6th Pacific Rim Conference on the Biotechnology of Bacillus thuringiensis *and its Environmental Impact,* Victoria BC, 2005
Côté, J.-C., Otvos, I.S, Schwartz, J.-L. and Vincent, C. (eds)

Bt Resistance Management: Have We Been Lucky or Smart?

Anthony M. Shelton[1], Jian-Zhou Zhao, and Ping Wang

Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY, USA*, 14456.*

Keywords: *Bacillus thuringiensis*, resistance, plant, insect

Prior to the development of plants genetically engineered to express insecticidal crystal proteins (ICP) from *Bacillus thuringiensis* (Bt), *Bt* had been a relatively minor insecticide, with total sales <2% in the annual $8 billion global market. However by 2004, 22.5 million hectares of *Bt* crops (maize and cotton) were grown worldwide, making *Bt* one of the most widely used insecticides. *Bt* plants were first commercialized in 1996 amid concern from some scientists, regulators and environmentalists that the widespread use of *Bt* crops would inevitably lead to resistance and the loss of a highly valued, safe insecticide. Ten years later, there has been no evidence of any insect species having developed resistance to *Bt* plants. The absence of resistance to *Bt* plants is remarkable and greatly exceeds the typical period of time before resistance first occurs to most conventional insecticides. However, caution is warranted since the ability of some species of agriculturally important insects to develop resistance to an ICP has been documented in the field with the diamondback moth and in the greenhouse with the cabbage looper.

Bacillus thuringiensis (Bt) is the most successfully used pathogen for the biological control of both agriculturally and medically important insects in the orders of Lepidoptera, Diptera and Coleoptera (38). Bt formulations have been widely applied in agriculture for insect pest management. Since 1996, transgenic plants expressing Bt toxins have been commercialized and increasingly incorporated into integrated pest management programs (43). In 2004, the acreage of Bt crops worldwide reached 22.4 million hectares (17). However, the widespread and prolonged application of Bt formulations and planting of Bt transgenic plants have created the threat of evolution of Bt resistance in field insect populations (5,11,45). Although no insects have developed resistance in the field to Bt plants (50), it is clear that some insect species can develop resistance to some Bt proteins. Thus, it is fair to ask whether the lack of widespread resistance, especially to Bt plants, is due to being lucky or smart.

Occurrence of resistance to *Bt*

To date, Bt resistant populations of two agriculturally important insect pests, diamondback moth, *Plutella xylostella*, and the cabbage looper, *Trichoplusia ni*, have been identified in the fields or commercial vegetable greenhouses where sprayable Bt formulations were applied (2,18,37,42,45,46). In the case of *P. xylostella*, Bt products became widely used in large scale commercial crucifer vegetable production when resistance to many other insecticides had rendered them useless (40,42). It is likely that resistance to Bt in *T. ni* occurred because it was used extensively in greenhouses where human safety is stressed. The evolution of Bt-resistant insect populations has prompted an urgent need to understand the mechanisms and the genetic basis for Bt resistance in various insect species of agricultural importance in order to provide the fundamental knowledge needed for the development of strategies for Bt resistance management.

Mechanism(s) of resistance to *Bt*

The mechanism of Bt-resistance has been an important focus of study since the discovery of Bt-resistance in insects (5). In lepidopteran larvae, the most common type of resistance to Cry1A toxins is known as “Mode 1” resistance, which is characterized by a high level of resistance to one or more Cry1A toxins, recessive inheritance, reduced binding of one or more Cry1A toxins to the midgut brush border membrane and little or no cross-resistance to Cry1C toxin (48). This “Mode 1” resistance has been identified in Bt-resistant strains of *P. xylostella*, *P. interpunctella*, *H. virescens* and *P. gossypiella* (45,47,48). Observations made from the resistant strains suggested that “Mode 1” resistance results from an alteration of the midgut target sites, which leads to reduced binding of Cry1A toxins to the brush border membranes in homozygous resistant individuals, but has little or no effect on the binding in heterozygous individuals (5,45).

Corresponding author. Mailing adress: New York State Agricultural Experiment Station, 630 West North Street, Geneva, NY, USA, 14456. Tel: 315-787-2352. Fax: 315-787-2326. Email: ams5@cornell.edu

The pathogenesis of Bt toxins in insects involves multiple steps and alteration of any of the steps may affect the toxicity of Bt toxins in insects and can be potentially involved in Bt resistance. Therefore, various mechanisms have been suggested in laboratory selected Bt-resistant insects (5). Solubilization of Cry protein crystals in the midgut is a factor determining the toxicity in insects (1). Therefore, reduced solubilization could be a potential mechanism for Bt-resistance in insects (38). Midgut digestive proteinases are critically involved in both activation and inactivation (degradation) of Bt toxins in the midgut (7,30,31,32,39). Excessive degradation of Bt toxin by the midgut proteinases could contribute to low toxicity of Bt toxins in insensitive or Bt-resistant insect hosts (7,39). Similarly, insufficient activation of Bt toxins by midgut serine proteinases can also be a mechanism for Bt resistance (31). The alteration of midgut trypsin activities has been observed in Bt-resistant strains of *P. interpunctella* and *O. nubilalis* (24,25,31).

After activation of the protoxin, the active toxin must penetrate through the midgut peritrophic membrane (PM) to reach its target site, the midgut epithelium. The PM plays a role in the toxicity of Bt toxins in insects and can be an important factor for the toxicity of Bt (35). It has been shown that disruption of the PM with a PM protein-specific metalloprotease could increase the toxicity of Bt in insects (12). Recently, trapping of Bt toxin Cry1Ac in the PM was discovered in a Cry1Ac-resistant strain of *Bombyx mori* (15). These observations suggest that the PM may be involved in Bt resistance.

Upon contact with the midgut epithelium, the Bt toxin binds to the receptors on the midgut brush border. The specific binding of Bt toxins to the midgut receptors is a critical event for the toxicity of Bt toxins. Studies on interactions of Bt toxins with several Lepidoptera species demonstrated that reduced binding of Bt toxins to the midgut brush border membranes could result in reduced toxin activities in insects (23) and is a primary mechanism for Bt resistance (6,45,51). Identified midgut receptors for Bt toxins include midgut aminopeptidases N (APNs), cadherin-like proteins, membrane-bound alkaline phosphatase, an uncharacterized 252 kDa midgut glycoprotein and glycolipids (8,9,10,13,16,19 ,20,21,22,36,53,54). Recently, the functional role of APN as the receptor for Cry1Ac was demonstrated by transformation of *Drosophila*, which was not susceptible to Cry1Ac, with the gene for an APN from *Manduca sexta*. The resulting transgenic *Drosophila* with the *M. sexta* APN transgene became susceptible to Cry1Ac, indicating the functional role of the APN in Bt toxicity. In addition, the role of APN as the receptor for Bt toxins has been functionally demonstrated in *Spodoptera litura* by suppressing the APN gene expression using the RNAi technique (34). RNAi suppressed the APN expression and the larvae with APN expression suppressed showed a lower susceptibility to Cry1C. Moreover, Cry1Ac-induced pore formation in the midgut brush border membranes in *T. ni* larvae was found to depend on the APN activity on the brush border membranes (26). Clearly, alteration of APNs could be a mechanism of Bt resistance in insects.

Midgut cadherins are known Bt toxin receptor proteins in insects (8,21). Importantly, mutations of the cadherin gene in two insect species have been identified to be linked with resistance to the Bt toxin Cry1Ac. In a laboratory-selected Bt resistant *H. virescens* strain, the resistance was found to be associated with the disruption of the cadherin gene by insertion of a retrotransposon (9). More recently, cadherin mutations have also been identified in Bt resistant *P. gossypiella* strains (29). In these resistant strains, three mutant alleles (deletion mutants) were identified to be linked with the resistance to Cry1Ac.

Very recently, the midgut membrane-bound alkaline phosphatase from *H. virescens* has been identified as a receptor for Bt toxin Cry1Ac (20). Similar to the midgut APNs, the alkaline phosphatase is also GPI-anchored to the midgut brush border membranes and the terminal GalNAc on the alkaline phosphatase is the binding site for the toxin. More importantly, the decreased level of the alkaline phosphatase in the midgut directly correlated with resistance in *H. virescens* to Bt.

The interaction of midgut receptors with Bt toxins is complex and further investigations are desirable. In addition to the midgut APNs, the cadherin and alkaline phosphatase, other midgut proteins have been identified to bind to Bt toxins in vitro. In *M. sexta* midgut brush border, the actin was found to bind to the toxin Cry1Ac, although its role in the Bt toxicity is currently not clear (28). In *B. mori* midgut, a 252 kD protein was recently identified as a novel Bt toxin binding protein (16). A comparative proteomic study of the midgut proteins from Bt-resistant and susceptible *P. interpunctella* showed that the proteomic changes in the resistant strain could be very complex (4). It was suggested that the proteomic alterations in the resistant larvae might

result in increased utilization of glutathione, elevated metabolism of oxidants and differential maintenance of energy balance within the midgut cells in the Bt-resistant larvae (4). In addition, other mechanisms involved in Bt-resistance have been reported to include aggregation of Bt toxin proteins by over-production of midgut esterase (14), and elevated immune response of resistant insects (27,33). Clearly, mechanisms for Bt-resistance in insects are multifaceted. These results indicate that insect resistance to Bt can be multifaceted and mechanisms for Bt resistance may be diverse.

Resistance to *Bt* plants

While a considerable amount is known about resistance mechanisms to Bt, there is far less empirical evidence about the ability of insects to develop resistance to Bt plants. Much of this is due to the lack of ability of all but two insects (*P. xylostella* and *T. ni*) to survive well on high expressing Bt plants. Prior to the introduction of commercial Bt plants, several strategies were proposed for the construction and deployment of Bt plants to reduce the likelihood of resistance development. These included regulating the expression level of the Bt protein, using one or more Bt genes in the same plant, having the plant express the Bt protein only at specific times or in specific tissues, and using non-Bt plants as a refuge for susceptible alleles in the insect population. Because of the lack of field-developed resistance to the commercially available Bt plants (corn and cotton), however, it has been difficult to test some of the theoretical models used to evaluate these various management options.

Our laboratory has tested some of the theoretical models using a unique system of *P. xylostella* populations with resistance to specific Bt proteins and broccoli plants expressing those same proteins. Studies to data have indicated the value of refuges (41,51) used in conjunction with high expression of the toxin within the plant, i.e. the "high dose/refuge" strategy". Additional studies with this system indicate that plants containing two dissimilar Bt toxin genes ('pyramided') have the potential to significantly delay the evolution of insect resistance compared with single-gene Bt crops (55). This is particularly relevant since pyramided cotton plants ("Bollgard II") with two genes derived from Bt (Cry1Ac and Cry2Ab2) were approved for commercial use in Australia and the U.S. in 2002, and several companies are developing new cotton and corn varieties with pyramided Bt genes.

The absence of field resistance could be due to one or more of the following factors: large fitness costs or other disadvantages suffered by resistant individuals; an initial low frequency of resistant alleles; dilution of resistant alleles with susceptible individuals from non-Bt plants or "refuges", and; a high-dose of toxin delivered by plants (3). An analysis to date of the available data suggest that the lack of resistance is likely due to an initial low frequency of resistance alleles and that resistance behaves as completely to partially recessive (5). In a recent analysis of the situation of pink bollworm, *Pectinophora gossypiella*, over an 8-year period on Bt cotton in Arizona, the authors suggest their experimental and modeling data indicate that a delay in the evolution of resistance could be explained by the use of refuges and the recessive inheritance of resistance. However, to these factors they also add that fitness costs were associated with resistance and that resistance was incomplete resistance (49).

Conclusions

So the question about whether we were lucky or smart remains. It is clear that some species of insects can develop resistance to some Bt proteins when subjected to high selection pressure. Foliar sprays of Bt generally have not been used widely or intensively enough to generate such resistance in the field, except in the case of *P. xylostella* and, to a far lesser extent, *T. ni*. The wide use of Bt plants may generate such selection pressure for resistance, but this has not yet occurred…so we have been lucky. On the other hand, we have likely been smart because when Bt plants were created and deployed they were done so using a high dose/refuge strategy for the main target pests and now there is a shift to pyramided Bt plants which have a demonstrated effect in delaying resistance even more. We must continue to be smart by developing other strategies, such as the use of tissue-specific or temporal promoters, that will further delay resistance evolution (3).

References

1. **Aronson, A. I., E.-S. Han, W. McGaughey, and D. Johnson.** 1991. The solubility of inclusion proteins from *Bacillus thuringiensis* is dependent upon protoxin composition and is a factor in toxicity to insects. Appl. Environ. Microbiol. **57:** 981-986.
2. **Ballester, V., B. Escriche, J. L. Ménsua, G.W. Riethmacher, and J. Ferré.** 1994. Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to Cry1Ab. Biocontrol Sci. Technol. **4:** 437-443.
3. **Bates, S. L., J.Z. Zhao, R.T. Roush, and A. M. Shelton.** 2005. Insect resistance management in GM crops: past, present and future. Nat. Biotechnol. **23:** 57-62.
4. **Candas, M., O. Loseva, B. Oppert, P. Kosaraju, and L.A. Bulla, Jr.** 2003. Insect resistance to *Bacillus thuringiensis*, alterations in the Indianmeal moth larval gut proteome. Mol. Cell. Prot. **2:** 19-28.

5. **Ferré, J. M., and J. Van Rie.** 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. Ann. Rev. Entomol. **47:** 501-533.
6. **Ferré, J., M., D. Real, J. Van Rie, S. Jansens, and M. Peferoen.** 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. Proc. Natl. Acad. Sci. USA **88:** 5119-5123
7. **Forcada, C., E. Alcacer, M.D. Garcera, and R. Martinez.** 1996. Differences in the midgut proteolytic activity of two *Heliothis virescens* strains, one susceptible and one resistant to *Bacillus thuringiensis* toxins. Arch. Insect Biochem. Physiol. **31:** 257-272.
8. **Francis, B. R., and L. A. Bulla, Jr.** 1997. Further characterization of BT-R1, the cadherin-like receptor for Cry1Ab toxin in tobacco hornworm (*Manduca sexta*) midguts. Insect Biochem. Mol. Biol. **27:** 541-550.
9. **Gahan, L. J., F. Gould, and D. G. Heckel.** 2001. Identification of a gene associated with Bt resistance in *Heliothis virescens*. Science **293:** 857-860.
10. **Gill, S. S., E. A. Cowles, and V. Francis.** 1995. Identification, isolation and cloning of a *Bacillus thuringiensis* Cry1Ac toxin-binding protein from the midgut of the lepidopteran insect *Heliothis virescens.* J. Biol. Chem. **270:** 27277-27282.
11. **Gould, F.** 1998. Sustainability of transgenic insecticidal cultivars: integrated pest genetics and ecology. Ann. Rev. Entomol. **43:** 701-726.
12. **Granados, R. R., F. Yan, B. Cosaro, and P.R. Hughes.** 2001. Enhancement of *Bacillus thuringiensis* toxicity to lepidopterous species with the enhancing from *Trichoplusia ni* granulovirus. Biol. Contr. **20:** 153-159.
13. **Griffitts, J. S., S. M. Haslam, T. Yang, S. F. Garczynski, B. Mulloy, H. Morris, P. S. Cremer, A. Dell, M. J. Adang, and R. V. Aroian.** 2005. Glycolipids as receptors for *Bacillus thuringiensis* crystal toxin. Science **307:** 922-925.
14. **Gunning, R. V., H. T. Dang, F. C. Kemp, I. C. Nicholson, and G. D. Moores.** 2005. New resistance mechanism in *Helicoverpa armigera* threatens transgenic crops expressing *Bacillus thuringiensis* Cry1Ac toxin. Appl. Environ. Microbiol. **71:** 2558-2563.
15. **Hayakawa, T., Y. Shitomi, K. Miyamoto, and H. Hori. 2004.** GalNAc pretreatment inhibits trapping of *Bacillus thuringiensis* Cry1Ac on the peritrophic membrane of *Bombyx mori*. FEBS Lett. **576:** 331-335.
16. **Hossain, D. M., Y. Shitomi, K. Moriyama, M. Higuchi, T. Hayakawa, T. Mitsui, R. Sato, and H. Hori.** 2004. Characterization of a novel plasma membrane protein, expressed in the midgut epithelia of *Bombyx mori*, that binds to Cry1A toxins. Appl. Environ. Microbiol. **70:** 4604-4612.
17. **James, C.** 2004. Global status of commercialized biotech/ GM crops: 2004. ISAAA briefs no. **32**. International Service for the Acquisition of Agri-biotech Applications, Ithaca, NY.
18. **Janmaat, A. F., and J. Myers.** 2003. Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. Proc. R. Soc. Lond. B. Biol. Sci. **270:** 2263-2270.
19. **Jenkins, J. L., and D.H. Dean.** 2006 Binding specificity of *Bacillus thuringiensis* Cry1Aa for purified, native *Bombyx mori* aminopeptidase N and cadherin-like receptors. BMC Biochem. **2**:
20. **Jurat-Fuentes, J.L., and M.J. Adang.** 2004. Characterization of a Cry1Ac-receptor alkaline phosphatase in susceptible and resistant *Heliothis virescens* larvae. Eur J Biochem. **271:** 3127-3135.
21. **Keeton, T. P., and L. A. Bulla, Jr.** 1997. Ligand specificity and affinity of BT-R, the *Bacillus thuringiensis* Cry1A toxin receptor from *Manduca sexta,* expressed in mammalian and insect cell cultures. Appl. Environ. Microbiol. **63:** 3419-3425.
22. **Knight, P. J. K., N. Crickmore, and D. J. Ellar.** 1994. The receptor for *Bacillus thuringiensis* Cry1a(C) delta-endotoxin in the brush border membrane of the lepidopteran *Manduca sexta* is aminopeptidase N. Microbiol. **11:** 429-436.
23. **Lee, M. K., T. H. You, F. L. Gould, and D. H. Dean.** 1999. Identification of residues in domain III of Bacillus thuringiensis Cry1Ac toxin that affect binding and toxicity. Appl. Environ. Microbiol. **65:** 4513-4520.
24. **Li, H., B. Oppert, R. A. Higgins, F. Huang, K.Y. Zhu, and L. L. Buschman.** 2004. Comparative analysis of proteinase activities of *Bacillus thuringiensis*-resistant and -susceptible *Ostrinia nubilalis* (Lepidoptera: Crambidae). Insect Biochem. Mol. Biol. **34:** 753-762.
25. **Li, H., J. Gonzalez-Cabrera, B. Oppert, J. Ferré, R. A. Higgins, L.L. Buschman, G. A. Radke, K. Y. Zhu, and F. Huang.** 2004. Binding analyses of Cry1Ab and Cry1Ac with membrane vesicles from *Bacillus thuringiensis*-resistant and -susceptible *Ostrinia nubilalis*. Biochem. Biophys. Res. Commun. **323:** 52-57.
26. **Lorence, A., A. Darszon, and A. Bravo.** 1997. Aminopeptidase dependent pore formation of *Bacillus thuringiensis* Cry1Ac toxin on *Trichoplusia ni* membranes. FEBS Lett. **414:** 303-307.
27. **Ma, G., H. Roberts, M. Sarjan, N. Featherstone, J. Lahnstein, R. Akhurst, and O. Schmidt.** 2005. Is the mature endotoxin Cry1Ac from *Bacillus thuringiensis* inactivated by a coagulation reaction in the gut lumen of resistant *Helicoverpa armigera* larvae? Insect Biochem. Mol. Biol. **35:** 729-739.
28. **McNall, R. J., and M. J. Adang.** 2003. Identification of novel *Bacillus thuringiensis* Cry1Ac binding proteins in *Manduca sexta* midgut through proteomic analysis. Insect Biochem. Mol. Biol. **33:** 999-1010.
29. **Morin, S., R. W. Biggs, M. S. Sisterson, L. Shriver, C. Ellers-Kirk, D. Higginson, D. Holley, L. J. Gahan, D. G. Heckel, Y. Carriere, T.J. Dennehy, J. K. Brown, and B. E. Tabashnik.** 2003. Three cadherin alleles associated with resistance to *Bacillus thuringiesis* in pink bollworm. Proc. Natl. Acad. Sci. USA **100:** 5004-5009.
30. **Oppert, B., K. J. Kramer, D. E. Johnson, S. C. MacIntosh, and W. H. McGaughey.** 1994. Altered protoxin activation by midgut enzymes from a *Bacillus thuringiensis* resistant strain of *Plodia interpunctella.* Biochem. Biophys. Res. Comm. **198:** 940-947.
31. **Oppert, B., K. J. Kramer, R. W. Beeman, D. Johnston, and W. H. McGaughey.** 1997. Proteinase-mediated insect resistance to *Bacillus thuringiensis* toxins. J. Biol. Chem. **272:** 23473-23476.
32. **Oppert, B.** 1999. Proteases interactions with *Bacillus thuringiensis* insecticidal toxins. Arch. Insect Biochem. Physiol. **42:** 1-12.
33. Rahman M. M., H. L. Roberts, M. Sarjan, S. Asgari, and O. Schmidt. 2004. Induction and transmission of *Bacillus thuringiensis* tolerance in the flour moth *Ephestia kuehniella*. Proc. Natl. Acad. Sci. USA. **101:** 2696-2699.
34. **Rajagopal, R., N. Agrawal, A. Selvapandiyan, S. Sivakumar, S. Ahmad, and R. K. Bhatnagar.** 2003. Recombinantly expressed isoenzymic aminopeptidases from *Helicoverpa armigera* (American cotton bollworm) midgut display differential interaction with closely related *Bacillus thuringiensis* insecticidal proteins. Biochem. J. **370:** 971-978.
35. **Rees, J. S., P. Jarrett and D. J. Ellar.** 2002. The peritrophic membrane plays an additional role in protecting lepidopteran insects from *Bacillus thuringiensis* Cry δ-endotoxins. J. Insect Sci. **2:** 47-48.
36. **Sangadala, S., F. S. Walters, L. H. English, and M. J. Adang.** 1994. A mixture of *Manduca sexta* aminopeptidase and phosphatase enhances *Bacillus thuringiensis* insecticidal Cry1Ac toxin binding and $^{86}Rb^{+}$-K^{+} efflux in vitro. J. Biol. Chem**. 269:** 10088-10092.
37. **Sayyed, A. H., J. Ferré, J. and D. J. Wright.** 2000. Mode of inheritance and stability of resistance to *Bacillus thuringiensis var kurstaki* in a diamondback moth (*Plutella xylostella*) population from Malaysia. Pest Manag. Sci. **56:** 743-748.
38. **Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler, and D. H. Dean.** 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. **62:** 775-806.
39. **Shao, Z., Y. Cui, X. Liu, H. Yi, J. Ji, and Z. Yu.** 1998. Processing of delta-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 in *Heliothis armigera* midgut juice and the effects of protease inhibitors. J. Invertebr. Pathol. **72:** 73-81.
40. **Shelton, A. M., J. A. Wyman, N. L. Cushing, K. Apfelbeck, T.J. Dennehy, S.E.R. Mahr, and S.D. Eigenbrode.** 1993. Insecticide resistance of diamondback moth in North America. J. Econ. Entomol. **86:** 11-19
41. **Shelton, A. M., J. D. Tang, R. T. Roush, T. D. Metz, and E. D. Earle.** 2000. Field tests on managing resistance to Bt-engineered plants. Nat. Biotechnol. **18:** 339-342.
42. **Shelton, A. M., J. L. Roberson, J. D. Tang, C. Perez, S. D. Eigenbrode, H. K. Preisler, W. T. Wilsey, and R. J. Cooley.** 1993. Resistance of diamondback month (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. J. Econ. Entomol. **86:** 697-705.
43. **Shelton, A. M., J.-Z. Zhao, and R. T. Roush.** 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Ann. Rev. Entomol. **47:** 845-881.
44. **Tabashnik, B.E.** 1994. Evolution of resistance to *Bacillus thuringiensis.* Ann. Rev. Entomol. **39:** 47-79.
45. **Tabashnik, B. E., N. Finson, F. R. Groeters, W. J. Moar, M. W. Johnson, K. Luo, and M. J. Adang.** 1994. Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. Proc. Natl. Acad. Sci. USA **91:** 4120-4124.
46. **Tabashnik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson.** 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback month (Lepidoptera: Plutellidae). J. Econ. Entomol. **83:** 1671-1676.
47. **Tabashnik, B. E., Y. B. Liu, T. J. Dennehy, M. A. Sims, M. S. Sisterson, R. W. Biggs, and Y. Carrière.** 2002. Inheritance of

resistance to Bt toxin Cry1Ac in a field-derived strain of pink bollworm (Lepidoptera: Gelechiidae). J. Econ. Entomol. **95:** 1018-1026.
48. **Tabashnik, B. E., Y. B. Liu, T. Malvar, D, G. Heckel, L Masson, and J. Ferré.** 1998. Insect resistance to *Bacillus thuringiensis*: uniform or diverse? Phil. Trans. R. Soc. Lond. B. **353:**1751-1756.
49. **Tabashnik, B. E., T. J. Dennehy, and Y. Carrière.** 2005. Delayed resistance to transgenic cotton in pink bollworm. Proc. Natl. Acad. Sci. USA **102:** 15389-15393.
50. **Tabashnik, B. E., Y. Carriere, T. J. Dennehy, S. Morin, M. S.. Sisterson, R.T. Roush, A. M. Shelton, and J.-Z. Zhao.** 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. J. Econ. Entomol. **96:** 1031-1038.
51. **Tang, J. D., A. M. Shelton, J. van Rie, S. de Roeck, W. J. Moar, R. T. Roush, and M. Peferoen.** 1996. Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*). Appl. Environ. Microbiol. **62:** 564-569.
52. **Tang, J. D., H. L. Collins, T. D. Metz, E. D. Earle, J. Zhao, R. T. Roush, and A. M. Shelton.** 2001. Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies. J. Econ. Entomol. **94:** 240-247.
53**. Vadlamudi, R. K., E. Weber, T.H. Li, and L. A. Bulla.** 1995. Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis*. J. Biol. Chem. **270:** 5490-5494.
54. **Valaitis, A. P., M. K. Lee, F. Rajamohan, and D. H. Dean.** 1995. Brush border membrane aminopeptidase-N in the midgut of the gypsy moth serves as the receptor for the Cry1a(C) delta-endotoxin of *Bacillus thuringiensis*. Insect Biochem. Mol. Biol. **25:** 1143-1151.
55. **Zhao, J., J. Cao, Y. Li, H. L. Collins, R. T. Roush, E. D. Earle, and A. M. Shelton.** 2003. Plants expressing two *Bacillus thuringiensis* toxins delay insect resistance compared to single toxins used sequentially or in a mosaic. Nat. Biotechnol. **21:** 1493-7.