Transgenic Res (2016) 25:33–44
DOI 10.1007/s11248-015-9917-1

ORIGINAL PAPER

The interaction of two-spotted spider mites, *Tetranychus urticae* Koch, with Cry protein production and predation by *Amblyseius andersoni* (Chant) in Cry1Ac/Cry2Ab cotton and Cry1F maize

Yan-Yan Guo · Jun-Ce Tian · Wang-Peng Shi · Xue-Hui Dong · Jörg Romeis · Steven E. Naranjo · Richard L. Hellmich · Anthony M. Shelton

Received: 27 June 2015 / Accepted: 31 October 2015 / Published online: 6 November 2015

Abstract Crops producing insecticidal crystal (Cry) proteins from the bacterium, *Bacillus thuringiensis* (Bt), are an important tool for managing lepidopteran pests on cotton and maize. However, the effects of these Bt crops on non-target organisms, especially natural enemies that provide biological control services, are required to be addressed in an environmental risk assessment. *Amblyseius andersoni* (Acari: Phytoseiidae) is a cosmopolitan predator of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), a significant pest of cotton and maize. Tri-trophic studies were conducted to assess the potential effects of Cry1Ac/Cry2Ab cotton and Cry1F maize on life history parameters (survival rate, development time, fecundity and egg hatching rate) of *A. andersoni.* We confirmed that these Bt crops have no effects on the biology of *T. urticae* and, in turn, that there were no differences in any of the life history parameters of *A. andersoni* when it fed on *T. urticae* feeding on Cry1Ac/Cry2Ab or non-Bt cotton and Cry1F or non-Bt maize. Use of a susceptible insect assay demonstrated that *T. urticae* contained biologically active Cry proteins. Cry proteins concentrations declined greatly as they moved from plants to herbivores to predators and protein concentration did not appear to

Electronic supplementary material The online version of this article (doi:10.1007/s11248-015-9917-1) contains supplementary material, which is available to authorized users.

Y.-Y. Guo · A. M. Shelton (✉)
Department of Entomology, Cornell University, New York State Agricultural Experiment Station (NYSAES), Geneva, NY, USA
e-mail: ams5@cornell.edu

Y.-Y. Guo · W.-P. Shi
Department of Entomology, China Agricultural University, Beijing, China

J.-C. Tian
State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China

X.-H. Dong
Department of Agriculture Science, China Agricultural University, Beijing, China

J. Romeis
Agroscope, Institute for Sustainability Sciences ISS, Zurich, Switzerland

S. E. Naranjo
Arid-Land Agricultural Research Center, USDA-ARS, Maricopa, AZ 85138, USA

R. L. Hellmich
Corn Insects and Crop Genetics Research Unit, USDA-ARS, Ames, IA 50011, USA

R. L. Hellmich
Department of Entomology, Iowa State University, Ames, IA 50011, USA

be related to mite density. Free-choice experiments revealed that *A. andersoni* had no preference for Cry1Ac/Cry2Ab cotton or Cry1F maize-reared *T. urticae* compared with those reared on non-Bt cotton or maize. Collectively these results provide strong evidence that these crops can complement other integrated pest management tactics including biological control.

Keywords Tri-trophic exposure · Cry1Ac · Cry2Ab · Cry1F · Environmental risk assessment · Biological control

Introduction

Genetically engineered (GE) crops have been planted since 1995 and, in 2014, 18 million farmers in 28 countries planted GE crops (James 2014). Of the total 181.5 million ha of GE crops planted in 2014, 78.8 million ha were planted with insect-resistant varieties producing Cry proteins derived from *Bacillus thuringiensis* Berliner (Bt). Cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.) are important crops worldwide that are attacked by a complex of pest Lepidoptera (Naranjo et al. 2008; Hellmich et al. 2008). In the United States, more than 75 % of the land planted to each of these two crops utilizes Bt technology (Fernandez-Maizeejo et al. 2014). The primary Bt proteins utilized for control of Lepidoptera in maize are Cry1Ab and Cry1F and in cotton Cry1Ac and Cry2Ab.

In agricultural ecosystems, arthropods provide important ecological functions that can be disrupted by pest management practices. The use of Bt crops may have direct or indirect impact on non-target arthropods (NTAs) that may interfere with important functions such as biological control (Kennedy 2008; Romeis et al. 2008a). The risk that GE crops pose to valued NTAs and the functions that they provide are addressed in an environmental risk assessment that precedes the commercialization of any new GE crop (Romeis et al. 2008b). Although most studies have reported no unexpected and unacceptable adverse impact of Bt crops on NTAs (e.g., Romeis et al. 2006; Wolfenbarger et al. 2008; Naranjo 2009; Comas et al. 2014), concerns still persist and influence regulatory decisions (Romeis et al. 2013).

Amblyseius andersoni (Chant) (Acari: Phytoseiidae) is an important predator species found in many crops and countries worldwide (McMurtry 1982). Both nymphs and adults of *A. andersoni* are predaceous, feeding on various mite species (Amano and Chant 1977, 1978), thrips (van der Linden 2004), and pollen (Tsolakis and Ragusa di Chiara 1994). Thus, *A. andersoni* can be exposed to Bt proteins directly (through herbivory) or indirectly (through predation) when feeding in Bt crops.

Tri-trophic studies that aim to assess the impact of plant-produced Cry proteins on predators or parasitoids carry the risk that the plant-reared herbivores used as prey or hosts are themselves affected by the test substance. This could lead to reduced quality in these prey or hosts and consequently cause an effect on the natural enemy. Such so-called "prey-quality-mediated effects" have been observed in many tri-trophic studies with Bt crops (Romeis et al. 2006; Naranjo 2009) and have sometimes been misinterpreted as direct toxic effects of the Bt proteins under consideration (Lövei et al. 2009; but see the responses by Shelton et al. Shelton et al. 2009a, b, 2012). One way to eliminate these prey-quality-mediated effect is to use herbivores that have evolved resistance to the Bt proteins (Ferry et al. 2006; Chen et al. 2008; Lawo et al. 2010; Li et al. 2011; Tian et al. 2012, 2013, 2014a, b; Su et al. 2015) or species that contain the Bt proteins but are not susceptible to them (Bernal et al. 2002; Dutton et al. 2002; Bai et al. 2006; Meissle and Romeis 2009a; Li and Romeis 2010; Álvarez-Alfageme et al. 2008, 2011; García et al. 2010, 2012). In this way, the natural enemies can be exposed to actual levels of Bt proteins but not suffer from any prey-quality-mediated effects that would interfere with the assessment of direct Cry proteins effects.

Cry protein concentration in Bt crops is affected by crop variety and stage (Adamczyk and Sumerford 2001; Nguyen and Jehle 2007) as well as many abiotic factors, including light intensity (Dong and Li 2007), soil salinity (Luo et al. 2008), temperature (Zhou et al. 2009), and water availability (Benedict et al. 1996; Luo et al. 2008). Few studies have investigated whether herbivores affect Cry protein concentration in Bt plants. Olsen et al. (2005) observed that the effectiveness of Bt cotton against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) was increased by a factor of 4–15 when plants were injured by caterpillars. This increased efficacy, however, was not due to

changes in the Cry protein concentration but due to the induction of other cotton defense compounds. This fact was later confirmed for Bt cotton plants that displayed increased efficacy against *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) after induction with jasmonic acid (Mészáros et al. 2011). Recently, Prager et al. (2014) suggested that Cry1Ab and Cry3Bb1 concentrations decrease when maize plants were infested with *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) (which is suggested to be a synonym of *Tetranychus urticae* Koch; Auger et al. 2013). Unfortunately, the data are difficult to interpret and compare to previously published data because Cry protein content was only shown as absorbance values from the ELISA assay. The authors did not compile standard curves and express the Cry protein levels as μg/g leaf material.

We conducted tri-trophic studies to assess the potential impact of Cry1Ac/Cry2Ab cotton and Cry1F maize on *A. andersoni* by using the two-spotted spider mite, *T. urticae*, as a Bt protein carrier. *T. urticae* are important secondary pests on cotton and maize, and they can undermine the economic benefits of Bt crops (Archer and Bynum 1993; Reddall et al. 2004; Wilson 1993). Furthermore, we conducted a study to confirm that the quality of *T. urticae* was not affected by Cry1Ac/Cry2Ab cotton and Cry1F maize to eliminate possible prey-quality-mediated effects. Additionally, we quantified Cry protein levels in Cry1Ac/Cry2Ab cotton and Cry1F maize leaves with and without different levels of *T. urticae* infestation over time to determine whether the presence of the herbivore affects Cry protein concentrations. Lastly, we studied the preference of *A. andersoni* for *T. urticae* reared on Cry1Ac/Cry2Ab cotton or non-Bt cotton and Cry1F maize or non-Bt maize.

Materials and methods

Plants

Seeds of Bt cotton (BollGard II®, event 15895), which has genes coding for Cry1Ac and Cry2Ab, and the corresponding non-transformed near-isoline Stoneville 474, were obtained from Monsanto Company (St. Louis, MO). The Cry1Ac/Cry2Ab cotton and non-Bt cotton were grown in 6 L plastic pots with Cornell Mix potting soil (Boodley and Sheldrake 1977). Approximately 6 g Osmocote® Plus release fertilizer (Scotts, Marysville, OH) was placed in each pot and 500 ml Power-Gro liquid fertilizer (Wilson Laboratories Inc., Dundas, Ontario, Canada) was applied weekly. All plants were grown in the same greenhouse at 27 ± 2 °C with a photoperiod of 16L:8D.

Seeds of Bt maize (Mycogen 2A517), producing Cry1F, and the corresponding non-Bt near-isoline (Mycogen 2A496) were obtained from Dow AgroSciences (Indianapolis, IN). The Cry1F maize and non-Bt maize were both grown in Ray Leach Cone-tainer Cells (diameter 3.8 cm; depth 21 cm; volume 164 ml) (Stuewe & Sons, Tangent, OR) with Cornell Mix potting soil and 500 ml Power-Gro liquid fertilizer was applied weekly. All maize plants were grown in the same greenhouse at 21 ± 2 °C under a 16L:8D regime.

Seeds of dry Roman beans (*Phaseolus vulgaris* L.) were obtained from Goya (Secaucus, NJ). They were grown in a climatic chamber at 27 ± 1 °C, 50 ± 10 % relative humidity (RH) with a photoperiod of 16L:8D.

Insects

The *T. urticae* colony was collected in greenhouses at Cornell University's New York State Agricultural Experiment Station and reared for multiple generations on green beans (*P. vulgaris* L.) and was never exposed to Bt proteins.

The predator, *A. andersoni*, was obtained from Green Spot Ltd. (Notthingham, NH) in 2013 and maintained in our laboratory on green beans infested with *T. urticae*.

A Bt-susceptible strain of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), which was used to assess the bioactivity of Bt proteins, has been reared on artificial diet since 1988 (Shelton et al. 1991).

All the insects were maintained in a climatic chamber at 27 ± 1 °C, 50 ± 10 % RH and a 16L:8D photoperiod. All the experiments were conducted under these conditions as well.

Effects of Cry1Ac/Cry2Ab cotton and Cry1F maize on *T. urticae*

Newly hatched *T. urticae* were reared individually in 1.5 ml tubes on Bt or non-Bt cotton or maize leaves.

For cotton, the 3rd or 4th leaf (counting from base of plant) of a 5-leaf stage cotton plant was used and for maize the 5th or 6th leaf of a 7-leaf stage maize plant was used. Leaves were changed every other day and the mites were checked twice a day. The survival and development times of larvae, protonymphs, deutonymphs and adults were recorded. In total, 30 individuals were tested for each of the four treatments.

For assessing fecundity, ten pairs of newly hatched *T. urticae* adults from each treatment were kept in individual 1.5 ml tubes and allowed to mate. The number of eggs laid throughout adult life and adult longevity were recorded daily; eggs were removed daily.

To measure egg-hatching rates, 30 eggs from each treatment were selected randomly and monitored until the eggs hatched. Three replications, each with 30 eggs, were utilized. Eggs were collected from 5 to 7 day old *T. urticae* adults over 3 days.

Bioactivity of Bt proteins after ingestion by *T. urticae*

T. urticae that had fed on their respective plant types for ca. 20 days and used in the bioassays were collected and washed with PBST buffer five times, then crushed and diluted at a rate of 1:10 (mg sample: μL dH2O). Bond-spreader sticker (Loveland Industry, Loveland Co) was added at 0.1 % to each sample solution before being applied to cabbage leaf disks (diameter 3 cm). Ten 2nd instars of Bt-susceptible *P. xylostella* were placed on each leaf disk inside a 30-ml cup. Larval mortality was assessed after 72 h at 27 ± 1 °C. The experiment was conducted with five replications per treatment.

Preference of *A. andersoni* for *T. urticae* that have fed on Bt and non-Bt cotton or maize leaves

To examine predator preference for *T. urticae* reared on either Bt or non-Bt plants (Bt or non-Bt mites, respectively), we conducted a free-choice experiment with adult *A. andersoni* starved for 24 h prior to the experiments. This experiment was conducted in the lid of a 1.5 ml centrifuge tube covered with plastic wrap. One adult Bt and one adult non-Bt *T. urticae* that had fed on Bt or non-Bt plants for 10 days, respectively, were randomly dyed with blue or red fluorescent dyes in a 1.5 ml tube to differentiate each *T. urticae* type placed in the container. The first type, as identified by color, of *T. urticae* consumed by *A. andersoni* was recorded. The predator *A. andersoni* usually consumed the first *T. urticae* within 10 min after which the observation was terminated. The maximum observation time was set at 1 h. A total of 50 replications were conducted for both cotton and maize.

Prey-mediated effects of Cry1Ac/Cry2Ab cotton on *A. andersoni*

Newly-hatched larval *A. andersoni* were transferred to a fresh Bt or non-Bt cotton leaf disk (30 mm in diameter) infested with *T. urticae* that were placed on a water-saturated sponge in a Petri dish (90 mm in diameter). Leaf disks were changed daily and *A. andersoni* were checked twice daily (8 a.m. and 8 p.m.). Survival and development time of larvae, protonymph, deutonymph and adult were recorded. The experiment started with 30 *A. andersoni* larvae for each treatment.

Fecundity and egg hatching rates were assessed as described above. *A. andersoni* adults were placed in a 90 mm Petri dish with a fresh Bt or non-Bt cotton leaf disk infested with *T. urticae*.

The offspring (F2 of *A. andersoni*) underwent another generation of testing, as described above.

Prey-mediated effects of Cry1F maize on *A. andersoni*

The experiments were conducted as described above but using disks from Cry1F maize and non-Bt maize leaves.

Bt protein levels in Bt crops, *T. urticae* and *A. andersoni*

Three samples of Bt and non-Bt crop leaves (10 mg per replicate) were collected. For cotton, the 3rd or 4th leaf of a 5-leaf stage cotton plant was used and for maize the 5th or 6th leaf of a 7-leaf stage maize plant was used. In order to provide *A. andersoni* with *T. urticae* with a high Bt protein dose, we determined the Bt protein residue in different nymphal stages of *T. urticae*. Three samples (5–10 mg fresh weight as one replicate) from each nymph stage were collected and ground by hand using a plastic pestle. Three samples (5–10 mg fresh weight as one replicate) from each treatment were collected when *A. andersoni* reached the deutonymph

stage. Prior to assay, all insects were washed five times with phosphate-buffered saline with Tween 20 (PBST) buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, 0.05 % Tween-20, pH 7.4) to remove any Bt protein from the surface. Leaf samples were diluted at a rate of 1:1000 (mg sample: µL PBST buffer) and ground with a mortar and pestle. Insect samples were diluted at a rate of 1:10 (mg sample: µL PBST buffer) in 1.5 ml centrifuge tubes and ground by hand using a plastic pestle. The Bt protein concentrations in the samples were determined by ELISA using Cry1Ac (Catalog # PSP 06200) and Cry1F (Catalog # PSP 11700) detection kits from Agdia (Elkhart, IN), and Cry2Ab (Catalog # AP 005) detection kits from EnviroLogix (Portland, ME). ELISA was performed following the manufacturer's instructions. Because Cry1Ac protein of known purity was not provided with the detection kit, we obtained 1 mg (purity 94–96 %) from Marianne Pusztai-Carey (Department of Biochemistry, Case Western Reserve University, Cleveland, OH) for calibration.

Bt protein levels in Bt crops after infestation with *T. urticae*

Zero, 10, 50, or 100 *T. urticae* larvae were transferred to the 3rd leaf of a five-leaf stage Cry1Ac/Cry2Ab or non-Bt cotton, with three replications for each treatment. Plastic plant bags (length: 30 cm, width: 20 cm) were used to isolate mites on plants. Leaf samples were collected from the leaves colonized by the mites and non-Bt cotton leaves randomly on the 0 (before infestation), 4th, and 8th day after infestation with *T. urticae*. ELISA was performed on three replications (plant samples) to detect the quantity of Cry1Ac/Cry2Ab in the three types of cotton (Bt infested, Bt uninfested, non-Bt uninfested).

For maize, we used the same methods described for cotton but transferred *T. urticae* to the 6th leaf of a seven-leaf stage maize.

Statistical analysis

Prior to analysis, all percentage data were arcsine or square-root transformed as necessary, but untransformed means are presented. Data on survival of *T. urticae* and *A. andersoni* were analyzed using Log-Rank test for homogeneity. Data on other life table parameters of *T. urticae* and *A. andersoni* were analyzed using Student's *t* test. Bt protein residue in tissues of the plant or arthropod and bioactivity of Bt proteins were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple-range test, as appropriate. Predator preference was analyzed using Chi-square test. All these data analyses were performed in SPSS 18.0 Windows (SPSS 1988). Bt protein levels in Bt crops after infestation with *T. urticae* were analyzed using repeated-measures ANOVA and Tukey's multiple-range test (SAS Institute, Cary, NC). Statistical threshold for significance was 0.05 for all tests.

Results

Effects of Cry1Ac/Cry2Ab cotton and Cry1F maize on *T. urticae*

Newly-hatched *T. urticae* were provided with Bt plant leaves (Cry1Ac/Cry2Ab cotton or Cry1F maize) or the corresponding non-Bt plant leaves. No significant differences were detected for any life table parameter of *T. urticae* (survival rate, development time, fecundity and egg hatching rate) between Bt (Cry1Ac/Cry2Ab cotton or Cry1F maize) and non-Bt treatments (Tables 1, 2).

Bioactivity of Bt proteins after ingestion by *T. urticae*

In order to examine the bioactivity of Bt proteins after ingestion by *T. urticae*, Bt plant-fed and non-Bt plant-fed *T. urticae* were collected. Extracts of *T. urticae* that had fed on Cry1Ac/Cry2Ab cotton or Cry1F maize plants were toxic to Bt-susceptible *P. xylostella* larvae, indicating that the predator *A. andersoni* was exposed to biologically active Bt proteins in the tri-trophic bioassays (Table 3).

Preference of predator, *A. andersoni*, for *T. urticae* that have fed on Cry1Ac/Cry2Ab and non-Bt cotton or Cry1F and non-Bt maize

In free-choice experiments, *A. andersoni* showed no preference for Bt or non-Bt plant fed *T. urticae* they consumed (cotton: $\chi^2 = 0.08$; df = 1; $P = 0.78$; maize: $\chi^2 = 0.08$; df = 1; $P = 0.78$). This indicates that the tested Cry1Ac/Cry2Ab cotton and Cry1F maize did not affect the predator's choice of prey.

Table 1 Life table parameters (mean ± SE) of *Tetranychus urticae* when fed Cry1Ac/Cry2Ab or non-Bt near-isoline cotton leaves

Parameters	Non-Bt cotton	Cry1Ac/Cry2Ab cotton	Statistical analysis
Survival (%)[a]	86.67 (30)	90.00 (30)	$\chi^2 = 0.007$; df = 1; $P = 0.93$
Larval stage (days)[b]	2.36 ± 0.08 (29)	2.30 ± 0.12 (28)	$t = 0.40$; df = 55; $P = 0.69$
Protonymph stage (days)[b]	2.28 ± 0.08 (27)	2.27 ± 0.14 (26)	$t = 0.054$; df = 51; $P = 0.96$
Deutonymph stage (days)[b]	2.31 ± 0.10 (27)	2.37 ± 0.18 (26)	$t = -0.25$; df = 51; $P = 0.81$
Adult stage (days)[b]	22.26 ± 0.43 (27)	22.06 ± 0.63 (26)	$t = 0.27$; df = 51; $P = 0.79$
Total fecundity[b]	98.00 ± 3.83 (10)	94.40 ± 4.28 (10)	$t = 0.64$; df = 18; $P = 0.53$
Egg hatching rate (%)[b]	86.67 ± 4.41 (3)	85.00 ± 2.89 (3)	$t = 0.32$; df = 4; $P = 0.77$

Numbers of replications is given in parentheses

[a] Log-Rank test ($P < 0.05$)

[b] Student's t test ($P < 0.05$)

Table 2 Life table parameters (mean ± SE) of *Tetranychus urticae* when fed Cry1F or non-Bt near-isoline maize leaves

Parameters	Non-Bt maize	Cry1F maize	Statistical analysis
Survival (%)[a]	83.33 (30)	90.00 (30)	$\chi^2 = 0.45$; df = 1; $P = 0.51$
Larval stage (days)[b]	2.25 ± 0.10 (28)	2.29 ± 0.10 (28)	$t = -0.25$; df = 54; $P = 0.81$
Protonymph stage (days)[b]	2.26 ± 0.11 (27)	2.15 ± 0.12 (27)	$t = 0.70$; df = 52; $P = 0.49$
Deutonymph stage (days)[b]	2.26 ± 0.12 (25)	2.15 ± 0.16 (27)	$t = 0.57$; df = 50; $P = 0.58$
Adult stage (days)[b]	21.90 ± 0.50 (25)	20.91 ± 0.89 (27)	$t = 0.95$; df = 50; $P = 0.35$
Total fecundity[b]	89.10 ± 3.71 (10)	84.80 ± 3.31 (10)	$t = 0.86$; df = 18; $P = 0.40$
Egg hatching rate (%)[b]	86.67 ± 3.33 (3)	85.00 ± 2.89 (3)	$t = 0.38$; df = 4; $P = 0.73$

Numbers of replications is given in parentheses

[a] Log-Rank test ($P < 0.05$)

[b] Student's t test ($P < 0.05$)

Table 3 Bioactivity of Bt protein residues in *Tetranychus urticae* reared on Cry1Ac/Cry2Ab cotton or Cry1F maize for 48 h to Bt-susceptible *Plutella xylostella* larvae

Treatment	Mortality % (mean ± SE)
T. urticae reared on Cry1 Ac/Cry2Ab cotton leaf	48.0 ± 3.7b
T. urticae reared on non-Bt cotton leaf	10.0 ± 3.2a
T. urticae reared on Cry1F maize leaf	60.0 ± 4.5b
T. urticae reared on non-Bt maize leaf	8.0 ± 3.7a
dH_2O (control)	6.0 ± 2.5a
Statistical analysis	$F_{4,20} = 51.2$; $P < 0.001$

Larval mortality was assessed after 72 h

Means followed by different letters are significantly different (One-way ANOVA, $P < 0.05$), n = 5

Prey-mediated effects of Cry1Ac/Cry2Ab cotton on *A. andersoni*

No significant difference was detected for any *A. andersoni* life table parameter (survival rate, development time, fecundity and egg hatching rate) when fed with *T. urticae* reared on Bt or non-Bt cotton over two generations (Table 4).

Prey-mediated effects of Cry1F maize on *A. andersoni*

No significant difference was detected for any *A. andersoni* life table parameter (survival rate, development time, fecundity and egg hatching rate) when fed with *T. urticae* reared on Cry1F maize or non-Bt maize over two generations (Table 5).

Table 4 Tri-trophic effects on life table parameters (mean ± SE) of *Amblyseius andersoni* when fed *Tetranychus urticae* that were reared on Cry1Ac/Cry2Ab-expressing cotton leaves or non-Bt near-isoline cotton leaves over two generations

Parameters	Non-Bt cotton	Cry1Ac/Cry2Ab cotton	Statistical analysis
1st generation			
Survival (%)[a]	90.00 (30)	86.67 (30)	$\chi^2 = 0.017$; df = 1; $P = 0.90$
Larval stage[b]	1.59 ± 0.07 (29)	1.47 ± 0.07 (29)	$t = 1.22$; df = 56; $P = 0.23$
Protonymph stage[b]	1.93 ± 0.07 (27)	1.96 ± 0.08 (26)	$t = -0.33$; df = 51; $P = 0.74$
Deutonymph stage[b]	2.13 ± 0.07 (27)	1.98 ± 0.07 (26)	$t = 1.51$; df = 51; $P = 0.14$
Adult stage[b]	35.59 ± 1.28 (26)	35.81 ± 1.35 (26)	$t = -0.12$; df = 51; $P = 0.91$
Total fecundity[b]	32.10 ± 1.21 (10)	31.20 ± 1.05 (10)	$t = 0.56$; df = 18; $P = 0.58$
Egg hatching rate (%)[b]	84.44 ± 4.84 (3)	83.33 ± 1.92 (3)	$t = 0.21$; df = 4; $P = 0.84$
2nd generation			
Survival (%)[a]	90.00 (30)	86.67 (30)	$\chi^2 = 0.25$; df = 1; $P = 0.62$
Larval stage[b]	1.64 ± 0.06 (28)	1.66 ± 0.07 (29)	$t = -0.13$; df = 55; $P = 0.90$
Protonymph stage[b]	2.15 ± 0.07 (27)	1.98 ± 0.07 (27)	$t = 1.65$; df = 52; $P = 0.11$
Deutonymph stage[b]	1.94 ± 0.07 (27)	2.12 ± 0.06 (26)	$t = -1.77$; df = 51; $P = 0.08$
Adult stage[b]	33.63 ± 1.22 (27)	33.88 ± 1.28 (26)	$t = -0.14$; df = 51; $P = 0.89$
Total fecundity[b]	31.10 ± 1.05 (10)	30.10 ± 1.08 (10)	$t = 0.67$; df = 18; $P = 0.52$
Egg hatching rate (%)[b]	80.00 ± 1.92 (3)	81.11 ± 4.84 (3)	$t = -0.21$; df = 4; $P = 0.84$

Number of replications is given in parentheses

[a] Log-Rank test ($P < 0.05$)

[b] Student's t test ($P < 0.05$)

Bt protein levels in Bt crops, *T. urticae* and *A. andersoni*

Cry1Ac/Cry2Ab cotton leaves contained high levels of Cry1Ac and Cry2Ab proteins (Table 6). When averaged over all life stages, *T. urticae* contained ≈36-fold lower levels of Cry1Ac and 27-fold lower levels of Cry2Ab compared with Cry1Ac/Cry2Ab cotton leaves. Average concentration of Cry1Ac and Cry2Ab proteins in *A. andersoni* were 18- and 21-fold lower, respectively, than those in *T. urticae*.

Similar results were found for Cry1F maize (Table 6). Cry1F protein levels in *T. urticae* were ≈39-fold lower than those in Cry1F maize leaves. The average concentration of Cry1F proteins in *A. andersoni* were 24-fold lower compared with *T. urticae*.

No Bt proteins were detected in non-Bt crops, prey fed on non-Bt crops, or predators fed on prey from non-Bt crops.

Bt protein levels in Bt crops after infestation with *T. urticae*

On the 0, 4th and 8th day after infestation with *T. urticae*, leaf samples were collected from Cry1Ac/Cry2Ab cotton and Cry1F maize plants to detect the Bt protein changes. Concentration levels of Cry1F, Cry1Ac and Cry2Ab proteins in Bt plants varied on the 0, 4th and 8th day after infestation with *T. urticae* (Cry1F: $F = 88.7$, $df = 2, 11.8$, $P < 0.0001$, Cry1Ac: $F = 269.0$, $df = 2, 2.64$, $P = 0.0009$, Cry2Ab: $F = 37.3$, $df = 2, 2.59$, $P = 0.012$), but only for Cry2Ab did mite density affect protein levels ($F = 22.7$, $df = 2, 5.64$, $P = 0.002$) (Online Resource 1). There were no interactions between days after infestation and mite density for any Cry protein ($P > 0.05$). Concentrations of Cry1F and Cry2Ab were highest 4 days after infestation and lowest after 8 days with a mean change of 23 % for Cry1F and 11 % for Cry2Ab. In contrast, concentrations of Cry1Ac were highest 8 days after infestation and lowest after 4 days with a mean change of 42 %. Concentrations of Cry2Ab were lowest at a density of 50 mites with no difference between 10 and 100 mites (Online Resource 1). The mean change at 50 mites from 10 or 100 was 6 or 10 %, respectively. No Bt proteins were detected in non-Bt crops (not shown).

Discussion

The primary ecological concern related to Bt crops is their potential effects on NTAs (Conner et al. 2003),

Table 5 Tri-trophic effects on life table parameters (mean ± SE) of *Amblyseius andersoni* when fed *Tetranychus urticae* that were reared on Cry1F-expressing maize leaves or non-Bt near-isoline maize leaves over two generations

Parameters	Non-Bt maize	Cry1F maize	Statistical analysis
1st generation			
Survival (%)[a]	80.00 (30)	83.33 (30)	$\chi^2 = 0.26$; df = 1; $P = 0.61$
Larval stage[b]	1.02 ± 0.06 (26)	0.94 ± 0.06 (27)	$t = 0.88$; df = 51; $P = 0.38$
Protonymph stage[b]	1.58 ± 0.07 (24)	1.62 ± 0.08 (25)	$t = -0.36$; df = 47; $P = 0.72$
Deutonymph stage[b]	1.77 ± 0.09 (24)	1.72 ± 0.08 (25)	$t = 0.43$; df = 47; $P = 0.67$
Adult stage[b]	33.13 ± 1.41 (24)	32.80 ± 1.17 (25)	$t = 0.18$; df = 47; $P = 0.86$
Total fecundity[b]	33.00 ± 1.36 (10)	35.80 ± 1.45 (10)	$t = -1.41$; df = 18; $P = 0.18$
Egg hatching rate (%)[b]	84.44 ± 2.94 (3)	88.89 ± 2.22 (3)	$t = -1.21$; df = 4; $P = 0.29$
2nd generation			
Survival (%)[a]	83.33 (30)	86.67 (30)	$\chi^2 = 0.02$; df = 1; $P = 0.89$
Larval stage[b]	1.16 ± 0.06 (28)	1.11 ± 0.07 (27)	$t = 0.56$; df = 53; $P = 0.58$
Protonymph stage[b]	1.67 ± 0.07 (26)	1.6 ± 0.09 (26)	$t = 0.67$; df = 50; $P = 0.50$
Deutonymph stage[b]	1.80 ± 0.08 (25)	1.85 ± 0.08 (26)	$t = -0.41$; df = 49; $P = 0.68$
Adult stage[b]	35.04 ± 1.49 (25)	36.15 ± 1.37 (26)	$t = -0.06$; df = 49; $P = 0.59$
Total fecundity[b]	34.20 ± 1.04 (10)	33.80 ± 1.40 (10)	$t = 0.23$; df = 18; $P = 0.82$
Egg hatching rate (%)[b]	84.44 ± 2.94 (3)	85.56 ± 4.84 (3)	$t = -0.20$; df = 4; $P = 0.85$

Number of replications is given in parentheses

[a] Log-Rank test ($P < 0.05$)

[b] Student's t test ($P < 0.05$)

Table 6 Cry protein levels (ng/g FW) in Bt crops (cotton and maize), prey (*Tetranychus urticae*) and the predator *Amblyseius andersoni* (deutonymph stage)

Sample	Cotton		Maize
	Cry1Ac	Cry2Ab	Cry1F
Leaves	2084.7 ± 106.8a	23950.5 ± 682.7a	3404.7 ± 255.6a
Prey (larva)	48.0 ± 10.1b	858.0 ± 4.6b	87.5 ± 1.2b
Prey (protonymph)	58.0 ± 5.2b	862.2 ± 13.2b	85.7 ± 2.4b
Prey (deutonymph)	61.1 ± 4.7b	887.1 ± 20.6b	89.5 ± 0.7b
Prey (adult)	67.0 ± 5.3b	899.2 ± 13.0b	87.8 ± 2.3b
Predator (deutonymph)	3.3 ± 0.3c	41.8 ± 2.0c	3.57 ± 0.43c
Statistical analysis	$F_{5,12} = 358.7$; $P < 0.001$	$F_{5,12} = 1158.3$; $P < 0.001$	$F_{5,12} = 170.3$; $P < 0.001$

Mean (±SE) within a column followed by different letters are significantly different (Tukey's multiple range test, $P < 0.05$), n = 3, FW: Fresh weight

especially natural enemies that play an important role in pest regulation and are considered economically and ecologically valuable (Dutton et al. 2003; Naranjo et al. 2015). Despite concerns with large-scale cultivation of transgenic Bt cotton and maize, research suggests NTA effects from Bt crops are negligible or nonexistent (Romeis et al. 2006; Wolfenbarger et al. 2008; Naranjo 2009; Comas et al. 2014). This lack of effect is important to maintain natural enemy diversity and abundance and because the preservation of natural enemies by Bt crops has been shown to benefit control of non-target pests (Lu et al. 2012) and to delay the evolution of resistance to Bt crops (Onstad et al. 2013; Liu et al. 2014).

Our study tested tri-trophic effects of Cry1Ac/Cry2Ab cotton and Cry1F maize on *A. andersoni*

when fed with *T. urticae* that were reared on these Bt crops. An important issue under a tri-trophic exposure scenario is that prey-quality-mediated effects are controlled. Here we demonstrated that *T. urticae* reared on Cry1Ac/Cry2Ab and non-Bt cotton or Cry1F and non-Bt maize did not significantly differ in any developmental or reproductive life history parameters even though they had ingested relevant levels of Bt proteins from maize and cotton. The data confirm that *T. urticae* is not susceptible to Cry1Ac, Cry2Ab or Cry1F, thus no prey-quality-mediated effects are expected. Previous studies with Cry1Ac-expressing cotton (Esteves et al. 2010) and Cry3Bb1-, and Cry1Ab-expressing maize (Dutton et al. 2002; Li and Romeis 2010) also revealed no effects on *T. urticae*.

Our bioassay with Bt-sensitive *P. xylostella* larvae also confirm the Cry1Ac, Cry2Ab and Cry1F contained in *T. urticae* retained biological activity, which is in agreement with the results for Cry1Ab (Obrist et al. 2006b) and Cry3Bb1 (Meissle and Romeis 2009b).

Choice experiments demonstrated that *A. andersoni* did not display any preferences in prey that had fed on Bt or non-Bt cotton (Cry1Ac/Cry2Ab) or maize (Cry1F) plants. Our prey preference results were consistent with Esteves et al. (2010) who found that the predatory mite *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) had no preference for *T. urticae* reared on Cry1Ac cotton or on non-Bt cotton. Two other studies reported contrasting results. When given a choice between *T. urticae* that had consumed Cry3Bb-transgenic eggplants and *T. urticae* from an untransformed control, the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) showed a preference for the non-Bt-eggplant fed prey (Zemková Rovensaká et al. 2005). Prager et al. (2014) reported that under choice conditions, *P. persimilis* spent more time in the vicinity of *T. cinnabarius* that had consumed non-Bt maize when compared to mites that had consumed Bt (Cry1Ab or Cry3Bb1) maize.

Once prey-quality-mediated effects were removed and it was demonstrated that the Cry proteins contained in the spider mites were bioactive and that there was no prey preference, this confirmed that neither Cry1Ac/Cry2Ab cotton or Cry1F maize affected multiple life history parameters of *A. andersoni*. We believe this is the first study to combine these factors for a predatory mite and provides clear evidence that Cry1Ac/Cry2Ab cotton and Cry1F maize does not affect *A. andersoni* when it is continuously exposed to realistic levels of biologically active Cry proteins through their prey.

The risk of Bt crops to NTAs depends on the toxicity of the transgenic product and the concentrations to which they are exposed (Garcia-Alonso et al. 2006; Romeis et al. 2008b). Thus, the concentration of Cry proteins in Bt crops is an important factor to determine when assessing the effects of Bt crops. Our ELISA results show that the concentrations of Cry1Ac, Cry2Ab and Cry1F proteins declined rapidly as they moved through the food chain. Concentrations of these proteins declined 28–36 fold from crop leaves to prey and another 18–24 fold in the predators. Our results are consistent with previous studies with *T. urticae* exposure to Cry1Ac cotton (Esteves et al. 2010; Torres and Ruberson 2008), Cry3Bb1 maize (Li and Romeis 2010; Álvarez-Alfageme et al. 2011; Garcia et al. 2012) and Cry1Ab maize (Obrist et al. 2006a, b; Álvarez-Alfageme et al. 2008, 2011; García et al. 2010). Other research has shown that some species of predators can pick up Cry proteins readily from their spider mite prey sources with lower protein levels as compared to plants or prey (Obrist et al. 2006b; Meissle and Romeis 2009a; Li and Romeis 2010; Garcia et al. 2010, 2012). We therefore conclude that *A. andersoni* was exposed to high levels of biologically active Cry1Ac, Cry2Ab and Cry1F proteins throughout the duration of the feeding assay.

A recent study found that concentrations of Cry1Ab and Cry3Bb1 proteins in maize leaves were significantly reduced after infestation with *T. urticae* (Prager et al. 2014). However, they did not delineate *T. urticae* densities on the Bt maize and did not examine the effect of exposure time nor quantify Cry protein concentrations. Here we exposed Cry1Ac/Cry2Ab cotton and Cry1F maize to various densities of *T. urticae* ranging from 10 to 100/leaf and found variable results. The concentrations of Cry1F and Cry1Ac were not altered by mite density but were affected by time of exposure, while concentrations of Cry2Ab varied by mite density and exposure time. Even then, the relationships between concentration and both time of exposure and mite density were non-linear. Densities of 10 or 100 mites/leaf did not affect protein levels in Cry2Ab but both differed from 50 mites/leaf. Likewise, all Cry protein concentrations changed over time but the patterns were inconsistent. For Cry1F and

Cry2Ab, 8 days of exposure reduced concentrations relative to no exposure while for Cry1Ac 4 days of exposure led to reduced concentrations. Thus, there is limited support for the findings of Prager et al. (2014) relative to changing Cry protein levels in the face of mite infestations, because the protein concentrations did not appear to be clearly related to mite density. Further investigation of this phenomenon may be warranted. Nonetheless, even the lowest Cry protein levels observed here are still sufficiently high to provide control of the target pest (Niu et al. 2013; Jalali et al. 2014), so it remains unclear if herbivore-related reductions in Cry protein concentrations hold any relevance to pest control.

In conclusion, our laboratory studies indicate that Cry1Ac/Cry2Ab-expressing cotton and Cry1F-expressing maize did not show any adverse effects on the two-spotted spider mite, *T. urticae* or *A. andersoni*. Our study eliminated prey-quality-mediated effects, prey preference effects and demonstrated that predators were exposed to and ingested realistic concentrations of bioactive Cry proteins found in currently cultivated Cry1Ac/Cry2Ab cottons and Cry1F maize. Our results provide further confidence that Bt crops can complement other IPM tactics such as biological control by natural enemies, especially in the management of primary and secondary pests not targeted by Bt crops.

Acknowledgments This project was supported by the China Scholarship Council and the Biotechnology Risk Assessment Program Competitive Grant No. 2010-33522-21772 from the USDA, National Institute of Food and Agriculture. We thank H. Collins, M. Cheung and D. Olmstead for technical assistance and J. Nyrop and K. Wentworth for advice and supplying the initial colony of *T. urticae*.

References

Adamczyk JJ, Sumerford DV (2001) Potential factors impacting season-long expression of Cry1Ac in 13 commercial varieties of Bollgard cotton. J Insect Sci 1:13

Álvarez-Alfageme F, Ferry N, Castanera P, Ortego F, Gatehouse AMR (2008) Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). Transgenic Res 17:943–954

Álvarez-Alfageme F, Bigler F, Romeis J (2011) Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design. Transgenic Res 20:467–479

Amano H, Chant DA (1977) Life-history and reproduction of 2 species of predacious mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius andersoni* (Chant) (Acarina: Phytoseiidae). Can J Zool 55:1978–1983

Amano H, Chant DA (1978) Some factors affecting reproduction and sex-ratios in 2 species of predacious mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius andersoni* (Chant) (Acarina: Phytoseiidae). Can J Zool 56:1593–1607

Archer TL, Bynum ED (1993) Yield loss to maize from feeding by the Banks grass mite and two-spotted spider mite (Acari: Tetranychidae). Exp Appl Acarol 17:895–903

Auger P, Migeon A, Ueckermann EA, Tiedt L, Navajas M (2013) Evidence for synonymy between *Tetranychus urticae* and *Tetranychus cinnabarinus* (Acari, Prostigmata, Tetranychidae): review and new data. Acarologia 53:383–415

Bai YY, Jiang MX, Cheng JA, Wang D (2006) Effects of Cry1Ab toxin on *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) through its prey, *Nilaparvata lugens* (Homoptera: Delphacidae), feeding on transgenic Bt rice. Environ Entomol 35:1130–1136

Benedict JH, Sachs ES, Altman DW, Deaton WR, Kohel RJ, Berberich SA (1996) Field performance of cottons expressing transgenic Cry1A insecticidal proteins for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). J Econ Entomol 89:230–238

Bernal CC, Aguda RM, Cohen MB (2002) Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis*. Entomol Exp Appl 102:21–28

Boodley JW, Sheldrake RJ (1977) Cornell peat-lite mixes for commercial plant growing. Cornell Information Bulletin 43, Ithaca, New York

Chen M, Zhao JZ, Collins HL, Earle ED, Cao J, Shelton AM (2008) A critical assessment of the effects of Bt transgenic plants on parasitoids. PLoS One 3:e2284

Comas C, Lumbierres B, Pons X, Albajes R (2014) No effects of *Bacillus thuringiensis* maize on nontarget organisms in the field in southern Europe: a meta-analysis of 26 arthropod taxa. Transgenic Res 23:135–143

Conner AJ, Glare TR, Nap JP (2003) The release of genetically modified crops into the environment. Part II: overview of ecological risk assessment. Plant J 33:19–46

Dong HZ, Li WJ (2007) Variability of endotoxin expression in Bt transgenic cotton. J Agron Crop Sci 193:21–29

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

Dutton A, Romeis J, Bigler F (2003) Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. Biocontrol 48:611–636

Esteves AB, de Oliveira JV, Torres JB, Gondim MGC (2010) Compared biology and behavior of *Tetranychus urticae* Koch (Acari: Tetranychidae) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) on BollGard (TM) and non-transgenic isoline cotton. Neotrop Entomol 39:338–344

Fernandez-Maizeejo J, Wechsler S, Livingston M, Mitchell L (2014) Genetically engineered crops in the United States. US Department of Agriculture Economic Research Service. http://www.ers.usda.gov/media/1282242/err162_summary.pdf

Ferry N, Mulligan EA, Stewart CN, Tabashnik BE, Port GR, Gatehouse AMR (2006) Prey-mediated effects of transgenic canola on a beneficial, non-target, carabid beetle. Transgenic Res 15:501–514

García M, Ortego F, Castañera P, Farinós GP (2010) Effects of exposure to the toxin Cry1Ab through Bt maize fed-prey on the performance and digestive physiology of the predatory rove beetle *Atheta coriaria*. Biol Control 55:225–233

García M, Ortego F, Castañera P, Farinós GP (2012) Assessment of prey-mediated effects of the coleopteran-specific toxin Cry3Bb1 on the generalist predator *Atheta coriaria* (Coleoptera: Staphylinidae). Bull Entomol Res 102:293–302

Garcia-Alonso M, Jacobs E, Raybould A, Nickson TE, Sowig P, Willekens H, Van der Kouwe P, Layton R, Amijee F, Fuentes AM, Tencalla F (2006) A tiered system for assessing the risk of genetically modified plants to non-target organisms. Environ Biosaf Res 5:57–65

Hellmich RL, Albajes R, Bergvinson D, Prasifka JR, Wang Z-Y, Weiss MJ (2008) The present and future role of insect-resistant genetically modified maize in IPM. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant, genetically modified crops within IPM programs. Springer, New York, pp 119–158

Jalali SK, Yadavalli L, Ojha R, Kumar P, Sulaikhabeevi SB, Sharma R, Nair R, Kadanur RC, Kamath SP, Komarlingam MS (2014) Baseline sensitivity of maize borers in India to the *Bacillus thuringiensis* insecticidal proteins Cry1A.105 and Cry2Ab2. Pest Manag Sci. doi:10.1002/ps.3888

James C (2014) Global status of commercialized biotech/GM crops: 2014 ISAAA Brief No. 49. ISAAA, Ithaca, NY

Kennedy GG (2008) Integration of insect-resistant genetically modified crops within IPM programs. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant, genetically modified crops within IPM Programs. Springer, New York, pp 1–26

Lawo NC, Wäckers FL, Romeis J (2010) Characterizing indirect prey-quality mediated effects of a Bt crop on predatory larvae of the green lacewing, *Chrysoperla carnea*. J Insect Physiol 56:1702–1710

Li YH, Romeis J (2010) Bt maize expressing Cry3Bb1 does not harm the spider mite, *Tetranychus urticae*, or its ladybird beetle predator, *Stethorus punctillum*. Biol Control 53: 337–344

Li YH, Romeis J, Wang P, Peng YF, Shelton AM (2011) A comprehensive assessment of the effects of Bt cotton on *Coleomegilla maculata* demonstrates no detrimental effects by Cry1Ac and Cry2Ab. PLoS One 6:e22185

Liu XX, Chen M, Collins HL, Onstad DW, Roush RT, Zhang Q, Earle ED, Shelton AM (2014) Natural enemies delay insect resistance to Bt crops. PLoS One 9:e90366

Lövei GL, Andow DA, Arpaia S (2009) Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. Environ Entomol 38:293–306

Lu YH, Wu KM, Jiang YY, Guo YY, Desneux N (2012) Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487:362–365

Luo Z, Dong HH, Li WJ, Ming Z, Zhu YQ (2008) Individual and combined effects of salinity and waterlogging on Cry1Ac expression and insecticidal efficacy of Bt cotton. Crop Prot 27:1485–1490

McMurtry J (1982) The use of phytoseiids for biological control: progress and future prospects. Recent advances in knowledge of the Phytoseiidae. University of California, Berkeley, pp 23–48

Meissle M, Romeis J (2009a) The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. Plant Biotechnol J 7:645–656

Meissle M, Romeis J (2009b) Insecticidal activity of Cry3Bb1 expressed in Bt maize on larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Entomol Exp Appl 131:308–319

Mészáros A, Beuzelin JM, Stout MJ, Bommireddy PL, Riggio RM, Leonard RB (2011) Jasmonic acid-induced resistance to the fall armyworm, *Spodoptera frugiperda*, in conventional and transgenic cottons expressing *Bacillus thuringiensis* insecticidal proteins. Entomol Exp Appl 144:226–237

Naranjo SE (2009) Impacts of Bt crops on non-target invertebrates and insecticide use patterns. CAB Rev Perspect Agric Vet Sci Nutrit Nat Resour 4(011):1–23

Naranjo SE, Ruberson JR, Sharma HC, Wilson L, Wu KM (2008) The present and future role of insect-resistant GM cotton in IPM. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant, genetically modified crops within IPM programs. Springer, New York, pp 159–194

Naranjo SE, Ellsworth PC, Frisvold GB (2015) Economics of biological control in integrated pest management of managed plant systems. Annu Rev Entomol 60:621–645

Nguyen HT, Jehle JA (2007) Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. J Plant Dis Prot 114:82–87

Niu Y, Meagher RL Jr, Yang F, Huang F (2013) Susceptibility of field populations of the fall armyworm (Lepidoptera: Noctuidae) from Florida and Puerto Rico to purified Cry1F protein and corn leaf tissue containing single and pyramided Bt genes. Florida Entomol 96:701–713

Obrist LB, Dutton A, Albajes R, Bigler F (2006a) Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecol Entomol 31:143–154

Obrist LB, Dutton A, Romeis J, Bigler F (2006b) Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. Biocontrol 51:31–48

Olsen KM, Daly JC, Finnegan EJ, Mahon RJ (2005) Changes in Cry1Ac Bt transgenic cotton inresponse to environmental factors: temperature and insect damage. J Econ Entomol 98:1382–1390

Onstad DW, Liu XX, Chen M, Roush R, Shelton AM (2013) Modeling the integration of parasitoid, insecticide and transgenic insecticidal crops for the long-term control of an insect pest. J Econ Entomol 106:1103–1111

Prager SM, Martini X, Guvvala H, Nansen C, Lundgren J (2014) Spider mite infestations reduce *Bacillus thuringiensis* toxin concentration in corn leaves and predators avoid spider mites that have fed on *Bacillus thuringiensis* corn. Ann Appl Biol 165:108–116

Reddall A, Sadras VO, Wilson LJ, Gregg PC (2004) Physiological responses of cotton to two-spotted spider mite damage. Crop Sci 44:835–846

Romeis J, Meissle M, Bigler F (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat Biotechnol 24:63–71

Romeis J, van Driesche RG, Barratt BIP, Bigler F (2008a) Insect-resistant transgenic crops and biological control. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant, genetically modified crops within IPM programs. Springer, Nwe York, pp 87–117

Romeis J, Bartsch D, Bigler F, Candolfi MP, Gielkins MC, Hartley SE, Hellmich RL, Huesing JE, Jepson PC, Layton R, Quemada H, Raybould A, Rose RI, Schiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD (2008b) Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. Nat Biotechnol 26:203–208

Romeis J, McLean MA, Shelton AM (2013) When bad science makes good headlines: the case of Bt crops. Nat Biotechnol 37:386–387

Shelton AM, Cooley RJ, Kroening MK, Wilsey WT, Eigenbrode SD (1991) Comparative analysis of two rearing procedures for diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). J Entomol Sci 26:17–26

Shelton AM, Naranjo SE, Romeis J, Hellmich RL, Wolt JD, Federici BA, Albajes R, Bigler F, Burgess EPJ, Dively GP, Gatehouse AMR, Malone LA, Roush RT, Sears MK, Sehnal F (2009a) Setting the record straight: a rebuttal to an erroneous analysis on transgenic insecticidal crops and natural enemies. Transgenic Res 18:317–322

Shelton AM, Naranjo SE, Romeis J, Hellmich RL, Wolt JD, Federici BA, Albajes R, Bigler F, Burgess EPJ, Dively GP, Gatehouse AMR, Malone LA, Roush RT, Sears MK, Sehnal F, Ferry N, Bell HA et al (2009b) Appropriate analytical methods are necessary to assess nontarget effects of insecticidal proteins in GM crops through meta-analysis (Response to Andow et al. 2009). Environ Entomol 38:1533–1538

Shelton AM, Naranjo SE, Romeis J, Hellmich RL (2012) Errors in logic and statistics plague a meta-analysis (response to Andow and Lovei 2012). Environ Entomol 41:1047–1049

SPSS (1988) SPSS user's guide. SPSS Inc, Chicago

Su HH, Tian JC, Naranjo SE, Romeis J, Hellmich RL, Shelton AM (2015) *Bacillus thuringiensis* plants expressing Cry1Ac, Cry2Ab and Cry1F are not toxic to the assassin bug, *Zelus renardii*. J Appl Entomol 139:23–30

Tian JC, Collins HL, Romeis J, Naranjo SE, Hellmich RL, Shelton AM (2012) Using field-evolved resistance to Cry1F maize in a lepidopteran pest to demonstrate no adverse effects of Cry1F on one of its major predators. Transgenic Res 21:1303–1310

Tian JC, Wang XP, Long LP, Romeis J, Naranjo SE, Hellmich RL, Wang P, Earle ED, Shelton AM (2013) Bt crops producing Cry1Ac, Cry2Ab and Cry1F do not harm the green lacewing, *Chrysoperla rufilabris*. PLoS One 8:e60125

Tian JC, Long LP, Wang XP, Naranjo SE, Romeis J, Hellmich RL, Wang P, Shelton AM (2014a) Using resistant prey demonstrates that Bt plants producing Cry1Ac, Cry2Ab, and Cry1F have no negative effects on *Geocoris punctipes* and *Orius insidiosus*. Environ Entomol 43:242–251

Tian JC, Wang XP, Long LP, Romeis J, Naranjo SE, Hellmich RL, Shelton AM (2014b) Eliminating host-mediated effects demonstrates Bt maize producing Cry1F has no adverse effects on the parasitoid *Cotesia marginiventris*. Transgenic Res 23:257–264

Torres JB, Ruberson JR (2008) Interactions of *Bacillus thuringiensis* Cry1Ac toxin in genetically engineered cotton with predatory heteropterans. Transgenic Res 17:345–354

Tsolakis H, Ragusa di Chiara S (1994) Biological and life table parameters of *Amblyseius andersoni* (Chant) (Parasitiformes, Phytoseiidae) on different kinds of food substances. Phytophaga (Palermo) 5:21–28

van der Linden A (2004) *Amblyseius andersoni* Chant (Acari: Phytoseiidae), a successful predatory mite on *Rosa* spp. Commun Agric Appl Biol Sci 69:157–163

Wilson LJ (1993) Spider mites (Acari: Tetranychidae) affect yield and fiber quality of cotton. J Econ Entomol 86:566–585

Wolfenbarger LL, Naranjo SE, Lundgren JG, Bitzer RJ, Watrud LS (2008) Bt crop effects on functional guilds of non-target arthropods: a meta-analysis. PLoS One 3:e2118

Zemková Rovensaká G, Zemek R, Schmidet JEU, Hilbeck A (2005) Altered host plant preference of *Tetranychus urticae* and prey preference of its predator *Phytoseiulus persimilis* (Acari: Tetranychidae, Phytoseiidae) on transgenic Cry3Bb-eggplants. Biol Control 33:293–300

Zhou GS, Zhou FC, Xie YM, Feng CN, Yang YZ (2009) Effects of temperature stress on Bt insecticidal protein expression in Bt transgenic cotton leaves and death rate of cotton bollworm. Cotton Sci 21:302–306