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Problems of insect resistance to *Bacillus thuringiensis*

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Abstract

Insect resistance to *Bacillus thuringiensis* (Bt) has been a recognised problem for only about 6 years. It now seriously threatens both conventional and gene transfer uses for this environmentally safe biological insecticide. Since 1985, the potential for resistance has been demonstrated in at least five insect species, and high levels of resistance among field populations have been reported in one species. In two moth species, *Plodia interpunctella* and *Plutella xylostella*, the potential for resistance is widespread among diverse populations and laboratory studies suggest that it can progress to high levels within only a few generations. The mechanism of resistance in these species involves a change in binding affinity of the insects' midgut membrane that is specific for the particular toxin type used in selecting the resistant population.

Recognition of the inevitability of Bt resistance in insects has led to increased research on deployment strategies that might delay or prevent its evolution. Although resistance to Bt toxins expressed in genetically engineered plants has not been reported yet, it is imperative that resistance management tactics be developed before resistance reduces the pest control value of Bt. Currently, the focus of strategies for managing resistance is on techniques that minimise selection pressure, such as providing untreated refuges, and on the use of multiple toxins in various mixture, mosaic, rotational, or sequential patterns. Experimental data are needed to support the value of these approaches in different pest and cropping systems.

1. Introduction

The possibility of insects developing resistance to the δ -endotoxins of *Bacillus thuringiensis* (Bt) has been considered a serious threat to the use of these toxins in insect control programmes for only about 6 years. Before 1985, all efforts to select strains of insects resistant to the δ -endotoxins had failed, although Harvey and Howell (1965) had succeeded in selecting house fly colonies for significant levels of resistance to the β -exotoxin of Bt. The reasons are unclear. Because Bt had been used in control programmes for many years without any resistance being reported, many scientists concluded that resistance was unlikely to occur in practice even

though it might be theoretically possible (Burgess, 1971; Boman, 1981; Briese, 1981, 1986). It was suggested that multiple effects on the host insects or evolutionary advantages of the pathogen might preclude or greatly reduce the likelihood of insects becoming resistant to Bt. However, as the last few years have shown, resistance to Bt δ -endotoxins develops readily in many species of pest insects, both in the field and in the laboratory. Although the mechanism of toxin activity is not yet fully understood, studies on mechanisms of resistance in two insect species, *Plodia interpunctella* and *Plutella xylostella*, provide convincing evidence that, however complex the mode of action, this apparently is not a signifi-

cant impediment to the development of resistance in insects.

2. Implications of resistance

The implications of insect resistance to Bt are far-reaching. Bt is the most widely used and best understood of all microbial products that have been proposed for insect control. It is widely used in mosquito control and in forestry, home gardens, and the production of a wide array of food and fibre crops for protection primarily against lepidopteran pests. Its use provides a safe and environmentally friendly alternative to extensive use of chemical insecticides to combat many serious pests. Widespread development of insect resistance would seriously set back efforts to reduce chemical insecticide use. Insect resistance also threatens to diminish seriously the value of gene transfer technology. This technology, which relies heavily upon the use of the δ -endotoxin genes from Bt, promises the unprecedented pest control option of planting crops that have been genetically transformed to produce their own insecticidal toxin (Gasser and Fraley, 1989; Boulter et al., 1990). At the same time, however, it creates a situation in which pest populations may be exposed continuously, or at least for much longer periods than when conventional chemical applications are used. The continuous exposure of pests is widely presumed to lead to resistance more quickly than does intermittent exposure (Roush, 1989). Thus, one of the characteristics that makes the gene transfer approach so appealing may turn out to be one of its primary weaknesses.

3. Resistance discovered to date

Although insect resistance to transgenic plants has not yet been reported, within the last 6 years there have been several reports of insects being selected for significant levels of resistance to native and cloned δ -endotoxins under both laboratory and field conditions. In each case, the pest species apparently had the capacity to develop

resistance within only a few generations. The author's laboratory has found high levels of resistance to subspecies *kurstaki* in *Plodia interpunctella* and *Cadra cautella* (McGaughey, 1985; McGaughey and Beeman, 1988). In other laboratories, a strain of *Heliothis virescens* has been selected for resistance to toxins from subspecies *kurstaki* produced by genetically transformed *Pseudomonas fluorescens* (Stone et al., 1989).

Perhaps more significantly, within the last 2 years resistance to subspecies *kurstaki* has been reported from field populations of *Plutella xylostella* from Hawaii (Tabashnik et al., 1990). This represents the first well documented instance of resistance occurring in the field, although earlier reports had suggested the possibility of Bt resistance in populations of this pest species in the Philippines (Kirsch and Schmutterer, 1988) and in populations of *Plodia interpunctella* in grain bins in the USA (McGaughey, 1985; McGaughey and Beeman, 1988).

Apparently, at least one strain of *Leptinotarsa decemlineata* has been selected for resistance to a Coleoptera-active strain of Bt (Miller et al., 1990). Statistically significant resistance to Bt subspecies *israelensis* has been reported in the mosquitoes *Culex quinquefasciatus* and *Aedes aegypti* (Georghiou et al., 1983; Goldman et al., 1986).

4. Genetics and characteristics of resistance

The genetics, mechanisms, and practical significance of Bt resistance are not completely understood, but are under investigation in several insect species. Most of the information so far has come from studies on *Plodia interpunctella*, *H. virescens*, and *Plutella xylostella*.

The capacity for resistance is widespread in some species. In *Plodia interpunctella*, resistance has been selected in six colonies obtained from six different grain storage sites in the midwestern US (McGaughey and Beeman, 1988). In fact, selection efforts in the author's laboratory on recently colonised strains of *Plodia interpunctella* have never failed. Similarly widespread capacity for resistance apparently occurs in *Plutella xylos-*

tella, with reports of resistance from Hawaii (Tabashnik et al., 1990, 1991), the Philippines (Kirsch and Schmutterer, 1988), and the continental US (Shelton and Wyman, 1992). Other species have been studied less intensively, but it will not be surprising if widespread capacity for resistance is eventually found in many species.

In *Plodia interpunctella* and *Plutella xylostella*, resistance appears to be partially recessive and probably because of a single factor (McGaughey, 1985; McGaughey and Beeman, 1988; Tabashnik et al., 1992). However, Sims and Stone (1991) have characterised Bt resistance in *H. virescens* as being incompletely dominant and controlled by several genetic factors. A significant obstacle in conducting definitive genetic studies thus far has been that most of the resistant insect colonies have been selected using DiPel, a commercial Bt formulation that reportedly contains a mixture of toxins (Höfte and Whiteley, 1989). The study on *H. virescens* is a notable exception in that a single gene protein expressed in a genetically engineered *Pseudomonas fluorescens* strain was used in that work. When toxin mixtures are used, results may be complicated by different gene frequencies and rates of resistance progression or modes of inheritance of resistance to the various components of the mixture.

Indeed, the results with *Plodia interpunctella* provide evidence for this possibility. Resistance progressed at different rates and the degree of recessiveness differed among the colonies that were compared (McGaughey and Beeman, 1988). Until studies can be done using purified, single-gene toxins, it will not be possible to determine whether the variable responses were a result of the mixture of toxins or the involvement of several genes or alleles in resistance to one toxin. In some cases, resistance is very stable once selection is discontinued. This seems to be true particularly when resistance has progressed to higher levels. At lower levels, there appears to be gradual reversion back to a more sensitive level. In *Plodia interpunctella*, there was a decline in field-selected resistance when colonies were first being reared in the laboratory, but in highly resistant laboratory colonies, resistance declined slowly or

not at all when selection pressure was discontinued (McGaughey, 1985; McGaughey and Beeman, 1988). Similar gradual declines in resistance of populations of *H. virescens* and *Plutella xylostella* that were only moderately resistant have been reported (Sims and Stone, 1991; Tabashnik et al., 1991). However, data are not available from field situations where immigration of susceptible insects or fitness of resistant individuals may be much more important in determining the stability of resistance.

5. Mechanism of resistance

The mechanism of resistance in *Plodia interpunctella* and *Plutella xylostella* apparently involves a change in binding affinity of receptors or binding sites on the brush border membrane of the insect midgut (Van Rie et al., 1990b; Ferre et al., 1991). This appears to be the same mechanism that is involved in the host specificity of Bt δ -endotoxins. Other possible mechanisms of specificity have been proposed, including biochemical differences in the activation of the toxins in the insect gut (Haider et al., 1986; Johnson et al., 1990). However, increasing evidence indicates that specificity and resistance involve differences in binding affinity of the toxin proteins to receptor sites on the midgut membrane that presumably are of a glycoprotein nature (Knowles and Ellar, 1986; Hofmann et al., 1988a,b; Van Rie et al., 1989, 1990a,b; Ferre et al., 1991).

Hofmann et al. (1988b) were the first to establish a relationship between binding affinity and differential toxicity in studies on the specificity of two δ -endotoxins toward *Pieris brassicae* and *Manduca sexta*. Their approach involved studies with ^{125}I -labelled δ -endotoxins and brush border membrane vesicles prepared from the midguts of the larvae. Subsequently, the same technique was used to demonstrate that a similar phenomenon was involved in Bt-resistant *Plodia interpunctella* (Van Rie et al., 1990b). In the Bt-resistant larvae, there was a change in binding affinity and a parallel change in susceptibility that was specific for a Bt subspecies *kur-*

staki type toxin that had been used in selecting for resistance. This work demonstrated that, in the normally Bt-sensitive *Plodia interpunctella* larvae, there were at least two kinds of binding sites. In the process of acquiring resistance, the binding sites for the ssp. *kurstaki*-type toxin somehow became defective and failed to bind the toxin. Binding sites for another type of toxin, however, remained functional and the insects were still susceptible to this other type of δ -endotoxin.

Very recently, the same kinds of binding experiments have been done on resistant and susceptible *Plutella xylostella* (Ferre et al., 1991). In that work, researchers found a change in binding affinity that was very specific for the ssp. *kurstaki*-type toxin that had been used to select the resistant insects, whereas the insects remained sensitive and midgut membrane vesicles still bound two other types of δ -endotoxins. Similar binding experiments have been done on resistant and susceptible colonies of *H. virescens* and the results were not as conclusive, possibly because of the choice of toxins used in the experiments (MacIntosh et al., 1991).

The only evidence against involvement of the binding step in the mechanism of both resistance and specificity has been presented by Wolfersberger (1990). He found that, in *Lymantria dispar*, there was a negative relationship between binding affinity and toxicity of two different δ -endotoxins toward a single strain of insect. That is, the more toxic protein bound with less affinity than the less toxic one. However, Wolfersberger's results are consistent with the idea that there could be differences in toxicity as well as differences in binding affinity.

One of the most important points to emerge from these studies on strains of insects resistant to Bt is that in each case the resistance appears to be very specific for the toxin or toxins used in selection. The insects are not resistant to all δ -endotoxins. This is shown most clearly in studies on *Plodia interpunctella* that were selected for resistance to DiPel, a commercial formulation of the HD-1 isolate of ssp. *kurstaki* (McGaughey and Johnson, 1987). The insects were also resistant to δ -endotoxins of 32 isolates of ssp. *thurin-*

giensis, *kurstaki*, and *galleriae*. However, they remained susceptible to some degree to at least 15 isolates of ssp. *kenyae*, *entomocidus*, *aizawai*, *tolworthi*, and *darmstadiensis*. Apparently, the insects recognised something different about the structure and/or function of the δ -endotoxins from the latter group.

Insight into Bt toxin specificity and resistance is provided by the classification and nomenclature system presented by Höfte and Whiteley (1989) for the various insecticidal crystal protein genes of Bt. In general, they proposed a classification system in which the toxins produced by various strains of Bt were divided into four major groups that are distinguishable based upon structural and insecticidal spectra differences. The proteins they designated CryI are toxic toward Lepidoptera, the CryII proteins are toxic toward Lepidoptera and Diptera, the CryIII toward Coleoptera, and the CryIV toward Diptera. Groups I, II, and IV have been subdivided further based upon structural and spectral differences.

Production of these different proteins by Bt strains corresponds only very generally to the conventional taxonomic nomenclature that is commonly used. The nomenclature is based upon the serology of the vegetative cells. The type IV proteins are the ssp. *israelensis* toxins used in controlling certain aquatic Diptera. The type III proteins are produced by ssp. *tenebrionis* and *san diego* and are toxic to *Leptinotarsa decemlineata*. The type II proteins are produced by several subspecies, including *kurstaki*, *thuringiensis*, *tolworthi* and *kenyae*. Some are specific for Lepidoptera, but others also affect *Aedes aegypti*. The type I Lepidoptera-specific proteins are the most intensively studied and probably because of this are the largest and most heterogeneous group.

Höfte and Whiteley divided the type I proteins into six subgroups: CryIA(a), CryIA(b), CryIA(c), CryIB, CryIC, and CryID. A CryIE type has subsequently been added (Van Rie et al., 1990a). These type I proteins are produced by many different subspecies of Bt including *kurstaki*, *aizawai*, *berliner*, *thuringiensis*, *entomocidus* and *sotto*. Also, a particular isolate of

these subspecies often produces more than one of these subgroups of proteins. For example, ssp. *kurstaki* isolate HD-1 produces CryIA(a), CryIA(b) and CryIA(c) proteins.

In *Plodia interpunctella* and *Plutella xylostella*, resistance as indicated by both toxicity and midgut membrane binding assays has been shown to be specific for the CryIA type proteins that occur in the commercial formulation (DiPel, ssp. *kurstaki* HD-1) used in selecting the resistant insects (Van Rie et al., 1990b; Ferre et al., 1991). Although the response of CryIA-resistant larvae of these species toward all types of CryI proteins has not been determined, HD-1 resistant *Plodia interpunctella* remain sensitive to CryIC proteins and resistant *Plutella xylostella* remain sensitive to CryIB and CryIC proteins. These results indicate that different midgut receptors or binding sites might be involved with the major subgroups of CryI proteins and that resistance to one does not confer cross-resistance to the others. However, this has been studied only with CryIA-resistant insects. Until recently, strains of insects resistant to other protein types have not been available, but studies are now in progress on colonies of *Plodia interpunctella* that we have selected for resistance to several different protein types (McGaughey and Johnson, 1992).

Related studies by Van Rie et al. (1989, 1990a) suggest that this binding site specificity system may be highly complex. In studies of the specificity of CryIA(a), CryIA(b), CryIA(c), CryIC and CryIE toxin types toward *Spodoptera littoralis*, *M. sexta* and *H. virescens*, they observed a high degree of heterogeneity among binding sites. They suggested that different toxins may compete for the same binding sites in some cases, but not in others. Thus, a change at a site which binds more than one type of toxin would result in a degree of cross-resistance, while a change at a site which is specific for only one type of toxin would not. More recent studies have confirmed broad-spectrum resistance to Bt toxins in *H. virescens* selected for resistance to CryIA(c) (Gould et al., 1992). Obviously, additional research is needed in order to understand fully this system.

Based on the information now available, it can only be concluded that this binding site recogni-

tion or specificity system is relatively specific, but without certainty of the exact degree of specificity. The degree of specificity is, of course, of critical importance in designing deployment strategies which aim to prevent or delay the onset of resistance by using multiple toxins, either in mixtures or in a rotational system. Nothing would be gained if the toxins employed used the same midgut binding sites and thus posed cross-resistance problems. Rapid advances are likely in the immediate future from research in many laboratories to sort out the cross-resistance patterns among the various proteins.

6. Management of insect resistance to Bt

Now that the seriousness of insect resistance to Bt has been recognised and the mechanisms at least partially elucidated, attention is at last beginning to focus on developing deployment strategies that might delay or prevent its evolution. At present, most of the available guidance on strategies for managing insect resistance to Bt toxins is theoretical and lacking supporting experimental data. However, some workers (e.g. Roush, 1989) have suggested that such data may not be essential, as reasonably effective resistance management programmes can be developed based upon only a general knowledge of pest population biology and conservative assumptions about cross-resistance.

The problem of insect resistance to genetically engineered crop plants may present a somewhat unique situation, although there is an extensive body of literature on the durability of classical host plant resistance. Gould (1988) has discussed at length some of the theoretical aspects of genetically engineering crops for durable resistance. Gould (1986a,b) has also used simulation models to evaluate the durability of multiple host-plant resistance factors when deployed sequentially or as a mixture combined in a pyramided resistant cultivar. Gould (1986a,b), Roush (1989), and others have discussed the potential for delaying resistance by interplanting susceptible plants to provide untreated refuges for susceptible insects. Limiting the expression

of toxins to particular plant tissues or developmental stages has also been suggested as a means for reducing selection pressure (Van Rie, 1991).

At present, much of the effort toward resistance management or avoidance seems to focus on the presumption that there is an almost unlimited number of different Bt toxins available in nature and that resistance can be managed by using these in various mixture, mosaic, rotational, or sequential systems (Höfte and Whiteley, 1989; Georghiou, 1990). The theoretical aspects of various multiple toxin approaches have been examined by Curtis (1985), Mani (1985), Gould (1986a,b), Roush (1989) and Tabashnik (1989), among others. In general, these studies have tended to favour mixtures over other tactics, although no single approach has been consistently best in all pest-crop systems. Provision of untreated refuges to ensure the survival of susceptible genotypes tended to improve the durability of mixtures.

Experimental data to support the value of these resistance management tactics are sparse. Regrettably, the available, albeit preliminary, experimental evidence suggests that the benefits of some of these approaches may be relatively small. In *Plutella xylostella*, the only pest in which field resistance to Bt has been studied, concerns have been raised by Tabashnik et al. (1991) regarding the effectiveness of rotational strategies for managing resistance. They found that resistance levels declined rather slowly if at all when treatment was discontinued. Because of this, they suggested that alternating or rotational strategies that rely upon rapid restoration of sensitivity in the population when treatments are discontinued or changed might not be very effective for managing Bt resistance in *Plutella xylostella*. The studies on *Plodia interpunctella* support a similar conclusion (McGaughey, 1985; McGaughey and Beeman, 1988). Resistance in *Plodia interpunctella* tends to be very stable once it reaches high levels. In both species, however, it appears that low levels of resistance, as might occur after only a few generations of selection, might be sufficiently unstable that susceptibility could be restored using a rotational or alternating approach.

Further studies are being conducted on *Plodia*

interpunctella in order to answer some important questions regarding the multiple toxin approach to resistance management (McGaughey and Johnson, 1992).

(1) All extreme cases of resistance reported to date in Lepidoptera are toward Bt ssp. *kurstaki*. Is that subspecies of Bt unique in eliciting insect resistance, or will resistance develop toward other strains just as readily?

(2) Once resistance has developed to one strain of Bt, will resistance develop as quickly to a second strain?

(3) Will simultaneous use of two toxins significantly delay the development of resistance?

In general, these authors have found that resistance develops quickly to other strains including ssp. *aizawai* and *entomocidus* that are toxic toward HD-1 resistant *Plodia interpunctella* colonies. Furthermore, resistance develops quite readily to a second strain of Bt. A colony that was already resistant to isolate HD-1 of ssp. *kurstaki* quickly evolved resistance to a second strain, isolate HD-133 of ssp. *aizawai*. Thus, the fact that an insect population was already highly resistant to one type of Bt toxin was no obstacle to the development of resistance to another type.

Simultaneous selection for resistance to a mixture of isolates HD-1 of ssp. *kurstaki* and HD-133 of ssp. *aizawai* also resulted in the rapid evolution of resistance toward both strains of Bt. The rate of resistance progression was generally the same toward the two components of the mixture, but somewhat slower toward the mixture. Superficially at least, it appears as one might expect that the rate of progression of resistance to a mixture could be related to some product of the frequency of genes for resistance to the individual Bt strains.

Studies are currently in progress to determine whether individual isolates of Bt that produce complex mixtures of toxins are more durable than those producing single toxins or less complex mixtures.

7. Conclusions

Based upon the evidence available to date, the likelihood of insects evolving resistance to Bt

toxins is very high. It is not yet known how the rate of resistance progression toward genetically engineered crops will compare with conventional deployment, but it could very possibly be much faster. Clearly, care must be taken over the use of these toxins. They are a valuable pest control resource that cannot be wasted by using them in ways that disregard or encourage the development of insect resistance. It is not yet clear how much information on the ecology and population genetics will be needed to effectively manage resistance. Much good may be accomplished using the information currently available. As a minimum, however, for the use of straightforward sequential or pyramided deployment strategies such as many are now advocating, a full understanding of cross-resistance patterns among the available Bt toxins for each pest insect species will be needed. Acquiring these data may depend upon progress in cloning each of the toxins and developing laboratory colonies of the various species of pest insects that are resistant to several if not all of the toxins. However, it is likely that studies on one or two model systems will establish general cross-resistance patterns that will be applicable to several if not most pest insect species.

Experimental data are needed to support the value of multiple toxin approaches to resistance management as well as management strategies which attempt to reduce selection pressure or ensure the survival of susceptible insects in the pest population.

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