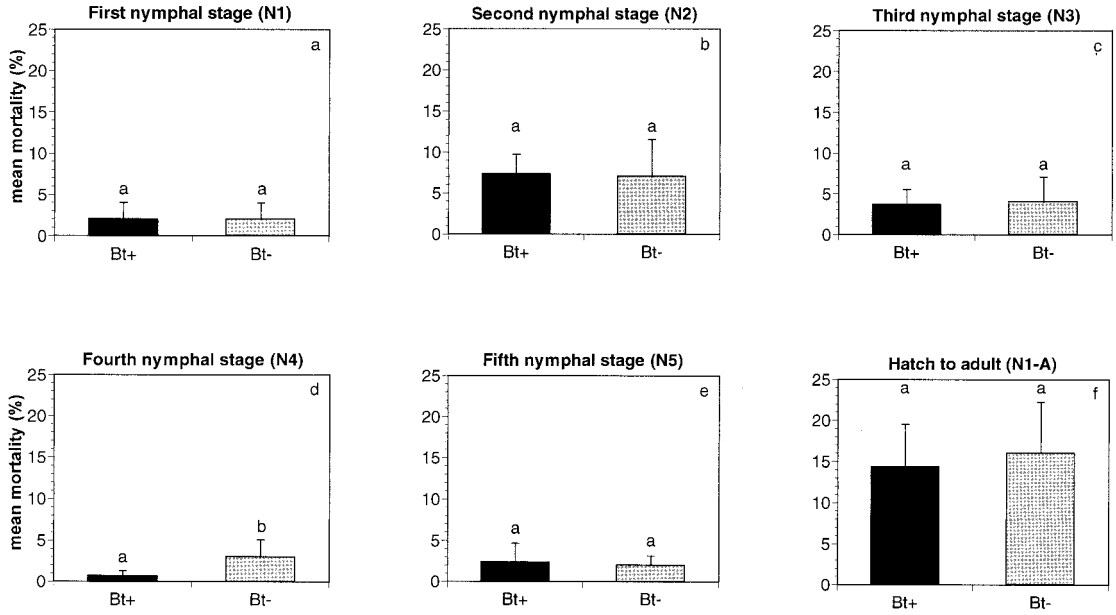

Fig. 2. Stage-specific mean mortality (including ±SE) of immature *O. majusculus* nymphs raised on *A. obscurus* fed transgenic *B. thuringiensis* corn (Bt+) or untransformed *B. thuringiensis*-free corn (Bt−). Columns with different letters represent treatment means that are significantly different at $P = 0.05$ (LSD).

and total mortality from hatch to adult did not differ significantly. There are several possible mechanisms to explain the observed results. First of all, *O. majusculus* could not simply be sensitive to Cry1Ab proteins, either because of lack of receptors or insufficient biochemical processing. Further, the amount of *B. thuringiensis*-protein ingested by the thrips could have been too low to be effective. No data exists on the levels of *B. thuringiensis*-ingestion by nontarget herbivores like the thrips, *A. obscurus*, used in this study.

There was also no significant difference in developmental time of *O. majusculus* on both treatments. Total developmental time observed in our study was 13.9 and 14.1 d for *O. majusculus* reared on *B. thuringiensis*-free and *B. thuringiensis*-fed thrips, respectively. This is in agreement with data by Husseini et al. (1993), who reported a developmental time for *O. majusculus* of ≈15.2 ± 1.2 d at 25.5 ± 0.5°C when fed with thrips of the species *F. occidentalis.*

Table 1. Mean percentage (%) (±SE) of immature *O. majusculus* that developed to the third nymphal stage (N3), fourth nymphal stage (N4), fifth nymphal stage (N5), and adult (A) on days 5, 8, 12, and 15 after hatch when reared on *B. thuringiensis* (Bt+)-fed prey or *B. thuringiensis* (Bt-)-free prey

Days	Prey	
	B.t.-fed (Bt+)	*B.t.*-free (Bt-)
N3 after 5 d	83.3 ± 7.8a	85.3 ± 1.7a
N4 after 8 d	78.7 ± 13.0a	87.0 ± 8.5a
N5 after 12 d	88.7 ± 9.4a	90.0 ± 10.0a
A after 15 d	74.7 ± 22.9a	83.3 ± 15.9a

Means with the same letters within rows (*B. thuringiensis*-fed versus *B. thuringiensis*-free) are not significantly different at $P < 0.05$ significance level (LSD). Data represent the mean of three replications.

Also Pilcher et al. (1997) reported no significant difference in survival and developmental time of the related species *Orius insidiosus* (Say) when reared on *B. thuringiensis* corn pollen. However, the prolonged developmental time and the low survival rate of the predators in their experiments indicate that corn pollen was a suboptimal food source (see Richards and Schmidt 1996). Although some predator species, including *Orius* spp., also occasionally feed on pollen, this type of diet serves primarily as a supplement to their otherwise carnivorous diet. Therefore, to test for long-term effects of plant compounds present in leaf tissues at high concentrations, tritrophic trials seem to be the most realistic route of exposure. The low mortality rates and short developmental time observed in our studies for both the control and the *B. thuringiensis*-treatment suggested that corn-fed *A. obscurus* thrips were an optimal prey for *O. majusculus.* It also demonstrated that the methodology used was appropriate. The efficient arrangement allowed testing of a large number of predator individuals during their entire immature development. Therefore, this methodology could be proposed as a standardized testing procedure for preregistrational testing of side effects of transgenic plants on this important biocontrol organism.

Because of the extended expression of the *B. thuringiensis* proteins in transgenic crops the debate about resistance of target insects is of great concern. One resistance management strategy is to develop transgenic crop plants with very high levels of *B. thuringiensis* expression combined with refuges planted with the nontransgenic crop variety (Mellon and Rissler 1998). Therefore, the current trend in

plant molecular biology is to increase expression levels of *B. thuringiensis* proteins in plants through chloroplast transformation (McBride et al. 1995; Kota et al. 1999). Using this technology, McBride et al. (1995) reported that an unprecedented 3–5% of total soluble protein in tobacco leaves was Cry1Ac protein. If chloroplast-transformed plants expressing *B. thuringiensis* proteins at such high levels become commercially available, their compatibility with important biocontrol organisms such as *Orius* spp. will have to be verified again.

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References Cited

Ahl Goy, P., G. Warren, J. White, L. Privalle, P. Fearing, and D. Vlachos. 1995. Interaction of an insect tolerant maize with organisms in the ecosystem, pp. 50–53. *In* Proceedings, Key Biosafety Aspects of Genetically Modified Organisms, 10–11 April 1995, Braunschweig, Germany. Blackwell, Berlin.

Brohmer, P., P. Ehrmann, and G. Ulmer (Hrsg.). 1966. Die Tierwelt Mitteleuropas. Insekten 1: Teil. Thysanoptera. Lief.2 (Heft IX). Quelle & Meyer, Leipzig.

Bühl, R., and R. Bässler. 1992. Biologische Bekämpfung von Thrips, Blattläusen und Miniерfliegen. Gemüse 28(3): 155–158.

Chambers, R. J., and S. Long. 1992. New predators for biocontrol under glass. Phytoparasitica 20(suppl.): 57S–60S.

Croft, B. A. 1990. Arthropod biological control agents and pesticides. Wiley, New York.

Dogan, E. B., R. E. Berry, G. L. Reed, and P. A. Rossignol. 1996. Biological parameters of convergent lady beetle (Coleoptera: Coccinellidae) feeding on aphids (Homoptera: Aphididae) on transgenic potato. J. Econ. Entomol. 89: 1105–1108.

[EPA] Environmental Protection Agency. 1997. Pesticide fact sheet: *Bacillus thuringiensis* CryIA(b) delta-endotoxin and the genetic material necessary for its production in corn (http://www.epa.gov/docs/fedrgstr/EPA-PEST/1997/September/Day-10/bac.htm).

Gould, F., and B. Tabashnik. 1998. Bt-cotton resistance management, pp. 65–105. *In* M. Mellon and J. Rissler 1998 [eds.], Now or never. Serious new plans to save a natural pest control. Union of Concerned Scientists (UCS), Cambridge, MA.

Hilbeck, A., M. Baumgartner, P. Fried, and F. Bigler. 1998a. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and developmental time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27: 480–487.

Hilbeck, A., W. J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1998b. Toxicity of the *Bacillus thuringiensis* Cry1Ab toxin on the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae) using diet incorporated bioassays. Environ. Entomol. 27: 1255–1263.

Hilbeck, A., W. J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea.* Entomol. Exp. Appl. 91: 305–316.

Höfte, H., and H. R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis.* Microbiol. Rev. 53: 242–255.

Husseini, M., K. Schumann, and H. Sermann. 1993. Rearing immature feeding stage of *Orius majesculus* Reut. (Het., Anthocoridae) on the acarid mite *Tyrophagus putriscentia* Schr. as new alternative prey. J. Appl. Ent. 116: 113–117.

James, C. 1998. Global review of commercialized transgenic crops: 1998. ISAAA Briefs No. 8. ISAAA, Ithaca, NY.

Jepson, P. C., B. A. Croft, and G. E. Pratt. 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol. Ecol. 3: 81–89.

Kota, M., H. Daniell, S. Varma, S. F. Garczynski, F. Gould, and W. Moar. 1999. Overexpression of the *Bacillus thuringiensis* (Bt) Cry2Aa2 protein in chloroplasts confers resistance to plants against susceptible and Bt-resistant insects. Proc. Natl. Acad. Sci. U.S.A. 96: 1840–1845.

McBride, K. E., Z. Svab, D. J. Schaaf, P. S. Hogan, D. M. Stalker, and P. Maliga. 1995. Amplification of a chimeric *Bacillus* gene in chloroplasts leads to an extraordinary level of an insecticidal protein in tobacco. Biotechnology 13: 362–365.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischhoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebr. Pathol 56: 258–266.

Mellon, M., and J. Rissler. 1998. Now or Never. Serious new plans to save a natural pest control. Union of Concerned Scientists (UCS), Cambridge, MA.

Moritz, G. 1994. Pictorial key to the economically important species of Thysanoptera in central Europe. EPPO Bull. 24: 181–208.

Pilcher, C. D., J. J. Obrycki, M. E. Rice, and L. C. Lewis. 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environ. Entomol. 26: 446–454.

Richards, P. C., and J. M. Schmidt. 1996. The effects of selected dietary supplements on survival and reproduction of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Can. Entomol. 128: 171–176.

Riddick, E. W., and P. Barbosa. 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.

Salama, H. S., F. N. Zaki, and A. F. Sharaby. 1982. Effect of *Bacillus thuringiensis* Berl. on parasites and predators of the cotton leafworm *Spodoptera littoralis* (Boisd.). Z. Angew. Entomol. 94: 498–504.

SAS Institute. 1996. SAS/STAT software: changes and enhancements through release 6.11. SAS Institute, Cary, NC.

Thayer, A. M. 1999. Transforming agriculture. Chem. Eng. News 77(19 April): 21–35.

Van Frankenhuyzen, K. 1993. The challenge of *Bacillus thuringiensis,* pp. 1–23. *In* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds.], *Bacillus thuringiensis,* an environmental biopesticide: theory and practice. Wiley, Chichester, UK.

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