Effects of *Bacillus thuringiensis* Cry Toxins on Developmental and Reproductive Characteristics of the Predator *Orius albidipennis* (Hemiptera: Anthocoridae) Under Laboratory Conditions

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ABSTRACT The effects of Cry toxins from *Bacillus thuringiensis* (Berliner) (*Bt*) on the anthocorid *Orius albidipennis* Reuter were studied under laboratory conditions. Tritrophic experiments were performed, in which *Orius* nymphs were fed *Helicoverpa armigera* (Hübner) larvae reared on a diet with Cry1Ac, Cry1Ab, or Cry2Ab toxins at different concentrations (0, 1, and 10 μg/ml), when supplemented with *Ephestia kuehniella* Zeller eggs. In complementary experiments, the *Bt* Cry1Ac toxin was directly fed to *Orius* nymphs at a very high concentration (1 mg/ml). No effects on prey consumption, developmental time, nymph survival, fecundity, and egg hatching of *O. albidipennis* were found in either experiment. It can be concluded that the toxins tested do not seem to pose a risk for the anthocorid *O. albidipennis*, especially when it is exposed through the prey.

KEY WORDS *Orius albidipennis*, *Bacillus thuringiensis* toxins, nontarget organisms

The use of *Bacillus thuringiensis* (*Bt*) crops is increasing worldwide (James 2006), especially by offering the advantage of reducing the use of synthetic insecticides to control some of the most important pests of several crops, such as cotton and maize (Romeis et al. 2006, Sanvido et al. 2006). However, the generalized use of these plants has led to a growing concern about the long-term effects of *Bt* toxins that are produced continuously throughout the growing season. *Bt* toxins can accumulate in soil, affect nontarget herbivores, or be acquired by predators and parasitoids at a higher trophic level through the prey/host, by direct feeding on plant material, or even by feeding on other substances (droppings, honeydew) (Obrist et al. 2005, O'Callaghan et al. 2005, Sanvido et al. 2006, Torres et al. 2006).

The most widely used method to assess the environmental risk posed by these crops is on the basis of a stepwise (tiered) approach, where each assessment increases in complexity and realism, based on knowledge gained in previous tests. With nontarget arthropods, risk assessment of insecticidal transgenic crops starts with laboratory tests (first tier), which are designed to determine if an organism is susceptible to the toxin under worst case conditions. In other words, the organism is directly exposed to high doses of the toxin or the assay can be conducted in a way that simulates the usual mode of toxin acquisition, especially through the prey/host, but also through various plant material (Dutton et al. 2003). The risk assessment can stop at this point if the results obtained under the worst case scenario show the toxin to be of low risk. Otherwise, higher tier tests will follow, in which the nontarget organisms are exposed to the toxin under more realistic conditions in semifield or field experiments.

Cotton and maize are the two major *Bt* crops, with different *Bt* toxins expressed in different commercial events (Cry1Ac, Cry2Ab, and Cry1 F in cotton and Cry1Ab, Cry1Fa, and Cry3Bb in maize) (AGBIOS 2007) against caterpillars and some beetles. These *Bt* toxins have been tested in laboratory experiments with different nontarget organisms, most often the predators *Chrysoperla carnea* (Stephens), various heteropterans (*Orius* spp., *Geocoris* spp., and some other species), and coccinellids (*Coccinella septempunctata* L., *Hippodamia convergens* Gurin-Meneville, and other species), together with different parasitoid species (O'Callaghan et al. 2005, Romeis et al. 2006). These experiments have been conducted in different ways, but mainly in tritrophic experiments with the prey/host feeding on the *Bt* plant. When the *Bt* plant is not avalaible, the tritrophic experiments have been carried out with the prey/host feeding on an artificial diet with a *Bt* toxin concentration similar to that of plant tissues. In other cases, a predator or parasitoid ingests the *Bt* toxin directly from the plant (including pollen) or from a solution of the toxin at a known (and normally high) concentration (Sims 1995, Obrist et al. 2006a, b, Rodrigo-Simón et al. 2006, see Romeis et al. 2006 for an extensive review, Torres et al. 2006). Although negative effects of *Bt* toxins have been re-

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ported for some predators, most of these have been attributed to indirect effects on the prey in tritrophic experiments rather than to a direct effect of the toxin itself (Dutton et al. 2002, Obrist et al. 2006a, Romeis et al. 2006).

The nontarget organisms should be selected from among the most important predators/parasitoids in the different crops and areas (Dutton et al. 2003). In our case, *Orius albidipennis* Reuter (Hemiptera, Anthocoridae) is a common anthocorid in southern Spain and is present in many crops (Ferragut and González-Zamora 1994). Like other anthocorids, *O. albidipennis* is polyphagous, feeding on several different prey, such as immature insects and acari. They also are great consumers of eggs of lepidopterans and tetranichyds in cotton and sweet peppers, playing an important role in the biological control of pests like *Frankliniella occidentalis* (Pergande) and *Helicoverpa armigera* (Hübner) (Alvarado et al. 1998, Sánchez and Lacasa 2006).

Different studies have examined the effects of *Bt* toxins on direct mortality of *H. armigera* and indirect effects on weight and development (Liao et al. 2002, Jalali et al. 2004, Avilla et al. 2005). They showed that the larvae ingest enough quantities of *Bt* toxins to die, or at least to reduce its weight and development, depending on the toxin and conditions of the experiment. Others have shown and quantified ingestion on *Bt* toxins by larvae of other lepidopterans [Head et al. 2001 with *Ostrinia nubilalis* (Hübner), *Helicoverpa zea* (Boddie) and *Agrotis ipsilon* (Hufnagel); Dutton et al. 2002 with *Spodoptera littoralis* (Boisduval); Torres et al. 2006 with *Spodoptera exigua* (Hübner)], which were at lower concentrations than in the plant tissues where they fed. Although, to our knowledge, no study of *H. armigera* has been conducted on this subject, Rodrigo-Simón et al. (2006) found that the Cry1Ac toxin ingested by *H. armigera* larvae binds to the brush border membrane of the midgut.

The objective of this work was to study the effect of three *Bt* toxins (Cry1Ac, Cry1Ab, and Cry2Ab) that are generally expressed in cotton and maize on different biological parameters of the predator *O. albidipennis* ingesting the *Bt* toxins through the prey *H. armigera* under laboratory conditions. This is thus a tritrophic experiment conducted as a first step preliminary to more complex studies at the field scale. Because the prey is susceptible to the toxins tested, and prey-mediated effects could result, a second experiment of direct feeding from a solution containing the *Bt* toxin Cry1Ac also was carried out. These types of studies are complex, because they need to follow the life story of the organism tested to obtain adequate information about its biological parameters. To our knowledge, this is the first time this predator has been tested with *Bt* toxins, making this an important contribution to understanding the possible risks that *Bt* crops can pose to the environment.

Materials and Methods

Insect Material. The *H. armigera* colony was established from larvae and eggs collected in cotton fields from June to September 2001–2002, periodically introducing new wild individuals collected each year. The *O. albidipennis* colony was started from individuals collected in cotton fields in September 2004 and also received new introductions periodically. Both species were obtained from the Seville province (Andalusia, Spain). Insects were maintained in the laboratory under controlled conditions with a photoperiod consisting of 16 h of light and 8 h of darkness and a relative humidity of 40–60% at 26 ± 2°C.

Helicoverpa armigera larvae were reared on a corn flour–based artificial diet (Poitout and Bues 1974), whereas adults were maintained with a 10% honey solution. The diet for both nymphs and adults of *O. albidipennis* consisted of *Ephestia kuehniella* Zeller eggs (Koppert Biological Systems, Berkelen Rodenrijs, The Netherlands) added every 2 d to the rearing units. The *O. albidipennis* population was reared in 2-liter square plastic boxes with a hole in the lid covered with mesh. The insects were provided with moistened cotton in a glass vial to maintain humidity and also for drinking. Bean (*Phaseolus vulgaris* L.) pods were introduced periodically in the rearing units where adults were kept to allow egg laying. The pods were removed after 4–5 d and transferred to another rearing unit to obtain nymphs.

Voucher specimens of the two species of insects are maintained by the first author in the department collection (Department of Ciencias Agroforestales, University of Seville).

Preparation of Cry Toxins. Cry1Ac and Cry1Ab toxins were obtained from recombinant *Bt* strains EG11070 and EG7077, respectively (Ecogen, Langhorne, PA). Cry2Ab was prepared from recombinant *Bt* strain EG7699 (Monsanto, Chesterfield, MO). Toxin preparation, solubilization, activation, and purification were performed as described previously (Estela et al. 2004), except that Cry2Ab was dissolved in carbonate buffer (50 mM Na_2CO_3, 0.1 M NaCl, 10 mM dithiothreitol; pH 12).

Toxins were trypsin activated and purified. The purity of toxin samples was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the toxin concentration was determined by densitometric analysis using bovine serum albumin as a standard. Before the toxin preparations were used, toxic activity was verified with larvae of the susceptible insect *Plutella xylostella* L.

***Orius albidipennis* Bioassays Using Treated Prey.** All experiments were carried out in a controlled-environment chamber as described above for insect rearing. Neonate larvae of *H. armigera* were reared for 1 d at 26 ± 2°C on an artificial diet containing no toxin or 1 or 10 μg/ml of toxin Cry1Ab, Cry1Ac, or Cry2Ab. Afterward, they were kept at 6–8°C to prevent growth. Third/fourth instars of *O. albidipennis* were separated just after molting and kept individually in plastic cages (diameter, 4 cm; height, 2 cm) with a

Table 1. Effect of Cry toxins on *O. albidipennis* prey consumption

Toxins	Treatment (μg toxin/ml diet)			H[a]	*P*
	0	1	10		
H. armigera larvae consumption[b]					
Cry1Ac-First	1.7 ± 0.20	2.9 ± 0.40	3.1 ± 0.91	4.62	0.10
Cry1Ac-Second	3.3 ± 0.47	3.6 ± 0.95	2.9 ± 0.34	0.62	0.73
Cry1Ab	2.9 ± 0.30	2.9 ± 0.35	2.1 ± 0.31	2.40	0.30
Cry2Ab	5.6 ± 0.25	3.1 ± 0.11	4.8 ± 0.52	5.80	0.06
H. armigera larvae consumption per day[b]					
Cry1Ac-First	0.4 ± 0.03	0.6 ± 0.12	0.8 ± 0.16	3.29	0.19
Cry1Ac-Second	0.5 ± 0.05	0.5 ± 0.16	0.5 ± 0.14	0.36	0.84
Cry1Ab	0.4 ± 0.06	0.3 ± 0.05	0.2 ± 0.06	1.87	0.39
Cry2Ab	0.9 ± 0.11	0.5 ± 0.04	0.7 ± 0.10	5.96	0.05

[a] Kruskal-Wallis test. The differences between treatments within a row (toxin) are not statistically significant ($P \geq 0.05$).
[b] The values are means ± SE for consumption of *H. armigera* larvae for each toxin, based on three replicates.

ventilation hole in the lid covered with mesh. A small amount of vermiculite was put inside the cages and moistened every day. The cages were placed in a plastic tray containing a layer of moistened cotton to maintain humidity. Nymphs were fed daily with three treated *H. armigera* larvae, and every 2 d, this diet was supplemented with *E. kuehniella* eggs for 24 h and removed completely, which allowed nymphs to complete development (in preliminar experiments, in which *Orius* nymphs were fed only with *H. armigera* larvae, *Orius* mortality was very high). The experiments with each toxin consisted of three treatments (0, 1, and 10 μg/ml) with three replicates per treatment. Ten nymphs were used in each replicate, with a total of 30 nymphs per treatment and 90 nymphs per toxin. When the adults emerged, pairs of one male and one female were placed together in plastic cages with a bean pod to allow egg laying for 2 d. The number of eggs per female was recorded at three consecutive periods of 48 h each, and the bean pods were kept in different plastic cages to allow egg hatching.

Orius albidipennis nymphs were checked daily to evaluate the following parameters: nymph survival, adult molting, development time, number of *H. armigera* larvae ingested, fecundity, and egg hatching. The experiment with the toxin Cry1Ac was conducted twice, because the first time the acceptability of the egg laying substrate (*Pelargonium peltatum* L. leafs) was very poor. Thus, fecundity and egg hatching data from this first experiment with the toxin Cry1Ac are not presented, but the rest of the parameters are.

The prey consumption data did not follow a normal distribution, so the data were analyzed with the Kruskal-Wallis test. The remaining parameters fit a normal distribution, so analysis of variance (one-way ANOVA) was used to determine whether significant differences existed among the three treatments for each toxin. Data were transformed using arcsine of the square root for variables recorded as percentages. All analyses were performed using the Statgraphics package (Statistical Graphics Corporation 2000).

***Orius albidipennis* Bioassays Using Water-Supplied Cry1Ac.** In preliminary experiments, it was observed that nymphs of *O. albidipennis* were reluctant to feed on a drop of water, so it was necessary to starve them for 2 d before offering them the solution of Cry1Ac toxin. Second/third instars were kept individually in the previously described cages for 2 d without water or food. Afterward, they were provided with a drop (4 μl) of fluorella blue–stained water containing either 0 or 1 μg/μl of the toxin Cry1Ac. Nymphs were allowed to feed on the drop ad libitum and then fed with *E. kuehniella* eggs for 24 h. Nymphs were left to starve for 24–48 h and again provided with a drop of blue-stained water (with or without Cry1Ac). This cycle was repeated (one to three times) until adult emergence. Nymphs were considered to acquire the toxin when they turned blue after drinking from the drop. Nymphs that did not drink from the water drop were excluded from further analysis. The experiment was repeated three times with at least 15 nymphs per treatment and experiment, making a minimum of 45 nymphs total for each treatment. The parameters evaluated were the same as in the previous section. Data of development and fecundity were compared using Student's *t*-test, whereas nymphal survival, adult emergence, and egg hatching were compared between treatments using contingency table analysis (χ^2 test), with the Yates correction for continuity.

Results

Effect of Treated Prey on *O. albidipennis*. The number of prey consumed by nymphs of *O. albidipennis* was low, far below the three larvae of *H. armigera* offered every day (Table 1). No statistical differences were found between the different concentrations of the *Bt* toxins tested, either for the total mean or the daily mean ingestion.

Development time showed no differences between concentrations of each toxin, with *P* always >0.05 (Table 2). The other parameters yielded a similar conclusion. No differences were found between the different concentrations of the *Bt* toxins for the proportion of individuals that reached fifth instar (Fig. 1A and Table 3 for summary statistics), with values ranging between 40 and 100%, or that reached the adult stage (Fig. 1B and Table 3 for summary statistics), with values ranging between 37.5 and 100%. The were no differences in fecundity and percent egg hatching for

Table 2. Effect of *Bt* toxin–treated prey on *O. albidipennis* development time

Toxin	Instar	Treatment (μg toxin/ml diet)			F^a	P
		0	1	10		
Cry1Ac-first	N3	—[b]	—	—		
	N4	3.2 ± 0.24[c]	2.7 ± 0.09	2.6 ± 0.19	1.94	0.22
	N5	1.2 ± 0.14	1.2 ± 0.02	1.2 ± 0.03	0.05	0.96
	Adult	3.9 ± 0.35	3.1 ± 0.15	3.3 ± 0.32	1.14	0.38
Cry1Ac-second	N3	3.2 ± 0.35	4.0 ± 0.62	3.5 ± 0.82	0.31	0.75
	N4	2.0 ± 0.01	2.1 ± 0.09	2.2 ± 0.30	0.20	0.83
	N5	1.6 ± 0.19	2.3 ± 0.22	1.7 ± 0.15	2.90	0.13
	Adult	6.7 ± 0.53	7.7 ± 0.77	6.3 ± 0.35	0.99	0.43
Cry1Ab	N3	4.1 ± 0.13	3.7 ± 0.10	4.6 ± 0.48	1.62	0.27
	N4	3.2 ± 0.20	2.4 ± 0.15	2.1 ± 0.32	4.48	0.06
	N5	2.1 ± 0.12	2.3 ± 0.14	3.2 ± 0.64	1.53	0.29
	Adult	9.0 ± 0.49	8.0 ± 0.21	9.9 ± 0.90	1.67	0.27
Cry2Ab	N3	3.0 ± 0.32	3.7 ± 0.35	3.2 ± 0.09	1.10	0.39
	N4	2.2 ± 0.14	1.6 ± 0.30	3.2 ± 0.51	3.44	0.10
	N5	2.1 ± 0.20	1.3 ± 0.09	1.5 ± 0.27	2.68	0.15
	Adult	7.0 ± 0.55	6.5 ± 0.09	7.8 ± 0.53	1.28	0.35

[a] One-way ANOVA with 2 and 6 df. The differences between treatments within a row and Cry toxin are not statistically significant ($P \geq 0.05$).

[b] The first exp with the toxin Cry1Ac started with fourth instars.

[c] The values are means ± SE for the no. development days and were calculated for each instar (N3, N4, N5) and adults from three replicates.

the *Bt* toxins tested (Fig. 2 and Table 3 for summary statistics).

Effect of Directly Supplied Cry1Ac on *O. albidipennis*. Ingestion of the toxin through the prey can mask possible effects of the toxin on the target species. For example, degradation of the toxin in the digestive system of the prey, or reduction of prey quality, can make the amount of toxin ingested by the predator very low. To avoid these potential effects, the toxin was supplied directly to *O. albidipennis* at a high concentration in an aqueous solution together with a dye agent. The nymphs were allowed to feed on it ad libitum.

No differences were found between the two treatments (with the toxin Cry1Ac and without the toxin) in the development time for any stages (Table 4). The percentage of individuals that reached the fifth instar or adult stage also showed no differences between the treatments, with $\chi^2 = 1.01$ (df = 1, $P = 0.31$) and $\chi^2 = 0.75$ (df = 1, $P = 0.39$), respectively (Fig. 3A), with proportions of individuals that ranged between 31 and 70% for fifth instar and between 31 and 56% for adults. Finally, no differences were observed for either of the other two parameters studied: fecundity (Fig. 3B) and egg hatching (Fig. 3A; fecundity: $t = -0.63$, df = 4, $P = 0.57$; egg hatching: $\chi^2 = 0.96$, df = 1, $P = 0.33$).

Discussion

It is necessary to study the effect of *Bt* toxins to assess the risk of transgenic *Bt* crops for nontarget organisms (Dutton et al. 2003, Romeis et al. 2006). Appropriate scenarios must be developed together with representative species, following a protocol with different levels or tiers. The first is the worst case tier, in which the experimental conditions are as unfavorable as possible to the organisms tested, using the pure insecticidal toxin in artificial diets (or through the prey that had previously ingested the toxin) and transgenic plant material. If no negative effects are found, further analyses may not be needed. Tritrophic experiments in which the prey previously ingests the toxin from the plant or from an artificial diet are a more realistic simulation of what actually happens in the crop. However, to elucidate if the toxin alone has any effect on the entomophagous predator, it should be ingested directly, because possible alterations in the toxins can occur when they pass through the prey (Romeis et al. 2006).

Since *Bt* crops started to appear in the mid-1990s, many studies have been carried out to determine their possible effects on different entomophagous arthropods, both in the laboratory and the field, as part of

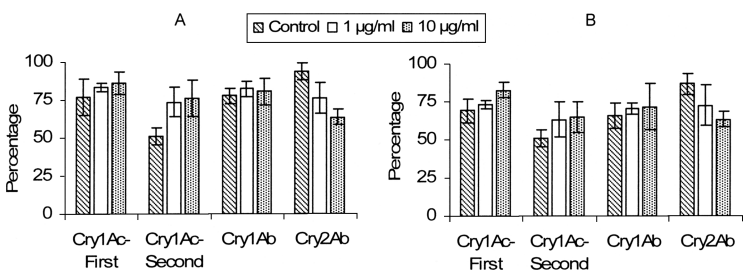

Fig. 1. Effect of *Bt*-treated prey on survival of *O. albidipennis*. (A) Percentage of individuals that reached fifth instar and (B) percentage of individuals that reached the adult stage. Values are mean ± SE from three replicates.

Table 3. Summary statistics of the effect of *Bt* toxin–treated prey on different parameters of *O. albidipennis:* percentage of individuals that reached fifth instar (N5), percentage of individuals that reached the adult stage, fecundity, and egg hatching

Toxins	Percentage individuals in N5		Percentage individuals in Adult		Fecundity		Percentage egg hatching	
	F^a	P	F	P	F	P	F	P
Cry1Ac-first	0.15	0.87	1.13	0.38	—[b]	—	—	—
Cry1Ac-second	1.30	0.34	0.45	0.65	0.79	0.49	1.27	0.35
Cry1Ab	0.17	0.85	0.22	0.81	1.63	0.27	0.14	0.87
Cry2Ab	3.76	0.09	1.41	0.32	0.96	0.44	4.47	0.07

[a] One-way ANOVA with 2 and 6 df.
[b] Fecundity and egg hatching were not calculated for the first experiment with the toxin Cry1Ac.

Table 4. Effect of directly supplied Cry1Ac toxin (1 mg/ml) on *O. albidipennis* development time

Instar	Treatment		t^a	P
	Control	Treated		
N3	3.4 ± 0.21[b]	2.6 ± 0.28	−1.83	0.14
N4	2.9 ± 0.26	2.3 ± 0.41	−0.94	0.40
N5	1.5 ± 0.15	2.0 ± 0.11	2.22	0.09
Adult	7.2 ± 0.56	6.4 ± 0.62	−0.83	0.45

[a] Student's t-test with 4 df. The differences between treatments within a row are not statistically significant ($P \geq 0.05$).
[b] The values are means ± SE for the no. development days and were calculated for each instar (N3, N4, N5) and adults from three replicates.

assessing the impact of these crops on the environment (Romeis et al. 2006). Anthocorids are one of the nontarget groups selected for testing because of its importance in predation of different pests (Lattin 1999) in the most important *Bt* crops, cotton and maize.

The results presented here showed no negative effect on the predator *O. albidipennis* when fed with prey, *H. armigera* larvae, that had previously ingested Cry1Ac, Cry1Ab, or Cry2Ab toxins at different concentrations. The concentrations of *Bt* toxins used in the experiments are in the range at which they can be found in cotton and maize tissues (Adamczyk et al. 2001, Dutton et al. 2002, 2003). *O. albidipennis* nymphs were fed only with *H. armigera* larvae in initial experiments, but they performed poorly, with a very high mortality. This is not a normal situation, and this predator (like other *Orius* species) consumes a wide range of prey (Pericart 1972, Alvarado et al. 1998) in its natural environment. For this reason, during the experiments, the nymphs were supplemented with *E. kuehniella* eggs, resembling the usual feeding behavior of this predator, as was also done, for example, with *C. carnea* larvae in similar experiments (Rodrigo-Simón et al. 2006).

Another interesting result is the low consumption of *H. armigera* larvae by nymphs of *O. albidipennis* compared with a predator like *C. carnea*, which in similar experimental conditions ingested about eight times more *H. armigera* larvae (Rodrigo-Simón et al. 2006). Nymphs of the *Orius* genus are smaller than *Chrysoperla* larvae, and it can be difficult for them to kill a 1- to 3-d-old *H. armigera* larva. Moreover, nymphs of another heteropteran, *Podisus maculiventris* (Say) (Hemiptera, Pentatomidae), are larger than *Orius* nymphs and killed 9-d-old *S. exigua* larvae, consuming a weight of larvae 338-fold greater than the amount consumed by *Orius* (Torres et al. 2006).

Several studies have shown that predators with sucking mouth parts can ingest *Bt* toxins from their prey (Obrist et al. 2006a, b, Torres et al. 2006). Most of the tritrophic laboratory studies carried out involving anthocorids and some other species of heteropterans showed no effects of *Bt* toxins. Examples include *Orius majusculus* (Reuter) fed with *Anaphothrips obscurus* (Müller) as prey, reared in maize with the *Bt* toxin Cry1Ab (Zwahlen et al. 2000), or *Orius insidiosus* (Say) fed with *O. nubilalis* larvae that had previously been reared on a meridic diet containing a mixture of *Bt* toxins (Al-Deeb et al. 2001). Only Ponsard et al. (2002) found a negative effect on the survival of adult *Orius tristicolor* (White) and *Geocoris punctipes* (Say) (Hemiptera, Lygaeidae) when fed with *S. exigua* larvae that had previously been on cotton leaves that contained the toxin Cry1Ac. A possible explanation for this effect could be the higher intake of toxin by the prey in the smaller instars, resulting in a high concentration in its body (Ponsard et al. 2002), which could affect the predators, although a sublethal effect on the prey cannot be disregarded (Ashfaq et al. 2000).

Development times also showed no statistical differences between the concentrations of each toxin (Table 2). Differences in development time among the controls for the toxin Cry1Ab compared with

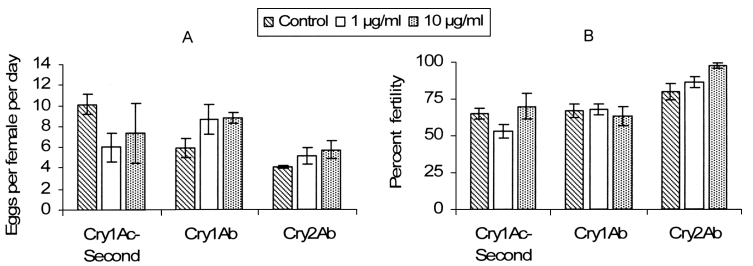

Fig. 2. Effect of *Bt*-treated prey on (A) fecundity and (B) egg hatching of *O. albidipennis*. Values are mean ± SE from three replicates.

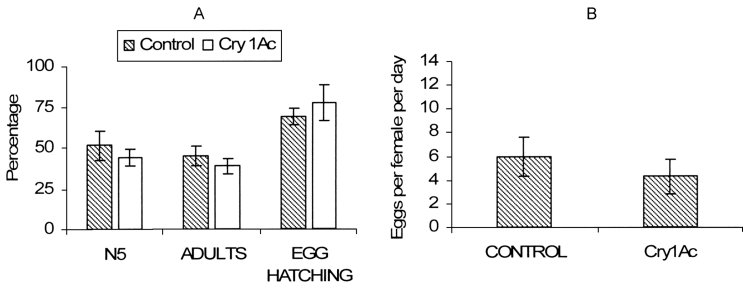

Fig. 3. Effect of directly supplied Cry1Ac at a concentration of 1 mg/ml on *O. albidipennis* fitness. (A) Percentages of individuals that reached fifth instar, adulthood, and egg hatching. (B) Fecundity. Values are mean ± SE from three replicates.

Cry1Ac-s and Cry2Ab can be explained by slight differences in temperature and management of the insects during the course of the experiments that were conducted over a period of 1.5 yr.

However, *Bt* toxins ingested by prey do not modify its activity, at least that of Cry1Ab (Head et al. 2001, Obrist et al. 2006a), which is important when drawing conclusions about possible effects on nontarget organisms such as predators. The quantity of *Bt* toxins ingested by caterpillars when they feed on *Bt* plant tissues can be low, not enough to produce detrimental effects even on other populations of the same species (Head et al. 2001), but can produce a suboptimal quality in the prey that could affect the predator (Dutton et al. 2002).

It is interesting to note that Torres et al. (2006) did not detect the Cry1Ac toxin in the predator *O. insidiosus* after it fed on *S. exigua* prey that were kept on *Bt* cotton plants, probably because the intake by the predator was very low compared with a larger heteropteran like *P. maculiventris*, in which the toxin was positively detected. In direct ingestion experiments with the species *G. punctipes*, Torres et al. (2006) found that the rate of dilution of the Cry1Ac toxin was ≈100-fold. Thus, depending on the original concentration, the ingested toxin can be below the detection limit, which could account for the negative results obtained.

Direct ingestion of *Bt* toxins from solutions using very high concentrations of the toxins, which largely surpass those found in plant and prey tissues (a worst case scenario), have been studied in laboratory experiments with *C. carnea* (Hilbeck et al. 1998, Romeis et al. 2004, Rodrigo-Simón et al. 2006). In general, no harmful effects were found on different biological traits of the predator. Hilbeck et al. (1998) reported detrimental effects on survival and development of second- and third-instar *C. carnea*, but it has been argued that this could be caused by the suboptimal quality of the artificial diet used and possible interactions with the *Bt* toxin (Romeis et al. 2004). Torres et al. (2006) showed that *G. punctipes* (a heteropteran like *O. albidipennis*) ingests the Cry1Ac toxin from solution, although they did not study any effects on biological parameters. Our results on direct ingestion of the purified Cry1Ac toxin by *O. albidipennis* revealed no negative effects on its development, survival, fecundity, or egg hatching compared with the control. The low proportion of individuals that reached the adult stage was caused by the high mortality when nymphs were starved to force them to drink from both solutions (with and without toxin). The toxin was tested at a concentration of 1 mg/ml (equivalent to 1,000 ppm), which is much higher than the average concentration of this toxin in *Bt* cotton plant tissues (Greenplate 1999, Adamczyk et al. 2001, Torres et al. 2006), where it is normally expressed.

Many species of anthocorids and other heteropterans feed directly on plant tissues or ingest pollen (Lattin 1999), so it is also important to know if they can ingest *Bt* toxins in this way and if the toxins affect them. Obrist et al. (2006b) found that *O. majusculus* ingested great quantities of the toxin Cry1Ab through pollen in maize fields, and no effect has been found on development or different biological parameters of species like *O. insidiosus* and other predators (Pilcher et al. 1997) or *O. majusculus* (Pons et al. 2004) when fed with pollen of *Bt* maize.

In conclusion, the results obtained here showed that the three *Bt* toxins (Cry1Ac, Cry1Ab, and Cry2Ab) tested in laboratory trials do not seem to pose a risk for the anthocorid *O. albidipennis* when it is exposed through prey. The scenario roughly reflects what happens in the field, with the prey consuming an artificial diet containing a range of concentrations of *Bt* toxins similar to those found in plant tissues, and the predator supplementing its diet with *E. kuehniella* eggs as an alternative food. In these conditions, this ancothorid's intake of *Bt* toxins would be expected to be very low (Torres et al. 2006). Equally, direct ingestion of the Cry1Ac toxin at high concentrations by *O. albidipennis* nymphs did not produce negative effects on any of the parameters examined. This seems to confirm the general pattern that populations of anthocorids, as well as other predators, occurring in *Bt* crops such as cotton and maize are not endangered by *Bt* toxins, as has been shown in diferent field studies (Naranjo 2005a, b, Romeis et al. 2006, Sanvido et al. 2006).

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