

Implications of Cross-resistance among *Bacillus thuringiensis* Toxins in Resistance Management

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*Insect resistance is now recognized as a serious threat to the long-term usefulness of *Bacillus thuringiensis* (Bt) toxins in pest management. Because of the great diversity among Bt toxins found in nature, one of the most tempting resistance management strategies is to use two or more of these toxins in mixtures, rotations or sequences. Cross-resistance among toxins and the ability of insects to develop resistance to multiple toxins will limit the success of this approach. Studies have shown that there are large differences in the cross-resistance spectrum of the insect species that have been selected for resistance using single toxins or simple mixtures. Other studies have demonstrated that some species can readily develop resistance to mixtures of toxins, and that the use of strains of Bt that produce a wide array of toxins can cause broad-spectrum resistance to most Bt toxins. These factors tend to be specific to individual insect species and must be considered when attempting to manage insect resistance using multiple toxin strategies. Polygenic inheritance and the existence of multiple mechanisms of resistance may be involved in broad-spectrum resistance, and may limit the use of multiple toxin strategies for managing resistance.*

Keywords: *insecticide resistance, biological control, *Bacillus thuringiensis*, host plant resistance, transgenic plants*

INTRODUCTION

The potential for insect resistance is a primary concern in both conventional and transgenic plant applications of *Bacillus thuringiensis* (Bt) toxins. So far, at least six species of pest insects have been selected for high levels of resistance to Bt in the laboratory; two of these species have developed resistance to conventionally applied Bt outside the laboratory on growing crops or stored grain (Table 1). Resistance development on transgenic plants expressing Bt toxins has not been reported, but it could occur unless appropriate resistance management strategies are used.

Specific deployment strategies to ensure the durability of Bt toxins are being widely discussed. These tactics are generally patterned after those used or proposed for use in managing chemical insecticide resistance, and typically involve variations of:

- (1) rotation or alternation of toxins;
- (2) mixtures or sequences of toxins;
- (3) high or low doses of toxins;

TABLE 1. Insect species with high levels of resistance to *Bt* δ -endotoxins

Species	Author	Type of resistance
<i>Plodia interpunctella</i>	McGaughey (1985)	Laboratory, stored grain
<i>Cadra cautella</i>	McGaughey & Beeman (1988)	Laboratory
<i>Plutella xylostella</i>	Kirsch & Schmutterer (1988)	Growing crops
	Tabashnik <i>et al.</i> (1990)	Growing crops
<i>Heliothis virescens</i>	Stone <i>et al.</i> (1989)	Laboratory
<i>Leptinotarsa decemlineata</i>	Whalon <i>et al.</i> (1993)	Laboratory
<i>Chrysomela scripta</i>	Bauer <i>et al.</i> (1993)	Laboratory

- (4) encouraging survival of susceptible insects by providing refuges or promoting immigration; and
- (5) alteration of the tissue, timing or induction of *Bt* toxin expression in transgenic plants.

Each of these approaches has genetic, ecological and practical constraints. Readers are referred to the papers of Gould (1988a,b), Brunke and Meeusen (1991), Stone *et al.* (1991), Denholm and Rowland (1992), McGaughey and Whalon (1992), Whalon and McGaughey (1993) and others for reviews of the rationale and requirements for their use.

Perhaps the simplest resistance management strategies to implement are multiple toxin approaches that involve alternating or mixing toxins, or using one toxin until effectiveness is lost and then introducing another toxin (sequences). Mixtures may involve δ -endotoxins that naturally contain multiple toxins, tank mixes, multiple genes in transgenic plants, or the use of mixtures of plants that have been engineered with different genes. Such approaches have value if they are used with due regard for the behavior and genetics of the pest insect (see Curtis, 1985; Mani, 1985; Comins, 1986; Roush, 1989; Tabashnik, 1989; Tabashnik *et al.*, 1991; Mallet & Porter, 1992; Ferro, 1993; Whalon & McGaughey, 1993). Basically, multiple toxin tactics require two or more toxins that do not induce cross-resistance to one another.

Ideally, multiple toxin approaches should involve the use of *Bt* toxins in combination with other, unrelated toxins. However, since we now recognize extensive diversity among the *Bt* toxins found in nature, some workers have proposed that multiple toxin resistance management strategies might be developed using two or more different *Bt* toxins or genes (Georghiou, 1990; Stone *et al.*, 1991; Van Rie, 1991; Marrone & MacIntosh, 1993). Currently, we recognize 20 or more different insecticidal crystal proteins that are produced by *Bt*, and it is safe to assume that others will be discovered in the search for more effective insecticides (Table 2). Twelve of these, the CryI and CryII toxins, are toxic towards various pest species of Lepidoptera. The CryIII and CryV toxins are toxic towards certain Coleoptera. One of the CryII toxins and the four CryIV toxins are toxic towards certain Diptera. Recent reviews provide information on the *Bt* strains producing the various toxins, their molecular structure and the insect species affected (Hofte & Whiteley, 1989; Adang, 1991; Lereclus *et al.*, 1993; Yamamoto & Powell, 1993).

While the number of known toxins is not unlimited, the current array does represent substantial diversity, particularly for Lepidoptera. Several studies over the last 6 years have investigated mechanisms of insect resistance to *Bt* toxins and the extent to which resistant insects were resistant to different *Bt* toxins. This review examines that literature and suggests that the potential for using multiple *Bt* toxins in resistance management programmes may be limited in some cases by problems of cross-resistance among these toxins.

SPECIFICITY OF RESISTANCE

Most of the research on the specificity of resistance has been done on lepidopteran species. The initial reports of insect resistance to *Bt* toxins involved lepidopteran insects selected on laboratory diets, stored grain or growing crops treated with formulations of the HD-1 isolate of *Bt* subsp.

TABLE 2. Diversity of crystal proteins produced by *Bt*^a

Lepidoptera active	Coleoptera active
CryIA(a)	CryIIIA
CryIA(b)	CryIIIB
CryIA(c)	CryIIIB(b)
CryIB	CryVA
CryIC	
CryID	
CryIE	Diptera active
CryIF	CryIVA
CryIG	CryIVB
CryIIB	CryIVC
CryIIC	CryIVD
Lepidoptera/Diptera active	Non-specific
CryIIA	CytA

^aFrom Hofte and Whiteley (1989); Adang (1991); Yamamoto and Powell (1993); Lereclus *et al.* (1993).

kurstaki. This *Bt* strain reportedly produces five toxins: CryIA(a), CryIA(b), CryIA(c), CryIIA and CryIIB (Yamamoto & Iizuka, 1983; Whiteley & Schnepf, 1986; Hofte & Whiteley, 1989; Masson *et al.*, 1989; Widner & Whiteley, 1989). McGaughey and Johnson (1987) studied the response of *Plodia interpunctella*, selected for resistance to this *Bt* strain, to 57 isolates of eight different subspecies of *Bt* that were known to be toxic towards *P. interpunctella* (Table 3). The insects were resistant to virtually all isolates of subspp. *galleriae*, *kurstaki* and *thuringiensis*. However, the δ -endotoxins of 15 isolates of subspp. *aizawai*, *darmstadiensis*, *entomocidus*, *kenyae* and *tolworthi* were toxic towards the insects, suggesting that there was a degree of resistance specificity involved with *P. interpunctella* resistance to *Bt* subsp. *kurstaki*. Studies on the mechanism of resistance in *P. interpunctella* demonstrated greatly reduced binding affinity of midgut vesicles for CryIA(b) toxin, a primary component of the *Bt* formulation used in selecting the resistant insects, but no reduction in binding affinity for CryIC toxin (Van Rie *et al.*, 1990). Subsequent work on *Plutella xylostella* revealed similar results. Insects resistant to subsp. *kurstaki* showed reduced binding affinity and toxicity of CryIA(b) toxin, but no reduction in binding affinity or toxicity of CryIC or CryIB toxins (Ferré *et al.*, 1991). Tabashnik *et al.* (1993) reported that *P. xylostella* selected for resistance to subsp. *kurstaki* were only minimally cross-resistant to subsp. *aizawai*.

While Van Rie *et al.* (1990) and Ferré *et al.* (1991) did not investigate the binding of a wide range of toxins, it is likely that other CryI toxins remain active against *P. interpunctella* and *P. xylostella* resistant to subsp. *kurstaki*. Yamamoto and Powell (1993) indicate that production of CryIB, CryIC, CryID, CryIE, CryIF and CryIG toxins tends to be unique to subspp. *aizawai*, *entomocidus*, *tolworthi*, *darmstadiensis*, *kenyae* and *galleriae*. Several of these toxins could be involved in the activity of isolates of these subspecies against subsp. *kurstaki* resistant *P. interpunctella* and *P. xylostella*.

Resistance specificity in *Heliothis virescens* appears to be somewhat more complex than in *P. interpunctella* and *P. xylostella*. The initial studies on resistance mechanisms in this species by MacIntosh *et al.* (1991) demonstrated changes in toxin binding to midgut vesicles, but the work did not investigate binding of toxins other than CryIA. CryIA(b) and CryIA(c) toxins appeared to share the same binding site and the resistant insects were resistant to both toxins. However, the authors concluded that there was evidence of altered specificity of the receptor in the resistant insects.

Gould *et al.* (1992) carried out more extensive cross-resistance studies on a strain of *H. virescens* resistant to CryIA(c) toxin. They reported cross-resistance to a broad range of *Bt* toxins

TABLE 3. *Bt* isolates with activity against *Plodia interpunctella* resistant to isolate Hd-1, subsp. *kurstaki*^a

Subspecies	Number tested	Number effective
<i>aizawai</i>	14	10 ^b
<i>darmstadiensis</i>	4	4 ^b
<i>entomocidus</i>	1	1
<i>galleriae</i>	3	0
<i>kenyae</i>	2	2
<i>kurstaki</i>	17	0
<i>thuringiensis</i>	12	1 ^b
<i>tolworthi</i>	4	3

^aFrom McGaughey and Johnson (1987).

^bTwo of the active isolates of subsp. *aizawai*, three of subsp. *darmstadiensis* and the one active isolate of subsp. *thuringiensis* showed activity against housefly, which is evidence of β -exotoxin.

including CryIA(a), CryIA(b), CryIB, CryIC and CryIIA. The cross-resistance to other CryIA toxins was not surprising because the CryIA toxins are structurally and functionally very similar, and other studies have demonstrated that these toxins may bind to the same receptor in *H. virescens* (Van Rie *et al.*, 1989; MacIntosh *et al.*, 1991). The cross-resistance to the less closely related CryIB, CryIC and CryIIA toxins was surprising and in sharp contrast to findings of the previous studies on *P. interpunctella* and *P. xylostella*.

More recently, McGaughey and Johnson (1994) studied the resistance spectrum of *P. interpunctella* selected for resistance to *Bt* subspp. *aizawai*, *entomocidus* and *kurstaki*. The δ -endotoxins of subspp. *aizawai* and *entomocidus* contain a more diverse array of toxins than subsp. *kurstaki* (Table 4). The strains of subspp. *aizawai* and *entomocidus* studied produce CryIA(a), CryIA(b), CryIC, CryID and possibly other toxins, while subsp. *kurstaki* produces primarily the closely related CryIA toxins with small quantities of CryIIA and CryIIB. The resistance spectrum of selected *P. interpunctella* strains tended to reflect the toxin composition of the formulation used to select the insects, but there may also be some instances of the kind of cross-resistance reported by Gould *et al.* (1992). The subsp. *kurstaki* (CryIA)-selected insects showed low levels of cross-resistance to subspp. *aizawai* and *entomocidus* δ -endotoxins, which is to be expected since the subspp. *aizawai* and *entomocidus* δ -endotoxins contain substantial amounts of CryIA toxins as well as others. Hama *et al.* (1992), Tabashnik *et al.* (1993) and

TABLE 4. Cry protein composition of *Bt* isolates^a

Isolate	Cry proteins
HD-1 subsp. <i>kurstaki</i> ^b	CryIA(a), CryIA(b), CryIA(c), CryIIA, CryIIB
HD-112 subsp. <i>aizawai</i> ^c	CryIA(a), CryIA(b), CryIC, CryID, CryIG ^d , CryII ^e
HD-133 subsp. <i>aizawai</i> ^c	CryIA(a), CryIA(b), CryIC, CryID
HD-198 subsp. <i>entomocidus</i> ^c	CryIA(a), CryIA(b), CryIC, CryID

^aTable from McGaughey and Johnson (1994).

^bComposition reported by Yamamoto and Iizuka (1983); Whiteley and Schnepf (1986); Hofte and Whiteley (1989); Widner and Whiteley (1989); Masson *et al.* (1989).

^cDetermined by monoclonal antibody analysis (Hofte *et al.*, 1988) by Plant Genetic Systems, Ghent, Belgium.

^dPresence is uncertain.

^eNot determined whether CryIIA, CryIIB or CryIIC.

TABLE 5. Cross-resistance ratios of *P. interpunctella* colonies selected for resistance to isolates of *Bt* subsp. *aizawai*, *entomocidus* and *kurstaki* to various Cry toxins^a

Toxins	Colonies selected for resistance to				
	Dipel (<i>kurstaki</i>)	HD-112 (<i>aizawai</i>)	HD-133 (<i>aizawai</i>)	Dipel + HD-133	HD-198 (<i>entomocidus</i>)
Dipel	70	4	45	33	7
CryIA(a)	6	4	17	4	10
CryIA(b)	263	24	226	253	27
CryIA(c)	2816	27	789	2267	150
CryIB	13	7	44	17	9
CryIC	2	14	19	32	5
CryIIA	5	5	24	10	20

^aData summarized from McGaughey and Johnson (1994). Resistance ratios are the LC₅₀ of the resistant colony divided by the LC₅₀ of the unselected colony.

Shelton *et al.* (1993) have reported similar findings with *P. xylostella* and suggested the same explanation.

In *P. interpunctella* selected for resistance to subsp. *aizawai* and *entomocidus* δ -endotoxins, resistance spectra reflected the more diverse toxin composition of these *Bt* δ -endotoxins (Tables 4 and 5). As expected, the insects were resistant to varying degrees to each of the toxins represented in the δ -endotoxins used for selection. They were also resistant to CryIA(c), CryIB, and, in some cases, to CryIIA toxins which are not reported to occur in subsp. *aizawai* and *entomocidus* δ -endotoxins. Cross-resistance to CryIA(c) is not surprising because the extensive work on *H. virescens* has already suggested that the closely related CryIA toxins probably share the same binding sites in some species. An explanation for the cross-resistance to CryIB and CryIIA, which is similar to that in *H. virescens*, is not obvious. *P. xylostella* resistant to CryIA toxins remained susceptible to CryIB, suggesting separate binding sites or mechanisms in that insect species (Ferré *et al.*, 1991). Therefore, receptor specificity in *P. interpunctella* may differ from that in *P. xylostella*, or the CryIB cross-resistance may have resulted from selection by other unknown toxins in the δ -endotoxins used for selection. No data on receptors or resistance mechanisms for CryIB, CryIC, CryID or CryII toxins in *P. interpunctella* have been reported.

Information on the cross-resistance of coleopteran species is very limited. Two species, *Chrysomela scripta* and *Leptinotarsa decemlineata*, have been selected for resistance to CryIIIA toxin from *Bt* subsp. *tenebrionis* (Bauer *et al.*, 1993; Whalon *et al.*, 1993). Both species apparently show cross-resistance to other CryIII toxins (M. E. Whalon, personal communication; Bauer *et al.*, 1993).

RESISTANCE TO MULTIPLE TOXINS

Beyond the apparent cross-resistance that has been observed, it appears that some insect species can be readily selected for resistance to several different *Bt* toxins and to mixtures of toxins. This could seriously diminish the value of using multiple toxins even where cross-resistance does not occur. The initial reports of resistance in *P. interpunctella* (McGaughey, 1985; McGaughey & Beeman, 1988) and *P. xylostella* (Tabashnik *et al.*, 1990) involved selection with subsp. *kurstaki* δ -endotoxin, which is primarily a mixture of three closely related toxins, CryIA(a), CryIA(b) and CryIA(c), with apparently minor quantities of CryIIA and CryIIB (Table 4). More recently, it has been shown that *P. interpunctella* can be readily selected for resistance to the more diverse array of toxins, including CryIA(a), CryIA(b), CryIC, CryID and possibly others, contained in subsp. *aizawai* and *entomocidus* δ -endotoxins (McGaughey & Johnson, 1992). *P. interpunctella* also

readily developed resistance towards a mixture of subspp. *aizawai* and *kurstaki* δ -endotoxins, although the addition of subspp. *kurstaki* to subspp. *aizawai* may not add much diversity to the toxin composition. Adding subspp. *kurstaki* would primarily increase the number and proportion of CryIA toxins. The progression of resistance towards the more diverse mixtures was little, if any, slower than towards the simple mixture of CryIA toxins contained in HD-1 (Tabashnik & McGaughey, 1994). Even if cross-resistance is not a concern, this rapid progression of resistance towards mixtures provides little encouragement for multiple toxin resistance management approaches.

IMPLICATIONS IN RESISTANCE MANAGEMENT

The evidence currently available suggests both good and bad news regarding the extent of cross-resistance among *Bt* toxins. For the coleopteran species, the number of available toxins is probably too limited at present to consider using multiple *Bt* toxin approaches for resistance management. However, the discovery of additional Coleoptera-active toxins could offer promise for multiple toxin approaches. For the lepidopteran species, the outlook is mixed. In *P. interpunctella* and *P. xylostella* strains resistant to subspp. *kurstaki*, resistance tends to be specific for the CryIA toxins, leaving the opportunity to use other Lepidoptera-active toxins in resistance management programs. In contrast, *H. virescens* strains resistant to CryIA(c) toxin exhibit broad-spectrum resistance to other Lepidoptera-active toxins, appearing to limit the potential of mixtures for resistance management in this species. At present, the reason for the broader spectrum resistance in *H. virescens* is not known. It may be the result of a mechanism that is inherently broad spectrum and unrelated to receptor binding, or it may relate to the apparent polygenic inheritance of resistance in this species.

The implications of polygenic inheritance of resistance in resistance management have not been addressed in studies reported so far. The relatively narrow-spectrum resistance to subspp. *kurstaki* in *P. interpunctella* and *P. xylostella* appears to be controlled by a single major gene (McGaughey & Beeman, 1988; Hama *et al.*, 1992; Tabashnik *et al.*, 1992). In contrast, the much broader-spectrum resistance in *H. virescens* is associated with multiple genes (Sims & Stone, 1991; Gould *et al.*, 1992). While studies have not attributed *H. virescens* resistance to changes in midgut binding (MacIntosh *et al.*, 1991; Gould *et al.*, 1992), polygenic inheritance is, nonetheless, consistent with the suggestion that in *H. virescens* there are multiple midgut receptors, each having affinity for a different, but probably overlapping, group of toxins (Van Rie *et al.*, 1989). It would seem reasonable to expect each of these receptors to be encoded by a different gene. The additive progression of resistance described by Gould *et al.* (1992) would not only result in progressively higher levels of resistance, but might also result in more complex cross-resistance patterns as additional genes which encode different toxin specificities are selected. This kind of result could be expected whether resistance is due to changes in midgut receptors, other mechanisms or a combination of mechanisms. The net effect might be more complex and possibly broader-spectrum resistance with polygenic than with monogenic inheritance of resistance.

The particular mechanism of resistance will undoubtedly have an important bearing on the extent of cross-resistance among *Bt* toxins. To date, the only substantiated mechanism of resistance implicates alterations in specificity or affinity of midgut receptors for toxin. However, other completely different kinds of resistance mechanisms should not be dismissed. The entire sequence of events in *Bt* toxicology from crystal dissolution and activation through post-binding cell disruption must surely be subject to selective pressure. The question is whether other mechanisms will tend to be specific to individual Cry toxins or general to many or all of them. Based on the existing theories that associate midgut binding with toxin specificity, it is tempting to speculate that changes in either pre- or post-binding events might be broader spectrum than the binding event itself. This issue can be clarified only with the discovery and description of additional resistance mechanisms.

Obviously, the appropriate sequence for using either individual toxins or mixtures of toxins is

an important consideration in both conventional and transgenic plant deployment of *Bt*. If a particular toxin induces cross-resistance to another or several other toxins, its use should be discouraged if other toxins that do not induce cross-resistance are available. A similar potentially serious situation exists in conventional spray programmes using strains of *Bt* that produce a diverse array of toxins. While such preparations may be desirable because of their greater initial activity or activity against a broader range of pest insects, research on *P. interpunctella* suggests that they may be no more durable than δ -endotoxins that have less diverse toxin mixtures. Moreover, once resistance has been selected towards one of these broad-spectrum δ -endotoxins, there may be few or no remaining *Bt* toxins to use.

While our current understanding of cross-resistance among *Bt* toxins is incomplete, there is increasing evidence that the use of multiple *Bt* toxins may provide little benefit in the management of resistance to *Bt* in some insect species. Attempts to use multiple *Bt* toxins in resistance management should proceed cautiously, and include research to gain a better understanding of resistance mechanisms and cross-resistance in the target pest species.

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