

## INSECT RESISTANCE TO *BACILLUS THURINGIENSIS* TOXINS

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### ABSTRACT

The long-term potential of the environmentally safe insecticidal toxins from *Bacillus thuringiensis* (Bt) are jeopardized by the increased use of Bt-transgenic plants in the field. Extensive cultivation of these plants will lead to escalated selection pressure for insects to develop resistance to Bt. Research on resistance mechanisms has shown that toxin binding to gut receptors is decreased in some resistant insects. However, proteinases are involved in determining the selectivity of toxins, and they are also involved in resistance development. Evidence is accumulating which demonstrates that different Bt preparations will select insect strains that resist Bt toxins in different ways, but how this happens is unclear. Effective resistance management depends on a full understanding of all selection factors that result in diverse resistance mechanisms. This article will briefly review the current data on receptor- and proteinase-mediated resistance mechanisms to Bt toxins.

### Introduction

The insecticidal toxins produced by the bacterium *Bacillus thuringiensis* (Bt) are an environmentally safe and effective way to control insects. Bt is relatively unique in the bacterial world because it shares the status of many chemical compounds designed for use in controlling economically and biomedically important insects. It is a crystalliferous spore-forming bacterium, ubiquitous in the soil, and is closely related to *B. cereus*. The term crystalliferous is applied to those *Bacillus* species that produce a discrete, characteristic inclusion body in addition to the endospore, which contains insecticidal crystal proteins (ICPs).

The various Bt subspecies contain approximately 50 distinct ICP genes that encode protoxins, denoted as Cry proteins, with different specificity for lepidopteran, coleopteran, or dipteran insects. An updated list of Bt toxin genes and a discussion of the latest accepted nomenclature can be accessed on the World Wide Web through a site maintained by Dr. Neil Crickmore at the University of Sussex in England ([http://epunix.biols.susx.ac.uk/Home/Neil\\_Crickmore/Bt/toxins.html](http://epunix.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/toxins.html)). In the case of lepidopteran-active Bt toxins, ICPs contain one or more protoxins in the Cry1 subclass with apparent molecular masses of approximately 130 kilodaltons (1).

For years, insects have developed immunity to many types of control products, and this has presented a serious challenge to their long-term use. Recent reports of resistance to Bt products, primarily spray formulations, indicates that this is also a problem

with microbial insecticides. Many insect species have been selected for resistance to Bt toxins (for a review, see 2). In addition, field resistance of the diamondback moth, *Plutella xylostella*, has been reported in locations where Bt sprays have been extensively used (3-6). These reports suggest that resistance development in the field is already a serious problem.

Plants that have been genetically engineered to express Bt toxins were developed for commercial use in 1996 and are now being cultivated in the field. The initial successes in insect control achieved with these transgenic plants will lead to expanded use of Bt crops. Concern has heightened that extensive planting of these crops will cause insects to encounter increased exposure to toxins, which could lead to additional selection pressure for resistance. Successful resistance management strategies will rely on a better understanding of the many mechanisms whereby insects develop resistance to Bt toxins used in both spray formulations and transgenic plants.

In order to understand how resistance may occur, it is helpful to examine what is known about the mode of action of Bt toxins. Ingestion of ICPs by a susceptible insect results in solubilization and processing by gut conditions, such as high pH, and proteinases. Lepidopteran-specific protoxins undergo limited proteolysis by midgut proteinases and are activated to toxins with sizes in the range of 60-70 kilodaltons, which are derived from the N-terminal portion of the protoxins. A cascade of events follows that leads to death of the insect, including binding of toxin to its

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midgut receptor(s), pore formation in the midgut cell membrane, ionic imbalance, lysis of the cells, and bacterial septicemia.

Our laboratory has been involved in studies to understand Bt resistance mechanisms. Much of the research on resistance mechanisms to Bt toxins implicates toxin receptors, but our laboratory and others are providing evidence that proteinases are also involved in resistance development. These diverse mechanisms must be fully characterized in order to develop effective Bt toxin-based control strategies that delay resistance development.

#### Receptor-Mediated Resistance To Bt

Resistance to Bt was first discovered in the Indian meal moth, *Plodia interpunctella* (7). Receptor-mediated resistance has since been substantiated by comparing the binding of various CryI toxins in *P. interpunctella* and several other insect species. In studies with the Indian meal moth, resistance to Bt toxin CryIAb was correlated with a 50-fold reduction in the affinity of toxin for its receptor in brush border membrane vesicles (8). Similarly, resistant strains of *P. xylostella* collected in the Philippines were insensitive to toxin CryIAb, and membrane binding and immunohistological studies showed a reduced binding affinity for that toxin (9, 10).

Other resistant strains of *P. xylostella* vary in their response to binding of CryI toxins. In one strain, the number of toxin binding sites was reduced, but no differences were observed in toxin binding affinity (11). Another strain that was resistant to three CryIA toxins exhibited reduced binding to only CryIAc in one type of binding assay (12) but showed binding to all three in another type (13). Studies using other strains have shown reduced binding to CryIAb and CryIAc (13, 14). Many of these strains were collected from different areas of the world, so that some of these conflicting data may be the result of genetic diversity among different populations of *P. xylostella*. Alternatively, these differences may reflect differences in assay procedures. However, they also argue for more than one mechanism of resistance operating within a single species.

Receptor-mediated resistance was proposed to occur in a strain of the tobacco budworm, *Heliothis virescens* (15), but resistance in another strain of *H. virescens* was not toxin-specific and could not be related to changes in midgut receptors (16). Data obtained recently suggest that there is a lack of binding to CryIAa toxin in a CryIAc-resistant strain of *H. vi-*

*rescens* (17). In the beet armyworm, *Spodoptera exigua*, reduced receptor binding to CryIC toxin was observed in a CryIC-resistant strain when compared with that of a susceptible strain (18). While no differences in binding site concentration were observed, non-specific binding to membrane proteins increased in the resistant strain, and it was proposed that non-specific binding sites competed with specific high-affinity toxin binding sites for the Bt toxin (18).

In summary, altered toxin binding to midgut receptors results in resistance development in some insects, but changes in binding cannot explain all cases of resistance. Information about alternate mechanisms is critical in developing effective resistance management plans for Bt-based control efforts.

#### Proteinase-Mediated Resistance To Bt

As previously discussed in the introduction, Bt ICPs are solubilized and processed in part by insect gut proteinases. Evidence indicates that the processing of ICPs can influence the toxin's spectrum of activity. Gut proteases from different insects process Bt protoxins differently, and this influences toxin selectivity (19-24).

Data to support a correlation between insect proteinases and toxin selectivity were provided by Haider *et al.* (19), in which they showed that protoxins from Bt subsp. *colmeri* (now designated *aizawai*) activated by dipteran proteases from mosquitoes were toxic only to dipteran larvae and cell lines, while those activated by lepidopteran proteases were toxic only to lepidopteran larvae and cells. Additional processing of the lepidopteran-selective toxin by dipteran gut proteases yielded a dipteran-selective form (22). This work was the first to emphasize the role of insect proteinases in determining the selectivity of Bt toxins.

Because proteinases are involved in the solubilization and activation of Bt toxins, they are suspected to control the degree of toxicity at an early step in the mechanism of action. In research on three lepidoptera, a direct correlation was found between the toxicity of Bt subsp. *thuringiensis* and both gut protein concentration and proteinase activity (25). It has also been proposed that accelerated toxin degradation in older larvae is due to an increase in gut protease specific activity, and this may account for the loss of toxin sensitivity in some older insect larvae (26).

Initial studies with *P. interpunctella* found no differences in midgut proteinase activity from susceptible insects and those that had been selected for resistance

using a Bt *kurstaki* preparation (27). However, a strain of resistant *P. interpunctella* insects that had been selected with another Bt preparation (subsp. *entomocidus*) had lower soluble gut proteinase activities (28, 29). Proteinases in gut extracts from the *entomocidus*-resistant insects processed the Bt protoxin less efficiently than those from the susceptible parent strain or the *kurstaki*-resistant strain. Recent data indicates a major serine proteinase is missing in the *entomocidus*-resistant strain (30). Our previous studies indicate that serine proteinases activate Bt protoxins in *P. interpunctella* (29). If the major serine proteinase missing in the *entomocidus*-resistant strain is one of the major Bt-activating enzymes, less protoxin would be activated in the guts of these insects, which could provide protection against Bt toxicity. Our laboratory is currently investigating in several lepidopteran species the role of this specific proteinase in Bt toxin activation and the possibility of a genetic linkage between the absence of this proteinase and the occurrence of resistance to Bt toxins.

Similar to what was observed in *P. interpunctella*, differences in Cry1Ab protoxin processing were also described between Bt-resistant and -susceptible strains of *H. virescens* (31). However, not only did enzymes from a resistant strain hydrolyze protoxin more slowly than those from a susceptible strain, but subsequent hydrolysis of the activated toxin was also faster with resistant enzymes. These data suggest that proteinases from some resistant insects, besides activating Bt toxins inefficiently, also degrade the activated toxin faster than enzymes from the susceptible strains.

Although protoxin activation was previously considered unique to lepidopteran-specific Cry proteins, recent evidence suggests that it also occurs in other insect orders. Activation of Cry3A by gut enzymes from the Colorado potato beetle, *Leptinotarsa decemlineata*, produced a protein that bound to midgut receptors in that insect (32). Thus, proteolytic processing of Coleoptera-active toxins occurs as well. A suspension of crystalline Cry3A was toxic to the potato aphid, *Macrosiphum euphorbiae*; yet Cry3A, which was solubilized and filtered to remove spores or crystalline protoxin, lacked activity (33). These researchers proposed that the toxin is more potent as a suspension due to a need for slow solubilization in the aphid midgut.

When these data are compared, it appears that activation/solubilization processes occur in orders other than Lepidoptera and might therefore be utilized by

different species to avoid toxicity. Other kinds of proteinase-mediated resistance mechanisms, such as proteinase interactions with Bt receptors, are yet to be explored. This type of information will be critical for understanding the importance of insect adaptation to Bt, which is mediated by either proteinase or receptor alterations.

### Conclusions

With the arrival of Bt-transgenic plants in the field, concern has increased about the possibility of widespread resistance by insects to these toxins. The long-range potential for these transgenic plants is questionable if effective resistance management strategies are not employed. All resistance mechanisms to Bt-toxins must be fully understood to develop successful resistance management plans. So far, two mechanisms have been identified, one receptor-mediated and the other proteinase-mediated. Others are anticipated.

From our studies with Bt-susceptible and -resistant strains of *P. interpunctella*, it appears that both the genetic makeup of the insect and specific components of the toxin preparation are important in the selection of resistance mechanisms. For example, we can select for reduced proteinase activity only with certain Bt preparations, but none of these preparations will select for the lower activity if insect populations do not have some individuals with the altered proteinase phenotype. Understanding the physiological bases for resistance development in different insect species exposed to different toxin formulations will provide for more effective toxin selection, utilization, and durability. Once all of the diverse resistance mechanisms are fully described, resistance management plans can be revised to specifically incorporate control measures that would prevent the selection of various types of resistance.

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Mention of a proprietary product does not constitute a recommendation or endorsement by the USDA. The USDA is an equal opportunity/affirmative action employer, and all agency services are available without discrimination.

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