EFFECTS OF CRY3Aa δ-ENDOTOXIN ON *PODISUS MACULIVENTRIS* (HEMIPTERA: PENTATOMIDAE) AND *COLEOMEGILLA MACULATA* (COLEOPTERA: COCCINELLIDAE).

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Department of Biology

Carleton University

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acceptance of the thesis

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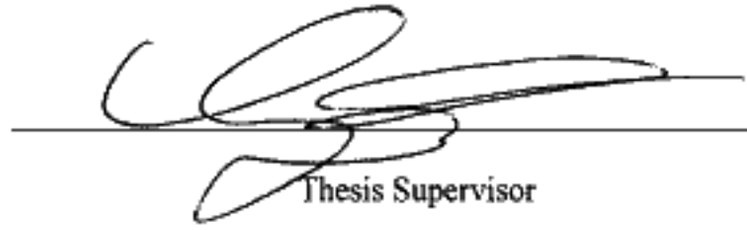

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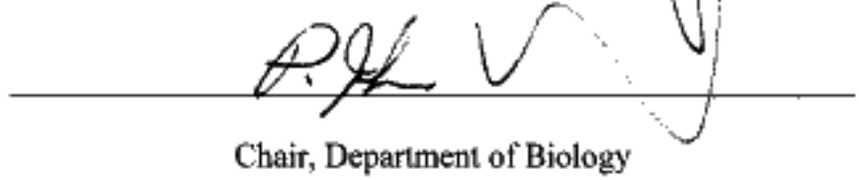

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ABSTRACT

In this study, I fed *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) and *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) different concentrations of purified Cry3Aa δ-endotoxin, the insecticidal protein expressed by NewLeaf™ transgenic potatoes. The maximum dose was 50µg Cry3Aa/g food for *P. maculiventris* and 200 µg Cry3Aa/g food for *C. maculata*. I found no effect on either developmental rate or survivorship for either species, even at the highest doses, strongly indicating that Cry3Aa poses virtually no risk to these predators. Frass from *C. maculata* in the highest dose treatment contained enough active Cry3Aa protein to cause significant mortality in early instar *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) (Colorado potato beetle). Colorado potato beetle showed no significant mortality when exposed to frass from *P. maculiventris* in either the highest dose treatment or an additional treatment of 500 µg Cry3Aa/g food, although some mortality was observed when they ingested *P. maculiventris* carcasses from the 500 µg/g treatment. This suggests that *P. maculiventris* digests Cry3Aa, while *C. maculata* does not.

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INTRODUCTION

Insect-resistant crops arrived in the agricultural marketplace in 1995 when Ciba Seeds released Maximizer™ corn with "built-in" Knockout™ control for European corn borers (ECB). Several companies followed suit with similar corn products, and in 1996 Monsanto Company released Bollgard® transgenic cotton and NewLeaf™ transgenic potatoes. All of these plants can be grouped as "Bt" transgenic crops—that is, plants that have been genetically engineered to express toxins that are ordinarily only found in subspecies of the bacteria, *Bacillus thuringiensis*[1].

B. thuringiensis is a group of bacteria that produces insecticidal crystal proteins (ICP) during sporulation. Different subspecies produce different ICPs, each of which contains the precursors (or protoxins) for one or more δ-endotoxins—the active proteins that can be acutely toxic to specific target insects. This specificity was the basis for grouping and naming Bt toxins (also known as Cry proteins) for almost 90 years, until Crickmore *et al.* developed the current nomenclature based on amino acid sequences (1993).

Transgenic corn, cotton, and potatoes, for example, each contain a different gene for expressing a δ-endotoxin that targets a different pest. Each introduced gene is a synthetic construct that has been optimized for expression in plants by increasing the cytosine:guanine base-pair ratio; these modifications, however, do not alter the final protein, which has an amino acid sequence identical to that of the wild-type δ-endotoxin (Perlak *et al.*, 1993). Several commercially-important Cry proteins and the *B. thuringiensis* subspecies in which they are found are shown in Table 1.

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[1] In this paper, *Bacillus thuringiensis* refers to the bacteria, while Bt refers to the bioinsecticide.

Table 1. Overview of some of the more widely used *Bacillus thuringiensis* toxins. (Crickmore *et al.*, 2002, and van Frankenhuyzen, K. and C. Nystrom, 2002)

Toxin	Bt subspecies	Target order	Target species for commercial applications	Commercial GM crops with toxin gene
Cry1Aa	*kurstaki, sotto, aizawai, entomocidus, dendrolimus*	Lepidoptera	diamondback moth, leafrollers	none
Cry1Ab	*berliner, kurstaki, aizawai*	Lepidoptera	European corn borer, corn earworm, spruce budworm, tent caterpillar, gypsy moth, diamondback moth, cabbage looper, tobacco budworm, and cabbage worm	corn
Cry1Ac	*kurstaki, kenyae*	Lepidoptera	cotton bollworm, tobacco budworm and pink bollworm, European corn borer	cotton, corn
Cry1Ca	*aizawai, entomocidus*	Diptera, Lepidoptera	mosquitoes	none
Cry2Aa	*kurstaki, sotto, kenyae*	Lepidoptera (Diptera?)	gypsy moth, tobacco budworm, European corn borer, mosquito	none
Cry3Aa	*san diego, tenebrionis, morrisoni*	Coleoptera	Colorado potato beetle	potato**
Cry4Aa	*israelensis*	Diptera	mosquitoes	none
Cry4Ba	*israelensis*	Diptera	mosquitoes	none
Cry9C	*tolworthi*	Lepidoptera	European corn borer	corn**

Insecticides containing cultures of *B. thuringiensis* subspecies and ICPs have been used as alternatives to chemical pesticides for decades. They degrade quickly in the presence of ultra-violet radiation (Pusztai *et al.*, 1991), thereby reducing the risks associated with environmental persistence. As well, they tend to be much more target-specific than synthetic insecticides because the Bt protein in the formulation cannot be processed into an active toxin or bound to an active site unless the environment inside the insect is suitable. Once an ICP has been ingested, it must dissolve in the insect's digestive fluids (requiring a specific pH), then be processed by specific enzymes into a truncated, active form that must bind to specific receptors before it can have any effect on the insect that consumed it (Oppert, 1999; Knowles, 1994; Gill, 1992; Li, 1991).

Elements of these environmental safeguards are lost when *B. thuringiensis* proteins are expressed in transgenic crop plants (e.g., see Jepson *et al.*, 1994; Schuler *et al.*, 1998; de Maagd *et al.*, 1999; Cannon, 2000; Hilbeck, 2001; and Obrycki *et al.*, 2001). Environmental persistence becomes a concern because the plants express the protein throughout the entire growing season, and possibly longer if residues are left in the soil (e.g. Stotzky, 2000). As well, the protein expressed by transgenic plants is several processing-steps closer to the active toxin than are the precursor forms applied in traditional Bt insecticides, possibly broadening the spectrum of activity (e.g. Hilbeck, 2001; Perlak, 1993).

Preserving healthy populations of beneficial, non-target insect species is a critical part of integrated pest management (IPM), which, in turn, is an essential strategy if farmers are to realize the economic and environmental benefits offered by transgenic crops. Predators and parasitoids can reduce the abundance of non-susceptible pest species

and stages, as well as help curb the rise of resistance in a population (Reed *et al.*, 2001; Riddick *et al.*, 2000; Arpaia *et al.*, 1997). It is, therefore, important to ensure that proteins expressed by transgenic crops do not accidentally intoxicate beneficial insects along with target pest species.

Naturally occurring predatory insects in potato fields include coccinellid and carabid beetles, tachinid flies, and predatory hemipterans, including members of the families Nabidae, Anthocoridae, Lygaeidae, and Pentatomidae. In this study, I examined the effects of chronic Cry3Aa exposure on nymphs of *Podisus maculiventris* Say (Hemiptera: Pentatomidae) and *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). Both species are native with ranges that span large areas in the United States and Canada. Both are also reared commercially as biocontrol agents and are considered important for controlling *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) (Colorado potato beetle (CPB)) ((Boiteau, 1988, in Biever and Chauvin, 1992; Groden *et al.* 1990; Arpaia, 1997).

I chose to focus on a hemipteran and a coccinellid because members of these groups possess digestive enzymes similar to those of chrysomelid beetles (including CPB): that is, they are cysteine-based (Terra and Ferreira, 1994). Tachinid flies and carabid beetles, on the other hand, have trypsin-based digestive enzymes, and therefore are less likely to possess the proteinases required to cleave the Cry3Aa protein into its active form.

The null hypothesis for this study is that there will be no difference in the developmental rate and the survival rate between predators reared on diet containing Cry3Aa δ-endotoxin and those reared on untreated diet. The alternative hypothesis is that

predators reared on Cry3Aa will take longer to develop or more will die before reaching maturity when they are reared on Cry3Aa.

I also conducted a much smaller investigation into the fate of Cry3Aa after ingestion by a predator. In particular, I wanted to know whether *P. maculiventris* or *C. maculata* were able to digest the toxin, thereby eliminating it from the environment, or if it passed right through, creating another route of exposure for non-target insects and possibly accumulating in the soil.

Background

Mechanism of action and CPB response to toxicity

Cry3Aa is one of the proteins produced by *B thuringiensis* subspecies *tenebrionis*, and *san diego* and has been genetically engineered into Monsanto Company's NewLeaf™ potato line[2]. It is toxic to insects from the families Tenebrionidae and Chrysomelidae, and is particularly effective against early-instar CPB, not only killing the insects but also quickly inhibiting feeding activity, thereby minimizing damage to foliage (Ferro and Gelernter, 1989).

Cry3Aa is typical of Bt toxins in that it damages the gut lining of insects (eg, Cannon, 1996; Gill *et al.*, 1992; Slaney *et al.*, 1992, Knowles, 1994). In susceptible insects like CPB, it recognizes specific receptors on the brush border membrane cells of the gut epithelium, binds, and penetrates the cell membrane. Aggregates form, creating pores that permit ions to rush into the cell, causing it to swell and eventually lyse. Within a matter of days (depending on the dose), sections of the midgut lining are destroyed and the insect dies.

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[2] This line was discontinued in 2001 when several fast food chains, including MacDonald's, bowed to public pressure and refused to buy genetically modified potatoes (Wall Street Journal REF).

Proteolytic processing

NewLeaf™ potatoes express a 68 kDa protein that appears to be the same as the δ-endotoxin from *B thuringiensis* subsp. *tenebrionis* (CFIA, 1996). Slaney *et al.* (1992) reported that this δ-endotoxin was "naturally truncated" and required no proteolytic processing to become active, based on their failure to find shorter, stable proteolytic fragments after treating it with CPB digestive juices. Later studies, however, have shown that digesting the protein with chrysomelid gut juices can produce smaller, stable fragments that are more soluble at chrysomelid gut pH (4.5-6.6) and more structurally suited to binding (Oppert, 1999; Carroll *et al.*, 1997).

Carroll *et al.* (1997) found that chymotrypsin from CPB midguts trimmed the δ-endotoxin to a 49 kDa protein that was soluble at pH 2.9 – 9.1 and bound readily to brush border membrane vesicles made from CPB midguts. This 49 kDa protein was not present in samples digested with yellow mealworm (*Tenebrio molitor*) gut extract; instead, a slightly larger (though still truncated) 55 kDa protein was found, closely associated with an 8-11 kDa fragment. This 55 kDa product was not soluble at the near-neutral pH of the mealworm midgut, even though mealworms are somewhat susceptible to Cry3Aa, suggesting that other factors may be involved. This raises the possibility that the final stages of processing and even the resulting activated toxin may differ between target species.

The degree to which the 68 kDa protein must be processed and the stability of the resulting products are important when considering non-target effects. If processing is required to activate a toxin, it is less likely to affect insects that do not possess the necessary proteases, or whose digestive tract is at the wrong pH. Current knowledge of insect digestive enzymes is rather sparse but, even so, it may be possible to identify

insects that may be at greater risk than others. *C. maculata* and *P. maculiventris*, for example, both have midguts at pH 5-6, which is in the same range as members of Chrysomelidae (Terra and Ferreira, 1994). As well, chrysomelids, coccinellids, and hemipterans all rely on cysteine proteinases as the predominant proteinases in their digestive systems—a physiological characteristic that is unique to Hemiptera and some members of Coleoptera (Terra and Ferreira, 1994).

Furthermore, if the final, activated toxin is stable, it might be passed on in its active form to non-target predators. This possibility has not been widely explored; however, Hilbeck *et al.* (1999) alluded to it in attempting to explain their findings that common green lacewings (*Chrysoperla carnea*) suffered higher mortality and greater delays in development when reared on Cry1Ab-fed prey compared to those fed Cry1Ab in artificial diet.

Effects of transgenic crop-fed prey on predators: previous studies

Given the number of insect species exposed to Bt toxins in the cause of pest control, there are few published studies assessing the toxicity of Bt toxins on non-target, predatory insect species (Tables 2 and 3). Of these, only 10 involve purified δ-endotoxins produced by transgenic crops (or the equivalent protein produced in *E. coli*), including findings from 2 groups that directly contradict each other.

In one study, Pilcher *et al.* (1997) found that ingesting pollen from transgenic corn plants (expressing Cry1Ab) had no effect on mortality of common green lacewings. Mortality rates were greater than 50% in both the treated and the control groups, however, possibly because the lacewings had to be coerced into eating the pollen by having their preferred food withheld for 24h periods at three points in their development. Even so, the authors concluded that lacewings were not susceptible to Cry1Ab.

Table 2. Studies investigating non-target effects of lepidopteran-specific Cry proteins on predaceous non-target species. L1=first instar; L2=second instar; L3=third instar; L4=fourth instar; A=adult; NS=not specified. Novo-biobit, Delfin, Dipel®, and Bactospeine are all commercial *B. thuringiensis* bioinsectides based on *B. thuringiensis* subsp. *kurstaki*.

Species	Order	Family	Life stage	Source of *cry* proteins	Exposure route	Response	Effect	Environment	Reference
Bembidion lampros	Coleoptera	Carabidae	A	Dipel	sprayed host plants	predation efficiency, mortality	0	field	Obadofin & Finlayson 1977
Coleomegilla maculata	Coleoptera	Coccinellidae	L1-A	transgenic corn (Cry1Ab)	pollen	development time, mortality	0	lab	Pilcher *et al.* 1997
C. maculata	Coleoptera	Coccinellidae	L1-A	transgenic corn (Cry1Ab)	pollen/prey	abundance	0	field	Pilcher *et al.* 1997
Hippodamia convergens	Coleoptera	Coccinellidae	A	Cry2A purified from *E. coli*	artificial diet	mortality	0	lab	Sims, 1995
H. convergens	Coleoptera	Coccinellidae	A	Cry1Ac purified from *E. coli*	artificial diet	mortality	0	lab	Sims, 1995
Orius insidious	Hemiptera	Anthocoridae	L1-A	transgenic corn (Cry1Ab)	pollen	development time, mortality	0	lab	Pilcher *et al.* 1997
O. majusculus	Hemiptera	Anthocoridae	L1-A	transgenic corn (Cry1Ab)	prey	development time, mortality	0	lab	Zwahlen *et al.* 2000
O. insidious	Hemiptera	Anthocoridae	L1-A	transgenic corn (Cry1Ab)	pollen/prey	abundance	0	field	Pilcher *et al.* 1997
Geocoris punctipes	Hemiptera	Lygaeidae	A	*B.t. kurstaki*: commercial preparation	prey (*Pseudoplusia includens*)	mortality	0	lab	Boyd & Boethel 1998
Dicyphus tamaninii	Hemiptera	Miridae	L3-L4	Novo-biobit® Delfin®	sprayed on foliage	mortality	0	lab	Castane *et al.* 1996
Nabis capsiformis	Hemiptera	Nabidae	A	*B.t. kurstaki*: commercial preparation	prey (*P. includens*)	mortality	0	lab	Boyd & Boethel 1998
Podisus maculiventris	Hemiptera	Pentatomidae	L3	*B.t. kurstaki*: commercial preparation	prey (*P. includens*)	mortality	0	lab	Boyd & Boethel 1998

Species	Order	Family	Life stage	Source of *cry* proteins	Exposure route	Response	Effect	Environment	Reference
P. maculiventris	Hemiptera	Pentatomidae	A	*B.t. kurstaki*: commercial preparation	prey (*P. includens*)	mortality	0	lab	Boyd & Boethel 1998
P. maculiventris	Hemiptera	Pentatomidae	A	Bactospeine	drinking water	mortality	0	lab	Mohaghegh *et al.* 2000
P. maculiventris	Hemiptera	Pentatomidae	L4	Bactospeine	drinking water	mortality	0	lab	Mohaghegh *et al.* 2000
Podisus. nigrispinus	Hemiptera	Pentatomidae	L2-A	Dipel	prey (*B. mori*)	fecundity	-	lab	Nascimento *et al.* 1998
P. nigrispinus	Hemiptera	Pentatomidae	L2-A	Dipel	prey (*B. mori*)	presence of *cry* proteins in gut and feces	+	lab	Nascimento *et al.* 1998
P. nigrispinus	Hemiptera	Pentatomidae	L2-A	Dipel	prey (*Bombyx mori*)	presence of *cry* proteins in hemolymph	0	lab	Nascimento *et al.* 1998
Tenodera aridifolia sinensis	Mantodea	Mantidae	L3-L4	*B.t. kurstaki*: commercial preparation	prey (*Trichoplusia ni*)	mortality	0	lab	Yousten 1973
Chrysoperla carnea	Neuroptera	Chrysopidae	nymphs	Cry1Ac purified from *E. coli*	mixed with *Sitotroga cerealella* eggs	mortality	0	lab	Sims, 1995
C. carnea	Neuroptera	Chrysopidae	nymphs	Cry2A purified from *E. coli*	mixed with *Sitotroga cerealella* eggs	mortality	0	lab	Sims, 1995
C. carnea	Neuroptera	Chrysopidae	L1-A	transgenic corn (Cry1Ab)	pollen	development time, mortality	0	lab	Pilcher *et al.* 1997
C. carnea	Neuroptera	Chrysopidae	L1-A	transgenic corn (Cry1Ab)	pollen/prey	abundance	0	field	Pilcher *et al.* 1997
C. carnea	Neuroptera	Chrysopidae	L1-A	transgenic corn (Cry1Ab)	prey (*Ostrinia nubilalis*)	development time, mortality	+	lab	Hilbeck *et al.* 1998a

Species	Order	Family	Life stage	Source of *cry* proteins	Exposure route	Response	Effect	Environment	Reference
C. carnea	Neuroptera	Chrysopidae	L1-A	transgenic corn (Cry1Ab)	prey (*Spodoptera littoralis*)	development time, mortality	+	lab	Hilbeck *et al.* 1998a
C. carnea	Neuroptera	Chrysopidae	L1-A	Cry1Ab purified from *B.t. kurstaki*	artificial diet	development time, mortality	+	lab	Hilbeck *et al.* 1998b
C. carnea	Neuroptera	Chrysopidae	L1-A	Cry1Ab purified from *B.t. kurstaki*	prey (*O. nubilalis*)	development time, mortality	+	lab	Hilbeck *et al.* 1999
C. carnea	Neuroptera	Chrysopidae	L1-A	Cry1Ab purified from *B.t. kurstaki*	prey (*S. littoralis*)	development time, mortality	+	lab	Hilbeck *et al.* 1999
C. carnea	Neuroptera	Chrysopidae	L1-A	Cry1Ab protoxin purfied from *B.t. kurstaki*	prey (*O. nubilalis*)	development time, mortality	+	lab	Hilbeck *et al.* 1999
C. carnea	Neuroptera	Chrysopidae	L1-A	Cry1Ab protoxin purfied from *B.t. kurstaki*	prey (*S. littoralis*)	development time, mortality	+	lab	Hilbeck *et al.* 1999
C. carnea	Neuroptera	Chrysopidae	L1-A	Cry2A purified from *B.t. kurstaki*	prey (*S. littoralis*)	development time, mortality	+	lab	Hilbeck *et al.* 1999
C. carnea	Neuroptera	Chrysopidae	L1-A	Cry2A purified from *B.t. kurstaki*	prey (*O. nubilalis*)	development time, mortality	+	lab	Hilbeck *et al.* 1999
C. carnea	Neuroptera	Chrysopidae	L1, L2	transgenic corn (Cry1Ab)	prey (*S. littoralis*)	prey preference	0	lab	Meier & Hilbeck 2001
C. carnea	Neuroptera	Chrysopidae	L3	transgenic corn (Cry1Ab)	prey (*S. littoralis*)	prey preference	-	lab	Meier & Hilbeck 2001
C. carnea	Neuroptera	Chrysopidae	L1, L2, L3	transgenic corn (Cry1Ab)	prey (*Rhopalosiphum padi*)	prey preference	0	lab	Meier & Hilbeck 2001

Table 3. Studies investigating non-target effects of coleopteran-specific Cry proteins on predaceous non-target species. N1=first instar; N2=second instar; N3=third instar; A=adult; NS=not specified. Novodor® and M-One® are commercial bioinsectides based on *B. thuringiensis* subsp. *tenebrionis*.

Species	Order	Family	Life stage	Source of *cry* proteins	Exposure route	Response	Effect	Environment	Reference
spider spp	NS	NS	NS	transgenic potatoes (Cry3Aa)	not specified	abundance	+	field	Riddick *et al.* 2000
Chauliognathus lugubris	Coleoptera	Cantharidae	A	Novodor	sprayed eggs (Chrysophtharta bimaculata)	predation efficiency	0	lab	Beveridge & Elek 1999
C. lugubris	Coleoptera	Cantharidae	A	Novodor	spray	mortality	0	lab	Beveridge & Elek 1999
Cicindela punctulata punctulata	Coleoptera	Carabidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
Poecilus chalcites	Coleoptera	Carabidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
Poecilus lucublandus lucublandus	Coleoptera	Carabidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
Scarites quadriceps	Coleoptera	Carabidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
Scarites subterraneous	Coleoptera	Carabidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
Coccinella septempunctata	Coleoptera	Coccinellidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
C. maculata	Coleoptera	Coccinellidae	N2-A	transgenic potatoes (Cry3Aa)	prey (L. decemlineata)	development time, live weight, fecundity,	0	lab	Riddick & Barbosa 1998
C. maculata	Coleoptera	Coccinellidae	N1-A	M-One	treated pollen	development time	+	lab	Giroux *et al.* 1994
C. maculata	Coleoptera	Coccinellidae	N1-A	M-One	treated pollen	mortality	0	lab	Giroux *et al.* 1994

Species	Order	Family	Life stage	Source of *cry* proteins	Exposure route	Response	Effect	Environment	Reference
C. maculata	Coleoptera	Coccinellidae	N3	M-One	treated eggs (L. decemlineata)	predation efficiency	-	lab	Giroux *et al.* 1994
Harmonia axyridis	Coleoptera	Coccinellidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
Hippodamia convergens	Coleoptera	Coccinellidae	N2-A	transgenic potatoes (Cry3Aa)	prey (Myzus persicae)N1-A	time between moults, pupal weight, fecundity, prey consumption, longevity	0	lab	Dogan *et al.* 1996
H. convergens	Coleoptera	Coccinellidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
O. insidious	Hemiptera	Anthocoridae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	Y1: + Y2: 0	field	Riddick *et al.* 2000
G. punctipes	Hemiptera	Lygaeidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000
Nabis spp	Hemiptera	Nabidae	NS	transgenic potatoes (Cry3Aa)	NS	abundance	0	field	Riddick *et al.* 2000

Another study, however, suggests that lacewings are susceptible to Cry1Ab. In 1998, Hilbeck *et al.* found that 62% of lacewings fed lepidopteran prey (ECB and *Spodoptera littoralis*) reared on Cry1Ab-transgenic corn foliage died before reaching adulthood, compared to 37% in the control group (fed lepidopteran prey reared on non-transgenic corn). In response to criticism from many sources, including the Select Committee on European Communities (1999), Hilbeck *et al.* conducted follow-up studies using prey reared on Cry1Ab +/- artificial diet (1999) and Cry1Ab +/- lacewing artificial diet (1998b). In both cases, lacewings exposed to Cry1Ab (in whatever form) experienced significantly higher mortality than those fed non-Cry1Ab diets.

Studies assessing non-target effects of Cry3Aa are especially scarce (Table 3). In 1994, Giroux *et al.* (1994) reared *C. maculata* on pollen and found that they developed significantly more slowly when the pollen had been treated with M-One™ , a commercial formulation of *B. thuringiensis* subspecies *san diego* from Mycogen Corporation. Dogan *et al.* (1996) fed aphids reared on Cry3Aa potatoes to ladybird beetles (*Hippodamia convergens*) and looked for effects on aphid consumption, development times (for each larval stage plus pupation), fecundity, and longevity. They found no difference between beetles fed aphids from transgenic potato plants expressing Cry3Aa and those in the control (i.e., fed non-transgenic potato plants). However, aphids feed on phloem sap and therefore may not ingest Cry3Aa at all (Raps *et al.*, 2001).

In 1998, Riddick and Barbosa conducted several experiments to determine whether CPB larvae reared on transgenic foliage expressing Cry3Aa had an effect on the appetite, development and fecundity of *C. maculata*. They found no effects, although their study has been criticized by Hilbeck *et al.* (2000) for several reasons, including very

high mortality in all treatments, including the controls (>50%) and failure to study first-instar *C. maculata*. In general, susceptibility to Bt toxins decreases with age (Wierenga *et al.*, 1996). Only second instars and older were used in this study.

Routes of exposure

C. maculata and *P. maculiventris* are not foliage feeders; however, pollen is an important element of *C. maculata*'s diet (Pilcher *et al.*, 1997), while *P. maculiventris* are polyphagous and will occasionally feed on plant juices. Consequently, there is a chance that they will ingest Cry3Aa directly, through plant matter. If they visit damaged transgenic plants, they might also become contaminated by plant juices and then ingest them while grooming.

More likely, the route of greatest exposure for generalist predators such as *P. maculiventris* and *C. maculata* will be by consuming prey that has fed on transgenic foliage. Hilbeck's studies with lacewings and lepidopteran prey (1998a and 1999) would seem to suggest that the toxin can be ingested by either a susceptible or a non-susceptible insect and still retain some activity. Head *et al.* (2001) demonstrated definitively that this was the case, at least with *Rhopalosiphum padi* and three lepidopteran species, including *Ostrinia nubilalis* (European corn borer (ECB)). They fed insects either transgenic or non-transgenic corn foliage for seven days, or artificial diet containing different concentrations of Cry1Ab for three days, and then measured the level of Cry1Ab in the insects' tissues using enzyme-linked immunosorbent assays (ELISA). Cry1Ab was detected in all species (although not in all treatments, depending on the concentration in the diet), with the highest levels found in the least susceptible species (i.e., the ones that fed more). They confirmed the activity of the toxin through bioassays in which aphids