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

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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, *Ploclia 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* 5-endotoxins. These include *Plodia interpunctella* (Hiibner) (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* (Hiibner) (Estada and Ferre, 1994), *Spodoptera littoralis* Boisduval (Muller-Cohn et al., 1994), *Spodoptera exigua* (Hiibner) (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 (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 ^{125}I-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)	*Bt* toxins used in binding assay	Assay methods	Difference observed from susceptible strain
Plodia interpunctella (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
Plutella xylostella-Philippines (*kurstaki;* 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)
Plutella xylostella-Hawaii (*kurstaki;* 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
Plutella xylostella-Florida (*kurstaki;* Escriche et al., 1995)	CryIA(b), CryIA(c)	Immunohistology	– No binding of CryIA(b) or CryIA(c)
Plutella xylostella-Florida (*kurstaki;* 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
Heliothis virescens (CryIA(b) in *Pseudomonas fluorescens;* 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
Heliothis virescens (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)
Spodoptera exigua (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 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 CryIIIA 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)

protoxin processing were described in *Bt*-resistant *Heliothis virescens* (Forcada et al., 1996). Not only did enzymes from an HD-73-resistant strain process the protoxin slower, they also hydrolyzed the toxin faster than those from a susceptible strain. Recent evidence indicates the increase in toxin degradation due to an increase in gut protease specific activity of fifth instar *Spodoptera littoralis* may account for the loss of sensitivity of fifth instar larvae to CryIC (Keller et al., 1996).

Other proteinase-mediated mechanisms, such as proteinase interaction with *Bt* receptors, have yet to be explored.

Implications in resistance management strategies

Microbial agents such as *Bt* have great potential in integrated pest management (IPM) programs. If they are solely used to replace existing pesticides, however, these agents could eventually induce resistance responses in insects. Transgenic plants may only be effective if IPM programs are designed to reduce selection pressure through reduced exposure to *Bt*. Strategies that have been proposed to slow the development of resistance include the use of high toxin doses, refugia for susceptible insects, and multiple toxins (Gould, 1988; McGaughey and Whalon, 1992). These specific recommendations are discussed in relationship to our current knowledge of resistance mechanisms.

High toxin dose

The use of very high doses of toxin evenly expressed in plants throughout the growing season may offer a viable means for managing resistance. This approach assumes that resistance is due to a single major gene and is recessively inherited. The strategy is to use a dose that is sufficient to kill 100% of the heterozygous insects, which are the most abundant carriers of resistance. Doses that are 20–30 times that needed to kill 99% of the susceptible insects are suggested. This approach is being recommended along with refuges to produce susceptible insects that will mate with the relatively rare homozygous resistant insects that occur.

Incorporation of sufficiently high amounts of toxins appears possible in some plants but perhaps not in others (Tabashnik, 1994). Also, in cotton there are indications that the toxin titer decreases toward the end of the growing season. This in itself could promote a late-season buildup of resistant insects. Another problem with the high toxin dose approach is that, while one concentration of *Bt* toxin may be the correct "high dose" for some target pests, other secondary or incidental pests may be innately more tolerant of the toxin (MacIntosh et al., 1990). These problems must be adequately addressed in order to use high toxin doses effectively in resistance management.

Refugia for susceptible insects

By minimizing the exposure of a target species to an insecticide, through both spatial and temporal refugia, its long-term efficacy may be preserved. Susceptible insects emerging from a refuge can reduce resistance development by diluting the gene pool of resistant insects. In order to be effective, the refuge must be arrayed in time and space to assure that susceptible insects are produced at the proper time and place to mate with selected populations from the transgenic crops.

Gould and Anderson (1991) suggested that planting resistant and susceptible plants in close proximity would provide a refuge from *Bt* toxin selection. There is some indication that *Bt*

TABLE 2
Insect cross resistance to *Bt* toxins

Insect (Reference)	Selection toxins	Response									
		aizawai	CryIA(a)	CryIA(b)	CryIA(c)	CryIB	CryIC	CryIE	CryIF	CryIH	CryIIA
Plodia interpunctella (McGaughey and Johnson, 1994)	*kurstaki*			+	+						
	aizawai		+	+	+	+	+				+
	entomocidus		+	+	+	+	+				+
Plutella xylostella (Hawaii) (Tabashnik et al., 1993)	*kurstaki*	+	+	+	+						+
(Tabashnik et al., 1994b)	*kurstaki*			+		–			+		
Plutella xylostella (Philippines) (Ferré et al., 1991)	*kurstaki*			+	–	–	–				
Ballester et al., 1994			–	+	–	–					
Trichoplusia ni Estada and Ferré, 1994	CryIA(b)		–	+	–						
Heliothis virescens Gould et al., 1992	CryIA(c)			+	+		(+)		+		(+)
Spodoptera exigua Moar et al., 1995	CryIC			+			+	+		+	+

+ = resistant; (+) = limited resistance; – = susceptible.

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-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 subsp. *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* subsp. *kurstaki* caused minimal cross resistance to subsp. *aizawai*, as expected because of the CryIA component of the subsp. *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* subsp. *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* subsp. *kurstaki* toxins tended to be resistant primarily to the CryIA toxins. However, those selected using *Bt* subsp. *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 subsp. *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.

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