

Israel Journal of Entomology
Vol. XXXII (1998) pp. 1-14

MECHANISMS OF INSECT RESISTANCE TO
BACILLUS THURINGIENSIS TOXINS (Keynote lecture)

WM. H. MCGAUGHEY AND BRENDA OPPERT

U.S. Grain Marketing Research Laboratory, ARS, USDA, Manhattan, Kansas 66502, USA

ABSTRACT

Insect resistance to *Bacillus thuringiensis* (*Bt*) toxins is of great concern because these toxins are being used in plant genetic transformation. Widespread use of the toxins in transgenic plants could lead to rapid onset of insect resistance not only to the toxins expressed in plants, but to conventional foliar applications of the toxins. This could seriously compromise the long-term value of this safe, environmentally-benign insecticide. Effective management of resistance depends in part on understanding the mechanisms involved in insect adaptation to these toxins. As research on insect responses to *Bt* toxins has increased, it appears likely that there are multiple resistance mechanisms. A large amount of data suggests that changes in toxin-binding events may lead to resistance development, either by changes in the receptor or in the affinity of binding. Recent evidence indicates that toxin solubility and/or proteinase activation in the insect midgut may be involved in some types of resistance. Postbinding events, such as receptor aggregation, pore formation, ionic fluxes, and insect recovery may also be involved. Multiple toxins, refugia, and high toxin doses have been proposed as strategies for minimizing resistance development. These practices will be discussed in light of what is known about resistance mechanisms, together with implications of cross resistance among *Bt* toxins.

KEY WORDS: *Bacillus thuringiensis*, insect resistance, *Plodia interpunctella*, review.

INTRODUCTION

Insect resistance to insecticides poses a serious agricultural and public health problem. More than 500 cases of pesticide resistant insects and mites have been described (Georghiou, 1990). *Bacillus thuringiensis* (*Bt*) has not escaped this problem. In the laboratory, at least eleven insect species have been selected for resistance to *Bt* δ -endotoxins. These include *Plodia interpunctella* (Hübner) (McGaughey, 1985), *Cadra cautella* (Walker) (McGaughey and Beeman, 1988), *Plutella xylostella* (Linnaeus) (Kirsch and Schmutterer, 1988), *Heliothis virescens* (Fabricius) (Stone et al., 1989; Gould et al., 1995), *Trichoplusia ni* (Hübner) (Estada and Ferré, 1994), *Spodoptera littoralis* Boisduval (Muller-Cohn et al., 1994), *Spodoptera exigua* (Hübner) (Moar et al., 1995), *Leptinotarsa decemlineata* (Say) (Whalon et al., 1993), *Chrysomela scripta* Fabricius (Bauer et al., 1994), *Culex quinquefasciatus* Say (Georghiou and Vasquez, 1982; Gill et al., 1992), and *Aedes aegypti* (Linnaeus) (Goldman et al., 1986). Of even greater concern is the recently reported field resistance of *Plutella xylostella* where *Bt* sprays have been intensively used (Kirsch and Schmutterer, 1988; Tabashnik et al., 1990; Hama et al., 1992; Shelton et al., 1993).

Genetically engineered *Bt*-plants are in commercial use. These plants offer several advantages over conventional insecticide application for controlling pests. However, widespread plantings of such genetically engineered crops will result in increased exposure to toxins, and may result in increased selection pressure for resistance. Continuous exposure to *Bt* probably accounts for the cases of resistance that have developed in the field. Resistance to *Bt* was first detected in *Plodia interpunctella*, an insect that infests stored grain products. Grain storage facilities provide a closed environment for the toxin and the pest, so that successive generations of the pest encounter a continuously high selection pressure. Discovery of resistance in the field in *Plutella xylostella* is also predictable in light of the ecology of the insect and the intensive use of *Bt* sprays. *Plutella xylostella* has low mobility and high reproductivity, and responded quickly to the selection pressures that were presented with repeated field applications of *Bt*. In species of insects that migrate freely and/or have low rates of reproduction, selection pressure for resistance may be less, at least delaying the onset of serious problems.

The mode of action of *Bt* is complex, and there are several different physiological or behavioral opportunities for resistance to occur. *Bt* produces insecticidal crystal proteins (ICP's) that must be solubilized and further processed by gut proteinases in order to bind to protein receptors in the guts of susceptible insects. Increasing evidence predicts that receptor/toxin aggregation leads to pore formation, followed by ionic imbalance and septicemia in the midgut cells. Although several different resistance mechanisms have been proposed (Gill et al., 1992), the most likely ones to date involve changes in *Bt* receptors or solubilization-activation of the crystal proteins. Receptor-mediated mechanisms may include the loss of *Bt* toxin binding sites, increases in non-specific binding not related to toxicity, and reduction in toxin/receptor aggregation associated with pore formation. Solubilization and/or proteinase-mediated resistance mechanisms could involve changes in gut pH or in proteinases involved in protoxin activation. Changes in gut physiology that cause a reduction in toxin solubility, ineffective toxin activation, or enhanced toxin and/or receptor-toxin degradation also would lead to insect adaptation to the toxins. Understanding the physiological basis for resistance development in different insect species exposed to different toxin formulations will provide the basis for more effective toxin selection and utilization.

Receptor mediated mechanisms

Studies in lepidopteran insects have indicated that toxin binding affinity to midgut receptors is responsible for toxin sensitivity or specificity among several different species (Knowles and Ellar, 1986; Haider and Ellar, 1987; Hofmann et al., 1988a,b; Van Rie et al., 1989, 1990a,b; Ferré et al., 1991; Ihara et al., 1993; Estada and Ferré, 1994; Ballester et al., 1994). Hofmann et al. (1988b) first established a relationship between binding affinity and differential toxicity in studies on the specificity of two δ -endotoxins toward *Pieris brassicae* (Linnaeus) and *Manduca sexta* (Linnaeus). Subsequent studies by Van Rie et al. (1989, 1990a) confirmed the role of specific receptors in determining susceptibility to lepidoptera-specific protoxins. In studies of the specificity of various toxins toward *Spodoptera littoralis*, *Manduca sexta*, and *Heliothis virescens*, a high degree of heterogeneity was found among binding sites. They proposed that some sites may bind a single toxin whereas others may bind two or more toxins. Similarly, specific toxins may bind to more than one site in some insect species.

It is generally assumed that *Bt* toxins must bind to the insect's gut membrane in order to be toxic (Ferré et al., 1991). There is growing evidence, however, that binding alone does not

account for toxicity (Gar 1995; Masson et al., 199; *Heliothis virescens* for s toxicity.

Evidence for receptor receptors that bind toxins used ^{125}I -labeled δ -endotoxin (BBMV) to correlate d CryIA(b). In resistant *Plu* and the insects were still

Examination of *Bt* toxin with *Plodia interpuncte xylostella* collected in th studies showed a reduced susceptible to CryIB and results were reported by)

However, other data Florida, and the Philippines technique called surface) the Philippines and Hawa in binding affinity for C *Plutella* that was resista BBMV assays (Tabashn: of all CryIA toxins in the indicated that resistance et al. (1995) found redu emphasizing the differ: may be an indication of that more than one mech

Initially, binding stud were involved in resista CryIA(b) that had been CryIA(b) and CryIA(c) concentration (MacIntos to CryIA(c) by *Heliothis* midgut receptors. Howe BBMV from a strain of *H* results suggest that alter of *Heliothis virescens*, b

Binding of CryIC tox binding affinity and ma: et al., 1995). No differ binding increased in the interfere in specific high

account for toxicity (Garczynski et al., 1991; Bravo et al., 1992; Ferré et al., 1995; Lee et al., 1995; Masson et al., 1995). For example, Lee et al. (1995) described multiple binding sites in *Heliothis virescens* for several *Bt* toxins, with some *Bt* receptors not actually involved in toxicity.

Evidence for receptor-mediated resistance development involves changes in the midgut receptors that bind toxins (Table 1). In studies of *Plodia interpunctella*, Van Rie et al. (1990b) used ¹²⁵I-labeled δ -endotoxins combined with larval midgut brush border membrane vesicles (BBMV) to correlate decreased receptor binding affinity and susceptibility to *Bt* toxin CryIA(b). In resistant *Plodia interpunctella*, binding sites for CryIC toxin remained functional and the insects were still susceptible to CryIC toxin.

Examination of *Bt* toxin binding in *Plutella xylostella*, using techniques similar to those used with *Plodia interpunctella*, revealed a similar resistance mechanism. Resistant *Plutella xylostella* collected in the Philippines were insensitive to CryIA(b), and membrane binding studies showed a reduced binding affinity for the toxin (Ferré et al., 1991). These insects were susceptible to CryIB and CryIC, with corresponding membrane binding to the toxins. Similar results were reported by Bravo et al. (1992) using immunohistology.

However, other data indicate that *Bt*-resistant strains of *Plutella xylostella* from Hawaii, Florida, and the Philippines differ in the nature or degree of changes in binding. Using a novel technique called surface plasmon resonance, toxin binding was studied in resistant insects from the Philippines and Hawaii. While there was a reduction in CryIA(c) binding sites, no difference in binding affinity for CryIA(c) was reported (Masson et al., 1995). A Hawaiian colony of *Plutella* that was resistant to all three CryIA toxins exhibited reduced binding of CryIA(c) in BBMV assays (Tabashnik et al., 1994a). However, histological examination revealed binding of all CryIA toxins in the same strain (Escriche et al., 1995). Yet, a study on insects from Florida indicated that resistance is due to changes in binding of CryIA(b) (Tang et al., 1996). Escriche et al. (1995) found reduced binding of CryIA(b) and CryIA(c) in this Florida strain, further emphasizing the differences between geographically isolated strains. These variable results may be an indication of biodiversity among different populations, and they may also indicate that more than one mechanism of resistance can occur within a single species.

Initially, binding studies in *Heliothis virescens* indicated that factors other than toxin binding were involved in resistance development to *Bt*. In a strain of *Heliothis virescens* resistant to CryIA(b) that had been expressed in *Pseudomonas fluorescens*, a decrease in binding of CryIA(b) and CryIA(c) was presumably compensated for by an increase in binding site concentration (MacIntosh et al., 1991). Gould et al. (1992) found that development of resistance to CryIA(c) by *Heliothis virescens* was not toxin-specific and could not be related to changes in midgut receptors. However, recently it was discovered that CryIA(a) toxin did not bind to BBMV from a strain of *Heliothis virescens* resistant to CryIA(c) (Lee et al., 1995). These mixed results suggest that altered binding may be involved in resistance development of some strains of *Heliothis virescens*, but other mechanisms may also be involved.

Binding of CryIC toxin to BBMV of CryIC-resistant *Spodoptera exigua* was lower in both binding affinity and maximum binding when compared to that of the susceptible strain (Moar et al., 1995). No differences in binding site concentration were observed, but non-specific binding increased in the resistant strain. Increases in non-specific binding could compete or interfere in specific high-affinity binding (Moar et al., 1995).

TABLE 1
Review of *Bt* toxin binding studies with gut proteins from resistant insects

Insect (toxin used for selection; reference)	<i>Bt</i> toxins used in binding assay	Assay methods	Difference observed from susceptible strain
<i>Plutella interpunctella</i> (Dipel; van Rie et al., 1990b)	CryIA(b), CryIC	¹²⁵ I-labeled toxins and BBMV	- Reduction in binding affinity of CryIA(b) - No difference in binding of CryIC
<i>Plutella xylostella</i> -Philippines (<i>kurstaki</i> ; Ferré et al., 1991)	CryIA(b), CryIB, CryIC	¹²⁵ I-labeled toxins and BBMV	- No binding of CryIA(b) - No differences in binding of CryIB and CryIC
(Bravo et al., 1992)	CryIA(b), CryIB	Immunohistology	- Reduced binding of CryIA(b) - No difference in binding of CryIB
(Masson et al., 1995)	CryIA(c)	Surface plasmon resonance	- Reduction in CryIA(c) binding sites - No difference in binding affinity for CryIA(c)
<i>Plutella xylostella</i> -Hawaii (<i>kurstaki</i> ; Tabashnik et al., 1994a)	CryIA(c), CryIC	¹²⁵ I-labeled toxins and BBMV	- Reduction in CryIA(c) binding - No differences in binding of CryIC - Revertant had restored binding of CryIA(c)
(Masson et al., 1995)	CryIA(c)	Surface plasmon resonance	- Reduction in CryIA(c) binding sites - No difference in binding affinity for CryIA(c)
(Escriche et al., 1995)	CryIA(a), CryIA(b), CryIA(c), CryIE	Immunohistology	- Specific binding of CryIA(a), CryIA(b), CryIA(c) - No binding of CryIE
<i>Plutella xylostella</i> -Florida (<i>kurstaki</i> ; Escriche et al., 1995)	CryIA(b), CryIA(c)	Immunohistology	- No binding of CryIA(b) or CryIA(c)
<i>Plutella xylostella</i> -Florida (<i>kurstaki</i> ; Tang et al., 1996)	CryIA(b), CryIB, CryIC	1) biotinylated toxin and tissue sections 2) biotinylated toxin and BBMV	- Reduction of CryIA(b) binding - No differences in binding of CryIB and CryIC
<i>Heliothis virescens</i> (CryIA(b) in <i>Pseudomonas fluorescens</i> ; MacIntosh et al., 1991)	CryIA(b), CryIA(c)	¹²⁵ I-labeled toxins and BBMV	- Reduction in binding affinity of CryIA(b) and CryIA(c) - Compensated by increase in binding site concentration
(HD-73; Gould et al., 1992)	CryIA(b), CryIA(c)	¹²⁵ I-labeled toxins and BBMV	- No difference in binding of either CryIA(b) or CryIA(c) toxin
<i>Heliothis virescens</i> (CryIA(c); Lee et al., 1995)	CryIA(a), CryIA(b), CryIA(c)	¹²⁵ I-labeled toxins and BBMV	- No binding of CryIA(a) - No differences in binding affinities of CryIA(b) and CryIA(c)
<i>Spodoptera exigua</i> (CryIC; Moar et al., 1995)	CryIC	¹²⁵ I-labeled toxin and BBMV	- 2-fold decrease in maximum binding of CryIC - 5-fold decrease in K _d - No difference in binding site concentration

These studies indicate resistance to *Bt* toxins, in some insects. Furthermore, this scenario greatly complicates resistance in the field.

Solubilization/proteinases

In addition to specific resistance is probably a factor in successful solubilization and activation of the toxin. It is considered unique to lepidopteran gut processing or activation (Ramirez and Real, 1996)

Gut proteases from dipteran target specificity (Haider and Ellar, 1987; Van Fan (1986) demonstrated that by gut proteases from mosquito gut proteases from *Pieris*. In a subsequent study, Haider and Ellar (1986) demonstrated that a protoxin yielded toxins in dipteran cells. When the proteases, a slightly smaller *albopictus* (Skuse) cells,

Others have also speculated at least in part the specificity of insect species, *Pieris brassicae*. They found a direct correlation between concentration of proteinases in the gut and resistance in *Pieris brassicae* (Jacquet et al., 1987, Lec

The first indirect evidence of *Bt* resistant strain of *Plutella* *kurstaki*-resistant strain. However, a study of resistant *Plutella* soluble gut proteinase activity showed that midgut proteinases in *kurstaki* (Oppert et al., 1996) the three strains indicate a resistant strain (unpublished data) (Oppert et al., 1996), lack of linkage of the absence of resistance is currently being investigated.

Some resistant insects also degrade the activated

¹²⁵ I-labeled toxins and BBMV	CryIA(b), CryIA(c)	- No difference in binding of either CryIA(b) or CryIA(c) toxin
¹²⁵ I-labeled toxins and BBMV	CryIA(a), CryIA(b), CryIA(c)	- No binding of CryIA(a) - No differences in binding affinities of CryIA(b) and CryIA(c)
¹²⁵ I-labeled toxin and BBMV	CryIC	- 2-fold decrease in maximum binding of CryIC - 5-fold decrease in K _d - No difference in binding site concentration
(HD-73; Gould et al., 1992)		
<i>Heliothis virescens</i> (CryIA(c); Lee et al., 1995)		
<i>Spodoptera exigua</i> (CryIC; Moar et al., 1995)		

These studies indicate that, while binding alterations may provide one mechanism of insect resistance to *Bt* toxins, other mechanisms may also contribute to resistance development in some insects. Furthermore, multiple mechanisms likely exist in some resistant strains. This scenario greatly complicates efforts to understand the genetics and progression of selection for resistance in the field.

Solubilization/proteinase mediated mechanisms

In addition to specific receptors for toxins, the initial processing of *Bt* toxins in the insects' guts is probably a factor in susceptibility to *Bt* toxins. Processing of ICP's in the insect gut involves solubilization and activation of the crystal proteins. Although activation was previously considered unique to lepidopteran-specific ICP's, recent evidence indicates that there may be some processing or activation involved with CryIII A Coleoptera-active toxins as well (Martinez-Ramirez and Real, 1996).

Gut proteases from different insects process *Bt* proteins differently and may influence their target specificity (Haider et al., 1986; Jaquet et al., 1987; Lecadet and Martouret, 1987; Haider and Ellar, 1987; Van Fankenhuyzen et al., 1991; Ogiwara et al., 1992). Studies by Haider et al. (1986) demonstrated that activation of *Bt* subsp. *colmeri* (now designated *aizawai*) inclusions by gut proteases from mosquitoes yielded proteins toxic primarily to mosquito cell lines, while gut proteases from *Pieris brassicae* yielded proteins toxic primarily to lepidopteran cells. In a subsequent study, Haider and Ellar (1987) found that trypsin activation of subsp. *aizawai* protoxin yielded toxins that bound to membrane proteins in lepidopteran cells, but not to dipteran cells. When the trypsin-activated toxins were further treated with *Aedes aegypti* gut proteases, a slightly smaller protein resulted that bound a membrane protein from *Aedes albopictus* (Skuse) cells, but not to lepidopteran cells.

Others have also speculated that the complement of proteinases in an insect gut determines at least in part the specificity of *Bt* toxins. Bai et al. (1990) studied the gut proteinases of three insect species, *Pieris brassicae*, *Mamestra brassicae* (Linnaeus), and *Spodoptera littoralis*. They found a direct correlation between the toxicity of *Bt* subsp. *thuringiensis* and gut protein concentration or proteinase activity. *In vivo* studies to determine whether insect-specific differences in gut proteases might influence the specificity of activated toxins have been inconclusive (Jacquet et al., 1987, Lecadet and Martouret, 1987, Van Frankenhuyzen et al., 1991).

The first indirect evidence of a proteinase involvement in *Bt* resistance was found in a *Bt* resistant strain of *Plodia interpunctella*. Midgut proteinase activity from susceptible and *Bt kurstaki*-resistant strains of *Plodia interpunctella* were similar (Johnson et al., 1990). However, a study of resistant insects selected with *Bt* subsp. *entomocidus* revealed much lower soluble gut proteinase activities, and these gut extracts processed *Bt* protoxin less efficiently than midgut proteinases from the susceptible parent strain or a strain resistant to *Bt* subsp. *kurstaki* (Oppert et al., 1994). Examination of the electrophoretic pattern of gut proteinases in the three strains indicates the absence of a major serine proteinase in the *entomocidus*-resistant strain (unpublished data). Since serine proteinases are involved in the activation of *Bt* protoxin (Oppert et al., 1996), lack of a critical *Bt*-activating enzyme could contribute to toxin resistance. The linkage of the absence of proteinase activity in the *entomocidus*-resistant strain with *Bt* resistance is currently being examined.

Some resistant insects may not only be able to retard the activation of *Bt* toxins, but they may also degrade the activated toxin faster than the susceptible strains. Differences in CryIA(b)

plants interspersed with non-*Bt* plants will not be as effective as separate plantings of non-*Bt* plants near a *Bt* field (Mallet and Porter, 1992; Halcomb et al., 1996), particularly where pests move between plants. The size of an effective refuge is currently being debated. Recommendations range from 4–25% of plantings to consist of non-*Bt* plants. Incorporation of smaller refuges will place more emphasis on such factors as synchronization of mating and distribution patterns of non-*Bt* plants in the field. More research is needed on actual *Bt* refugia in order to make effective decisions on their use in prevention of resistance.

The success of a temporal refuge, such as alternately planting *Bt* and non-*Bt* plants, depends on the stability of resistance once *Bt* selection is no longer applied. In general, susceptibility to *Bt* increases slowly once *Bt* selection is terminated, and the reversion to susceptibility is incomplete (Tabashnik et al., 1991, 1994a; Hama et al., 1992; McGaughey and Beeman, 1988; Sims and Stone, 1991; Rahardja and Whalon, 1995). However, Tabashnik et al. (1994a) found that reversal to susceptibility in one *kurstaki*-resistant strain of *Plutella xylostella* was rapid and complete. Yet this same strain was rapidly reselected for resistance, indicating that the genetic component for resistance was still present in the population. Rapid reselection for resistance has been recognized as a problem with chemical insecticides for over 30 years (Abedi and Brown, 1960; Keiding, 1967). Another study of *Plutella xylostella* found continued increases in resistance after repeated exposure to high concentrations of *Bt* subsp. *kurstaki*, and resistance in one of the selected colonies remained high after more than 20 generations without *Bt* selection (Tabashnik et al., 1995). This work not only implied that resistance was not due to a single locus, but it also suggested that at least one genotype responsible for resistance to *Bt* was stable.

Multiple toxin strategies and cross resistance

Multiple toxin strategies have been proposed to prevent resistance to *Bt* toxins (Georghiou, 1990; Stone et al., 1991; Van Rie, 1991). The number of *Bt* strains in public or private collections is estimated in the tens of thousands (Lambert and Peferoen, 1992), providing a wide choice of toxins. However, some *Bt* toxins that share sequence homology may have similar toxicity mechanisms. There is also a concern that multiple resistance may arise rapidly if individual resistance mechanisms have already been selected in different populations, mixing of resistance genes has occurred with mating, and then selection pressure is applied with more than one toxin (Grafius, 1995).

Genetic engineering has been employed to design *Bt* "hybrid" toxins that incorporate multiple toxin domains to enhance host spectrum and toxicity. Transfer of a CryIC toxicity domain to CryIE resulted in a new protein that had enhanced activity against *Spodoptera exigua* (Bosch et al., 1994). Domain III of CryIC, a major determinant of toxicity to *Spodoptera exigua* and *Mamestra brassicae*, was substituted into CryIA(b) (Maagd et al., 1996). The hybrid protein had enhanced activity against *Spodoptera exigua* when compared to the parental CryIA(b) and CryIC proteins. Hybrid toxins might also be useful in resistance management programs as alternatives to toxins that no longer kill insects.

The success of multiple toxins in preventing resistance primarily depends on the extent of cross resistance among the toxins (Table 2). A relatively narrow spectrum of cross resistance was described in *Plodia interpunctella* that were selected for resistance to Dipel, a commercial formulation of the HD-1 isolate of subsp. *kurstaki* (McGaughey and Johnson 1987). The insects

were resistant to δ -endotoxin but they remained susceptible to *entomocidus*, *aizawai*, *tc* subsp. *kurstaki*, have 82-1989). Therefore, cross-resistance is expected to be high. Apparent cross-resistance contained toxins other than

Unfortunately, subsequent studies on many if not all insect species already referred to on *H. zea* receptors bind multiple toxins for cross resistance to other

In cross resistance studies reported that resistance to *Bt* as expected because of the fact that CryIF is 70% identical to CryIA from *Plutella xylostella* from subsp. *kurstaki*, CryIA(a), CryIA(b) subsequent studies on *Plutella* insects readily developed resistance to *kurstaki* toxins tended to be rather different from the toxin composition of the

Selection for resistance to cross resistance. *Trichoplusia ni* to CryIA(a) or CryIA(c) demonstrated that a strain resistant to CryIA(a), CryIA(b) be resistant to CryIF (Grafius 1995). Interestingly, this resistance in field populations along with spores.

So far, no general pattern of toxin used for selection on *Bt* binding patterns may not be there probably are multiple mechanisms understand cross resistance will limit the effectiveness of resistance complicated and unpredictable

rate plantings of non-*Bt* particularly where pests debated. Recommendations incorporation of smaller mating and distribution of *Bt* refugia in order to

non-*Bt* plants, depends general, susceptibility to ion to susceptibility is hey and Beeman, 1988; nik et al. (1994a) found *xylostella* was rapid and licating that the genetic action for resistance has ars (Abedi and Brown, continued increases in *staki*, and resistance in ns without *Bt* selection as not due to a single stance to *Bt* was stable.

Bt toxins (Georghiou, s in public or private n, 1992), providing a homology may have nce may arise rapidly t populations, mixing e is applied with more

xins that incorporate r of a CryIC toxicity st *Spodoptera exigua* to *Spodoptera exigua* ., 1996). The hybrid are to the parental istance management

ends on the extent of n of cross resistance Dipel, a commercial n 1987). The insects

were resistant to δ -endotoxins of 32 isolates of subspp. *thuringiensis*, *kurstaki*, and *galleriae*, but they remained susceptible to some degree to at least 15 isolates of subspp. *kenyae*, *entomocidus*, *aizawai*, *tolworthi*, and *darmstadiensis*. CryIA toxins, a major component of subspp. *kurstaki*, have 82–90% homology in their amino acid sequences (Höfte and Whitley, 1989). Therefore, cross resistance among preparations containing CryIA toxins would be expected to be high. Apparently, the strains that were still active against the resistant insects contained toxins other than the CryIA-type.

Unfortunately, subsequent work has shown that cross resistance among toxins does occur in many if not all insect species, and general patterns of cross resistance may not exist. In work already referred to on *Heliothis virescens*, some toxins bind to multiple receptors and some receptors bind multiple toxins (Van Rie et al., 1989, 1990a). Thus, individual toxins can select for cross resistance to others.

In cross resistance studies on field populations of *Plutella xylostella*, Tabashnik et al. (1993) reported that resistance to *Bt* subspp. *kurstaki* caused minimal cross resistance to subspp. *aizawai*, as expected because of the CryIA component of the subspp. *aizawai* toxins. However, in a later study they reported significant cross resistance to CryIF (Tabashnik et al., 1994b), probably due to the fact that CryIF is 70–72% homologous to CryIA toxins (Bauer, 1995). A field population *Plutella xylostella* from the Philippines resistant to CryIA(b) was not resistant to *Bt* subspp. *kurstaki*, CryIA(a), CryIA(c), CryIB, or CryIC (Ferré et al., 1991; Ballester et al., 1994). In subsequent studies on *Plodia interpunctella* by McGaughey and Johnson (1987, 1992, 1994), insects readily developed resistance to mixtures of toxins. Strains selected using *Bt* subspp. *kurstaki* toxins tended to be resistant primarily to the CryIA toxins. However, those selected using *Bt* subspp. *aizawai* or *entomocidus*, which contain CryIC and CryID as well as CryIA toxins, tended to be rather broadly cross resistant to most *Bt* toxins, which reflected the broader toxin composition of the subspp. *aizawai* and *entomocidus* endotoxins.

Selection for resistance using individual toxins will more likely enable better understanding of cross resistance. *Trichoplusia ni* selected for resistance to CryIA(b) were not cross resistant to CryIA(a) or CryIA(c) toxins (Estada and Ferré, 1994). However Gould et al. (1992, 1995) demonstrated that a strain of *Heliothis virescens* selected for resistance to CryIA(c) was cross resistant to CryIA(a), CryIA(b), CryIB, CryIC, and CryIIA toxins. This strain was also found to be resistant to CryIF (Gould et al., 1995). A CryIC resistant strain of *Spodoptera exigua* was cross resistant to several toxins, including CryIA(b), CryIE, CryIH, and CryIIA (Moar et al., 1995). Interestingly, this same study found that insects were unable to develop resistance to a spore-crystal mixture. The authors suggested that transgenic plants could more readily induce resistance in field populations than formulated materials that contain multiple Cry proteins along with spores.

So far, no general patterns of cross resistance are apparent. Each insect species and each *Bt* toxin used for selection may present a different cross resistance pattern. Furthermore, receptor binding patterns may not always be indicative of cross resistance patterns. This suggests that there probably are multiple mechanisms of resistance which will greatly complicate efforts to understand cross resistance. Polygenic inheritance and the existence of multiple mechanisms of resistance will limit the use of multiple toxin strategies for managing resistance due to the complicated and unpredictable nature of cross resistance.

REFERENCES

- Abedi, Z.H. and Brown, A.W.A. 1960. Development and reversion of DDT-resistance in *Aedes aegypti*. *Canadian Journal of Genetics and Cytology* 2:252-261.
- Bai, C., Yi, S.-X., and Degheele, D. 1990. Determination of protease activity in regurgitated gut juice from larvae of *Pieris brassicae*, *Mamestra brassicae*, and *Spodoptera littoralis*. *Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent* 55:519-525.
- Ballester, V., Escriche, B., Mensua, J.L., Reithmacher, G.W. and Ferré, J. 1994. Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to CryIA(b). *Biocontrol Science and Technology* 4:437-443.
- Bauer, L.S. 1995. Resistance: A threat to the insecticidal crystal proteins of *Bacillus thuringiensis*. *Florida Entomologist* 78:414-443.
- Bauer, L.S., Koller, C.N., Miller, D.L. and Hollingworth, R.M. 1994. Laboratory selection of the cottonwood leaf beetles, *Chrysomela scripta*, for resistance to *Bacillus thuringiensis* var. *tenebrionis* δ -endotoxin. *Abstracts of the VIth International Colloquium on Invertebrate Pathology (Asheville, NC)*. p. 68.
- Bosch, D., Schipper, B., Van der Kleij, H., de Maagd, R.A. and Stiekema, W. 1994. Recombinant *Bacillus thuringiensis* crystal proteins with new properties: Possibilities for resistance management. *Bio-Technology (New York)* 12:915-918.
- Bravo, A., Hendrickx, K., Jansens, S. and Peferoen, M. 1992. Immunocytochemical analysis of specific binding of *Bacillus thuringiensis* insecticidal crystal proteins to Lepidopteran and Coleopteran midgut membranes. *Journal of Invertebrate Pathology* 60:247-253.
- Escriche, B., Tabashnik, B., Finson, N. and Ferré, J. 1995. Immunohistochemical detection of binding of CryIA crystal proteins of *Bacillus thuringiensis* in highly resistant strains of *Plutella xylostella* (L.) from Hawaii. *Biochemical and Biophysical Research Communications* 212:388-395.
- Estada, U. and Ferré, J. 1994. Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and selection for resistance to one of the crystal proteins. *Applied and Environmental Microbiology* 60:3840-3846.
- Ferré, J., Escriche, B., Bel, Y. and Van Rie, J. 1995. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins. *FEMS (Federation of European Microbiology Societies) Microbiology Letters* 132:1-7.
- Ferré, J., Real, M.D., Van Rie, J., Jansens, S. and Peferoen, M. 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. *Proceedings of the National Academy of Sciences of the United States of America* 88:5119-5123.
- Forcada, C., Alcácer, E., Garcerá, M. D. and Martínez, R. 1996. Differences in the midgut proteolytic activity of two *Heliothis virescens* strains, one susceptible and one resistant to *Bacillus thuringiensis* toxins. *Archives of Insect Biochemistry and Physiology* 31:257-272.
- Garczynski, S.F., Crim, J.W. and Adang, M.J. 1991. Identification of putative insect brush border membrane-binding molecules specific to *Bacillus thuringiensis* δ -endotoxin by protein blot analysis. *Applied and Environmental Microbiology* 57:2816-2820.
- Georghiou, G.P. 1990. Resistance potential to biopesticides and consideration of countermeasures. In: *Pesticides and Alternatives*. Edit. J.E. Casida. Elsevier, New York. pp. 409-420.
- Georghiou, G.P. and Vasquez, M.G. 1982. Assessing the potential for development of resistance to *Bacillus thuringiensis* var. *israelensis* toxin (BTI) by mosquitoes. *Mosquito Control Research Annual Report*. pp. 80-81.
- Gill, S.S., Cowles, E.A. and Pietrantonio, P.V. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. *Annual Review of Entomology* 37:615-636.
- Goldman, I.F., Arnold, J. subspecies *israelensis* *Invertebrate Pathology*
- Gould, F. 1988. Genetic e *Ecology & Evolution*
- Gould, F. and Anderson growth, behavior, & (Lepidoptera: Noctu
- Gould, F.A., Anderson, A analysis of a *Heliothis* some *Bacillus thuringiensis*
- Gould, F., Martinez-Ran spectrum resistance *National Academy of Sciences*
- Grafius, E.J. 1995. Is lo multiple insecticide
- Haider, M.Z. and Ellar, I *thuringiensis* crystal
- Haider, M.Z., Knowles, J insecticidal δ -endot larval gut proteases.
- Halcomb, J.L., Benedict, tobacco budworm o (Lepidoptera: Noctu
- Hama, H., Suzuki, K. ar *thuringiensis* formulatio Yponomeutidae). A
- Hofmann, C., Lüthy, P., *thuringiensis* to br *European Journal of Entomology*
- Hofmann, C., Vanderbrun Specificity of *Bacillus thuringiensis* binding sites in the *Academy of Science*
- Höfte, H. and Whiteley, J *cal Reviews* 53:242
- Ihara, H., Kuroda, E., *Bacillus thuringiensis*
- Jacquet, F., Hütter, R. *Applied and Environmental Microbiology*
- Johnson, D.E., Brookhouser Resistance to *Bacillus thuringiensis* midgut proteinases *55:235-244*.
- Keiding, J. 1967. Persistence of *Bacillus thuringiensis* *of Pest Control* 6:1
- Keller, M., Sneh, B., Strickland 1996. Digestion of *Bacillus thuringiensis* larvae of *Spodoptera*

- Goldman, I.F., Arnold, J. and Carlton, B.C. 1986. Selection for resistance to *Bacillus thuringiensis* subspecies *israelensis* in field and laboratory populations of the mosquito *Aedes aegypti*. *Journal of Invertebrate Pathology* 47:317-324.
- Gould, F. 1988. Genetic engineering, integrated pest management and the evolution of pests. *Trends in Ecology & Evolution* 3:S15-S18.
- Gould, F. and Anderson, A. 1991. Effects of *Bacillus thuringiensis* and HD-73 delta-endotoxin on growth, behavior, and fitness of susceptible and toxin-adapted strains of *Heliothis virescens* (Lepidoptera: Noctuidae). *Environmental Entomology* 20:30-38.
- Gould, F.A., Anderson, A., Reynolds, A., Bumgarner, L. and Moar, W.J. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to some *Bacillus thuringiensis* toxins. *Journal of Economic Entomology* 88:1545-1559.
- Gould, F., Martinez-Ramirez, A., Anderson, A., Ferré, J., Silva, F.J. and Moar, W.J. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. *Proceedings of the National Academy of Sciences of the United States of America* 89:7986-7990.
- Graffius, E.J. 1995. Is local selection followed by dispersal a mechanism for rapid development of multiple insecticide resistance in the Colorado potato beetle? *American Entomologist* 41:104-109.
- Haider, M.Z. and Ellar, D.J. 1987. Analysis of the molecular basis of insecticidal specificity of *Bacillus thuringiensis* crystal δ -endotoxin. *Biochemical Journal* 248:197-201.
- Haider, M.Z., Knowles, B.H. and Ellar, D.J. 1986. Specificity of *Bacillus thuringiensis* var. *colmeri* insecticidal δ -endotoxin is determined by differential proteolytic processing of the protoxin by larval gut proteases. *European Journal of Biochemistry* 156:531-540.
- Halcomb, J.L., Benedict, J.H., Cook, B. and Ring, D.R. 1996. Survival and growth of bollworm and tobacco budworm on nontransgenic and transgenic cotton expressing a CryIA insecticidal protein (Lepidoptera: Noctuidae). *Environmental Entomology* 25:250-255.
- Hama, H., Suzuki, K. and Tanaka, H. 1992. Inheritance and stability of resistance to *Bacillus thuringiensis* formulations of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* 27:355-362.
- Hofmann, C., Lüthy, P., Hütter, R. and Pliska, V. 1988a. Binding of the delta endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). *European Journal of Biochemistry* 173:85-91.
- Hofmann, C., Vanderbruggen, H., Höfte, H., Van Rie, J., Jansens, S. and Van Mellaert, H. 1988b. Specificity of *Bacillus thuringiensis* δ -endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. *Proceedings of the National Academy of Sciences of the United States of America* 85:7844-7848.
- Höfte, H. and Whiteley, H.R. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews* 53:242-255.
- Ihara, H., Kuroda, E., Wadano, A. and Himeno, M. 1993. Specific toxicity of δ -endotoxins from *Bacillus thuringiensis* to *Bombyx mori*. *Bioscience Biotechnology and Biochemistry* 57:200-204.
- Jacquet, F., Hütter, R. and Lüthy, P. 1987. Specificity of *Bacillus thuringiensis* delta-endotoxin. *Applied and Environmental Microbiology* 53:500-504.
- Johnson, D.E., Brookhart, G.L., Kramer, K.J., Barnett, B.D. and McGaughey, W.H. 1990. Resistance to *Bacillus thuringiensis* by the Indianmeal moth, *Plodia interpunctella*: Comparison of midgut proteinases from susceptible and resistant larvae. *Journal of Invertebrate Pathology* 55:235-244.
- Keiding, J. 1967. Persistence of resistant populations after relaxation of selection pressure. *World Review of Pest Control* 6:115-130.
- Keller, M., Sneh, B., Strizhov, N., Prudovsky, E., Regev, A., Koncz, C., Schell, J. and Zilberstein, A. 1996. Digestion of δ -endotoxin by gut proteases may explain reduced sensitivity of advanced instar larvae of *Spodoptera littoralis* to CryIC. *Insect Biochemistry and Molecular Biology* 26:365-373.

- Kirsch, K. and Schmutterer, H.** 1988. Low efficacy of a *Bacillus thuringiensis* (Berl.) formulation in controlling the diamondback moth, *Plutella xylostella* (L.), in the Philippines. *Journal of Applied Entomology* 105:249-255.
- Knowles, B.H. and Ellar, D.J.** 1986. Characterization and partial purification of a plasma membrane receptor for *Bacillus thuringiensis* var. *kurstaki* lepidopteran-specific δ -endotoxin. *Journal of Cell Science* 83:89-101.
- Lambert, B. and Peferoen, M.** 1992. Insecticidal promise of *Bacillus thuringiensis*. *Bioscience* 42:112-122.
- Lecadet, M.-M. and Martouret, D.** 1987. Host specificity of the *Bacillus thuringiensis* δ -endotoxin toward Lepidopteran species: *Spodoptera littoralis* Bois. and *Pieris brassicae* L. *Journal of Invertebrate Pathology* 49:37-48.
- Lee, M.K., Rajamohan, F., Gould, F. and Dean, D.H.** 1995. Resistance to *Bacillus thuringiensis* CryIA δ -endotoxins in a laboratory-selected *Heliothis virescens* strains is related to receptor alteration. *Applied and Environmental Microbiology* 61:3836-3842.
- Maagd, R.A., Kwa, M.S.G., Van der Klei, H., Yamamoto, T., Schipper, B., Vlask, J.M., Stiekema, W.J. and Bosch, D.** 1996. Domain III substitution in *Bacillus thuringiensis* delta-endotoxin CryIA(b) results in superior toxicity for *Spodoptera exigua* and altered membrane protein recognition. *Applied and Environmental Microbiology* 62:1537-1543.
- MacIntosh, S.C., Stone, T.B., Jokerst, R.S. and Fuchs, R.L.** 1991. Binding of *Bacillus thuringiensis* proteins to a laboratory-selected line of *Heliothis virescens*. *Proceedings of the National Academy of Sciences of the United States of America* 88:8930-8933.
- MacIntosh, S.C., Stone, T.B., Sims, S.R., Hunst, P.L., Greenplate, J.T., Marrone, P.G., Perlak, F.J., Fischhoff, D.A. and Fuchs, R.L.** 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. *Journal of Invertebrate Pathology* 6:258-266.
- Mallet, J. and Porter, P.** 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the strategy? *Proceedings of the Royal Society of London B. Biological Sciences* 250:165-169.
- Martinez-Ramirez, A.C. and Real, M.D.** 1996. Proteolytic processing of *Bacillus thuringiensis* CryIII A toxin and specific binding to brush-border membrane vesicles of *Leptinotarsa decemlineata* (Colorado potato beetle). *Pesticide Biochemistry and Physiology* 54:115-122.
- Masson, L., Mazza, A., Brousseau, R. and Tabashnik, B.** 1995. Kinetics of *Bacillus thuringiensis* toxin binding with brush border membrane vesicles from susceptible and resistant larvae of *Plutella xylostella*. *Journal of Biological Chemistry* 270:1-10.
- McGaughey, W.H.** 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science (Washington, DC)* 229:193-195.
- McGaughey, W.H. and Beeman, R.W.** 1988. Resistance to *Bacillus thuringiensis* in colonies of Indianmeal moth and almond moth (Lepidoptera: Pyralidae). *Journal of Economic Entomology* 81:28-33.
- McGaughey, W.H. and Johnson, D.E.** 1987. Toxicity of different serotypes and toxins of *Bacillus thuringiensis* to resistant and susceptible Indianmeal moths (Lepidoptera: Pyralidae). *Journal of Economic Entomology* 80:1122-1126.
- McGaughey, W.H. and Johnson, D.E.** 1992. Indianmeal moth (Lepidoptera: Pyralidae) resistance to different strains and mixtures of *Bacillus thuringiensis*. *Journal of Economic Entomology* 85:1594-1600.
- McGaughey, W.H. and Johnson, D.E.** 1994. Influence of crystal protein composition of *Bacillus thuringiensis* strains on cross-resistance in Indianmeal moths (Lepidoptera: Pyralidae). *Journal of Economic Entomology* 87:535-540.
- McGaughey, W.H. and Whal**
Science (Washington
- Moar, W.J., Pusztai-Cart**
Adang, M.J. 1995. I
(Hübner) (Lepidopte
- Muller-Cohn, J., Chau**
littoralis resistance t
Abstracts of the Vth
- Ogiwara, K., Indrasith, I**
thuringiensis subsp.
Invertebrate Pathol
- Oppert, B.S., Kramer, K.**
protoxin activation
interpunctella. Biocl
- Oppert, B., Kramer, K.J.,**
from *Plodia interpu*
Biochemistry and M
- Rahardja, U. and Whal**
tenebrionis CryIII A
of Economic Entom
- Shelton, A.M., Robertson**
and **Cooley, R.J.** 1
thuringiensis subsp
- Sims, S.R. and Stone, T**
Pseudomonas fluore
Invertebrate Pathol
- Stone, T.B., Sims, S.R., I**
Bacillus thuringien
Maramorosch. CRC
- Stone, T.B., Sims, S.R. :**
genetically enginee
ingiensis subsp. *kur*
- Tabashnik, B.E.** 1994. E
ogy 39:47-79.
- Tabashnik, B.E., Cushin**
to *Bacillus thuring*
Entomology 83:167
- Tabashnik, B.E., Finson**
1994a. Reversal of
National Academy
- Tabashnik, B.E., Finson**
Lessons from the d
84:49-55.
- Tabashnik, B.E., Finson**
thuringiensis toxir
mental Microbiolo

- (Berl.) formulation in es. *Journal of Applied*
- f a plasma membrane toxin. *Journal of Cell*
- s. *Bioscience* 42:112–
- ingiensis δ -endotoxin *issicae* L. *Journal of*
- us thuringiensis CryIA to receptor alteration.
- lak, J.M., Stiekema, 'ensis delta-endotoxin brane protein recogni-
- Bacillus thuringiensis* the *National Academy*
- ne, P.G., Perlak, F.J., *Bacillus thuringiensis* thology 6:258–266.
- s: are seed mixtures or *cal Sciences* 250:165–
- thuringiensis CryIIIa *notarsa decemlineata* 2.
- lus thuringiensis toxin ant larvae of *Plutella*
- thuringiensis. *Science*
- ziensis in colonies of *Economic Entomology*
- nd toxins of *Bacillus* Pyralidae). *Journal of*
- yalidae) resistance to *Entomology* 85:1594–
- omposition of *Bacillus* Pyralidae). *Journal of*
- McGaughey, W.H. and Whalon, M.E. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. *Science (Washington, DC)* 258:1451–1455.
- Moar, W.J., Puzsai-Carey, M., Van Faassen, H., Bosch, D., Frutos, R., Rang, C., Luo, K. and Adang, M.J. 1995. Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Applied and Environmental Microbiology* 61:2086–2092.
- Muller-Cohn, J., Chaufaux, J., Buisson, C., Gilois, N., Sanchis, V. and Lereclus, D. 1994. *Spodoptera littoralis* resistance to the *Bacillus thuringiensis* CryIC toxin and cross-resistance to other toxins. *Abstracts of the VIth International Colloquium on Invertebrate Pathology*. p. 66.
- Ogiwara, K., Indrasith, L.S., Asano, S. and Hori, H. 1992. Processing of δ -endotoxin from *Bacillus thuringiensis* subsp. *kurstaki* HD-1 and HD-73 by gut juices of various insect larvae. *Journal of Invertebrate Pathology* 60:121–126.
- Oppert, B.S., Kramer, K.J., Johnson, D.E., MacIntosh, S.C. and McGaughey, W.H. 1994. Altered protoxin activation by midgut enzymes from a *Bacillus thuringiensis* resistant strain of *Plodia interpunctella*. *Biochemical and Biophysical Research Communications* 198:940–947.
- Oppert, B., Kramer, K.J., Johnson, D., Upton, S.J. and McGaughey, W.H. 1996. Luminal proteinases from *Plodia interpunctella* and the hydrolysis of *Bacillus thuringiensis* CryIA(c) protoxin. *Insect Biochemistry and Molecular Biology* 26:571–583.
- Rahardja, U. and Whalon, M.E. 1995. Inheritance of resistance to *Bacillus thuringiensis* subsp. *tenebrionis* CryIIIa δ -endotoxin in Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 88:21–26.
- Shelton, A.M., Robertson, J.L., Tang, J.D., Perez, C., Eigenbrode, S.D., Preisler, H.K., Wilsey, W.T. and Cooley, R.J. 1993. Resistance of diamondback moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. *Journal of Economic Entomology* 86:697–705.
- Sims, S.R. and Stone, T.B. 1991. Genetic basis of tobacco budworm resistance to an engineered *Pseudomonas fluorescens* expressing the δ -endotoxin of *Bacillus thuringiensis* *kurstaki*. *Journal of Invertebrate Pathology* 57:206–210.
- Stone, T.B., Sims, S.R., MacIntosh, S.C., Fuchs, R.L. and Marrone, P.G. 1991. Insect resistance to *Bacillus thuringiensis*. In: *Biotechnology for Biological Control of Pests and Vectors*. Edit. K. Maramorosch. CRC Press, Boca Raton, FL. pp. 53–66.
- Stone, T.B., Sims, S.R. and Marrone, P.G. 1989. Selection of tobacco budworm for resistance to a genetically engineered *Pseudomonas fluorescens* containing the delta-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*. *Journal of Invertebrate Pathology* 53:228–234.
- Tabashnik, B.E. 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 39:47–79.
- Tabashnik, B.E., Cushing, N.L., Finson, N. and Johnson, M.W. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* 83:1671–1676.
- Tabashnik, B.E., Finson, N., Groeters, F.R., Moar, W.J., Johnson, M.W., Luo, K. and Adang, M.J. 1994a. Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Proceedings of the National Academy of Sciences of the United States of America* 91:4120–4124.
- Tabashnik, B.E., Finson, N. and Johnson, M.W. 1991. Managing resistance to *Bacillus thuringiensis*: Lessons from the diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* 84:49–55.
- Tabashnik, B.E., Finson, N., Johnson, M.W. and Heckel, D.G. 1994b. Cross-resistance to *Bacillus thuringiensis* toxin CryIF in the diamondback moth (*Plutella xylostella*). *Applied and Environmental Microbiology* 60:4627–4629.