RESISTANCE: A THREAT TO THE INSECTICIDAL CRYSTAL PROTEINS OF *BACILLUS THURINGIENSIS*

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ABSTRACT

Insecticidal crystal proteins (also known as δ-endotoxins) synthesized by the bacterium *Bacillus thuringiensis* Berliner (*Bt*) are the active ingredient of various environmentally friendly insecticides that are 1) highly compatible with natural enemies and other nontarget organisms due to narrow host specificity, 2) harmless to vertebrates, 3) biodegradable in the environment, and 4) highly amenable to genetic engineering. The use of transgenic plants expressing *Bt* δ-endotoxins has the potential to greatly reduce the environmental and health costs associated with the use of conventional insecticides. The complex mode of action of *Bt* is the subject of intensive research. When eaten by a susceptible insect δ-endotoxin crystals are solubilized in the midgut; proteases then cleave protoxin molecules into activated toxin which binds to receptors on the midgut brush border membrane. Part of the toxin molecule inserts into the membrane causing the midgut cells to leak, swell, and lyse; death results from bacterial septicemia. Insecticides formulated with *Bt* account for less than 1% of the total insecticides used each year worldwide because of high cost, narrow host range, and comparatively low efficacy. Environmental contamination, food safety concerns, and pest resistance to conventional insecticides have caused a steady increase in demand for *Bt*-based insecticides. The recent escalation of commercial interest in *Bt* has resulted in more persistent and efficacious formulations. For example, improved *Bt*-based insecticides have allowed management of the diamondback moth, *Plutella xylostella* (L.). Unfortunately this has resulted in the evolution of resistance to δ-endotoxins in *P. xylostella* populations worldwide. The recent appearance of *Bt* resistance in the field, corroborated by the results of laboratory selection experiments, demonstrates genetically-based resistance in several species of Lepidoptera, Diptera, and Coleoptera. The genetic capacity to evolve resistance to these toxins is probably

present in all insects, and the heritability, fitness costs, and stability of the resistance trait are documented in several insect populations. In two strains of *Bt*-resistant lepidopteran species, mechanisms of resistance involve reductions in the binding of toxin to midgut receptors. Research on other resistant strains suggests that other mechanisms are also involved. Unfortunately, the high stability of the resistance trait, as well as broad spectrum cross-resistance to other δ-endotoxins, undermines many potential options for resistance management. Genetically engineered plants, expressing δ-endotoxin continuously and at ultrahigh doses, ensure intense and rapid selection of the target insect population. The efficacy of transgenic plants can be preserved only by developing an integrated pest management program that is designed specifically to reduce selection pressure by minimizing exposure to *Bt* and increasing other mortality factors, thereby slowing the rate of pest adaptation to *Bt*.

Key Words: δ-endotoxin, cross-resistance, transgenic plants, resistance management

Resumen

Las proteínas de cristales insecticidas (también conocidas como δ-endotoxinas) sintetizadas por la bacteria *Bacillus thuringiensis* Berliner (*Bt*) son el ingrediente activo de varios insecticidas ambientalmente amistosos que son 1) altamente compatibles con los enemigos naturales y otros organismos no objetos de control debido a su estrecha especificidad de hospedante, 2) inocuos a los vertebrados, 3) biodegradables en el ambiente, y 4) altamente dóciles para la ingeniería genética. El uso de plantas transgénicas expesando δ-endotoxinas de *Bt* tiene la posibilidad de reducir grandemente los costos ambientales y de salud asociados con el uso de insecticidas convencionales. El modo complejo de acción de *Bt* es sujeto de investigación intensiva. Cuando son ingeridos por un insecto susceptible, los cristales de δ-endotoxina son disueltos en el intestino medio; las proteasas abren las moléculas de proteínas transformándolas en toxinas activadas que se unen a receptores en el cepillo de la membrana del intestino medio. Parte de la molécula de la toxina se inserta en la membrana causando que las células del intesitno medio pierdan el control de la permeabilidad, se hinchen y rompan, y la muerte ocurre luego por septicemia bacteriana. Los insecticidas formulados con *Bt* son menos del 1% del total de los insecticidas usados cada año en todo el mundo debido a su alto costo, estrecho rango de hospedantes, y eficacia comparativamente baja. La contaminación abiental y la resistencia de las plagas a los insecticidas convencionales han causado un incremento constante en la demanda de insecticidas de *Bt*. La reciente escalada de interés comercial en *Bt* ha provocado la aparición de formulaciones más persistentes y eficaces. Sin embargo, el uso intensivo de insecticidas mejorados a base de *Bt* autorizados para el manejo de la polilla de la col, *Plutella xyllostella* (L.), ha traído como reusltado la evolución de resistencia a las δ-endotoxinas en las poblaciones de *P. xyllostella* en todo el mundo. La reciente aparción de reistencia a *Bt* en el campo, corroborada por los resultados de experimentos de selección de laboratorio, demuestra que existe resistencia genética en varias especies de Lepidoptera, Diptera y Coleoptera. La capacidad genética de evolucionar la resistencia hacia esas toxinas está probablemente presente en todos los insectos, y la heredabilidad, costo de ajuste, y estabilidad de la resistencia son documentados en varias poblaciones de insectos. En dos especies de lepidópteros resistentes a *Bt*, los mecanismos de resistencia incluyen reducciones en la unión de la toxina a los receptores del intestino medio. La investigación sobre otras especies de insectos resistentes sugiere que otros mecanismos están también relacionados. Desafortunadamente, la alta estabilidad de la resistencia, así como la resistencia cruzada de amplio espectro a otras δ-endotoxinas, determina pocas opciones potenciales para el manejo de la resistencia. Las plantas transgénicas expesando δ-endotoxinas continuamente, y las dosis muy altas, aseguran la selección intensa y rápida de la población de insectos a controlar. La eficacia de las plantas transgénicas puede ser preservada sólo desarrollando un programa de manejo integrado de plagas diseñado específcamente para re-

ducir la presión de seleccción, minimizando la exposición a *Bt* e incrementando otros factores de mortalidad, para disminuir la velocidad de adaptación de la plaga a *Bt*.

The bacterium *Bacillus thuringiensis* Berliner (*Bt*) is a complex of subspecies characterized by their ability to synthesize crystalline inclusions during sporulation. These crystalline inclusions are comprised of relatively high quantities of one or more glycoproteins known as δ-endotoxins or Cry toxins (Table 1). The toxins produced by *Bt* play a vital role in the pathogenicity of this bacterium to insects and other invertebrates. The Cry toxins have enormous commercial value as safe, biodegradable pesticides. The specificity of *Bt* toxicity is highly desirable in integrated pest management (IPM) programs, particularly in sensitive aquatic and forest ecosystems where other life forms, including many beneficial and nontarget insects, must be conserved (May 1993). The selective toxicity, rapid environmental degradation, and vertebrate safety of *Bt*-based insecticides provide growers and the public with environmentally friendly and effective alternatives to conventional insecticides (Meadows 1993). Advances in biotechnology and genetic engineering, as well as the proteinaceous nature of the Cry toxins, led to the selection of the *cry* genes as the primary insect-resistance genes transferred into, and expressed in, plants and microbes (Gasser & Fraley 1989, Adang 1991, Peferoen 1992, Ely 1993, Gelernter & Schwab 1993).

Subspecies of *Bt* are distributed in a variety of diverse habitats worldwide (Martin & Travers 1989) and are typically isolated from soil, leaf surfaces, and environments rich with insects, such as grain bins and insectaries (Smith & Couche 1991, Burges & Hurst 1977). In fact, the first reports of *Bt* were from colonies of the silkworm *Bombyx mori* (L.) (Ishiwata 1901), and from the Mediterranean flour moth, *Ephestia kueh-*

TABLE 1. *BT* STRAINS, THEIR RESPECTIVE δ-ENDOTOXINS AND HOST RANGES, IN INSECT RESISTANCE STUDIES.

Bt Strain[1]	δ-Endotoxin[2]	Spectrum
kurstaki HD-1 (*Btk*)	CryIA(a), CryIA(b), CryIA(c), CryIIA, CryIIB	Lepidoptera Diptera[3]
kurstaki HD-73	CryIA(c)	Lepidoptera
aizawai HD-112 (*Bta*)	CryIA(a), CryIA(b), CryIC, CryID, CryIG, CryII[4]	Lepidoptera
aizawai HD-133 (*Bta*)	CryIA(a), CryIA(b), CryIC, CryID	Lepidoptera
thuringiensis HD-2	CryIA, CryIB	Lepidoptera Coleoptera[5]
entomocidus HD-198 (*Bte*)	CryIA(a), CryIA(b), CryIC, CryID	Lepidoptera
sotto (*Bts*)	CryIA(a)	Lepidoptera
israeliensis (*Bti*)	CryIVA, CryIVB, CryIVC, CryIVD, CytA	Diptera
tenebrionis (*Btt*)	CryIIIA	Coleoptera

[1]*Bt* strains may contain multiple toxins, and composition may differ slightly from those reported here.
[2]Organized by amino acid sequence by Höfte & Whiteley (1989).
[3]CryIIA accounts for the dipteran activity of this strain.
[4]Presence of CryIG uncertain, and CryII type unknown (McGaughey & Johnson 1994).
[5]CryIB is toxic to some coleopterans (Bradley et al. 1995).

niella (Zeller) (Berliner 1911). Ecological considerations of *Bt* as an insect pathogen and in the environment are discussed by Meadows (1993).

In the United States, commercial development of *Bt* into a formulated insecticide did not begin until the late 1950s. Most *Bt*-based insecticides are formulated mixtures of δ-endotoxin crystals and *Bt* spores, which are known to synergize the toxicity of the crystals. Although the effectiveness of these early *Bt*-based insecticides was often erratic, progress was slow in research and development of improved *Bt* formulation, delivery, and application technologies, as well as in the discovery of more active strains. Until the mid-1970s, it was generally accepted that lepidopterans were the only target of *Bt*.

The discovery of *Bt* subsp. *israelensis*, which is toxic to larval mosquitoes and black flies (Goldberg & Margalit 1977), and the discovery of *Bt* subsp. *tenebrionis* (Krieg et al. 1983), which is toxic to several beetle species, stimulated sudden and dramatic commercial interest in *Bt*. During the 1980s, new biotech companies and large agrochemical and pharmaceutical corporations initiated research programs to isolate *Bt* from various environmental samples and to screen for toxicity in agriculturally and medically-important target organisms (Van Frankenhuyzen 1993). Lambert & Peferoen (1992) estimated that 40,000 strains of *Bt* are now stored, mainly in private collections, worldwide. The spectrum of activity of Bt toxins has expanded from species in three insect orders (Lepidoptera, Diptera, and Coleoptera) to species in eight insect orders (Homoptera, Orthoptera, Mallophaga, Hymenoptera, Siphanoptera) (Bauer, unpublished) and various mites, nematodes, flukes, mollusks, and protozoans (Feitelson et al. 1992). Commercial products formulated with *Bt* are now registered for control of lepidopteran (Navon 1993), dipteran (Becker & Margalit 1993), and coleopteran pests (Keller & Langenbruch 1993). The short half-life of *Bt*, due to ultraviolet inactivation when topically applied, has stimulated considerable research into alternative delivery strategies. By far the most controversial strategy is the use of insect-resistant crops expressing *Bt* δ-endotoxin genes, which are already in the field in Asia and the United States where potato, cotton and corn are registered.

As concerns over environmental quality and food safety increase, *Bt*-based insecticides will become increasingly important in the development of IPM strategies. Currently, the use of *Bt* in insect control programs accounts for less than 1% of insecticides used worldwide each year. The comparatively high production cost of *Bt*-based insecticides is a primary impediment to more widespread usage. However, the major impetus for greater use of *Bt* in agriculture is the development of resistance to conventional insecticides (Georghiou 1994, Watkinson 1994). In fact, many growers typically add *Bt* to conventional sprays because of concerns about chemical control failure (Marrone & MacIntosh 1993). Because of their environmental safety, microbial insecticides are one of the few pesticides that can be developed and registered quickly and cheaply. In addition, resistance to conventional insecticides does not confer cross-resistance to *Bt* toxins due to the unique mode of action of δ-endotoxin (Stone et al. 1991, Tabashnik 1994a).

Resistance is a major problem associated with the intensive use of pesticides in agriculture and human health protection, and hundreds of insect and mite species can no longer be controlled by one or more pesticides (Georgiou & Lagunes 1988). Resistance is documented in diverse groups of insecticides, including neurotoxins, chitin synthesis inhibitors, juvenile hormone analogues (National Research Council 1986), and, most recently, *Bt* (Tabashnik et al. 1990). Adaptation to individual insecticides is a consequence of their intensive and prophylactic use, and many conventional insecticides are being lost to resistance faster than industry can replace them. Many researchers now predict that the use of *Bt cry* genes in the genetic engineering of insect-

resistant plants will expedite selection for resistance in the target organisms more rapidly than has occurred through conventional application methods (Gould 1988a, 1988b, Van Rie 1991, McGaughey & Whalon 1992, Marrone & MacIntosh 1993, May 1993, Whalon & McGaughey 1993, McGaughey 1994, Tabashnik 1994a).

Our understanding of the nature of *Bt* insecticidal crystal proteins has advanced rapidly since the mid-1980s, thanks to highly collaborative research programs involving entomologists, microbiologists, physiologists, geneticists, protein biochemists, and molecular biologists. Research on *Bt* is fast-paced, often involving worldwide liaisons between researchers in industry, university, and government, leading to an abundance of new research results, methodologies, and discoveries. In this paper, my objectives are to provide researchers, even those only peripherally involved or knowledgeable of *Bt* δ-endotoxins, with a greater understanding of 1) *Bt* mode of action, 2) resistance and cross-resistance to Cry toxins, and 3) resistance management, as they relate to the use of both improved *Bt* insecticides and genetically-engineered plants expressing δ-endotoxin genes. The research results presented here will compliment and update those reported in the comprehensive review by Tabashnik (1994a). In presenting this information, I hope to attract more researchers into the complex area of IPM and management of *Bt* resistance. The design, validation, and implementation of effective and sound IPM practices are some of the greatest challenges facing researchers in agriculture, agroforestry, and vector control today.

Bt Mode of Action

A generalized flow chart of the events leading to *Bt* intoxication by Cry toxins in a susceptible host reveals various levels at which resistance might evolve in an insect population (Fig. 1). The high degree of host specificity, as well as the complexity of *Bt* mode of action, results from the interaction of the toxin within the complex environment of the insect's midgut lumen and on the surface of the midgut epithelial cells (English & Slatin 1992). Although researchers discovered relatively early that the midgut was the primary site of δ-endotoxin activity (Heimpel & Angus 1959), the molecular mechanisms of *Bt* intoxication continue to be the subject of intensive research (for reviews see Gill et al. 1992, English & Slatin 1992, Lambert & Peferoen 1992, Aronson 1993, Honée & Visser 1993, Knowles & Dow 1993, Yamamoto & Powell 1993, Federici 1993, Visser et al. 1993). A small family of *Bt* δ-endotoxins known as the cytolytic or Cyt toxins, an important component of *Bt* subsp. *israelensis* (Table 1), is not covered in this review (see Chilcott et al. 1990, Koni & Ellar 1994, Wu et al. 1994).

Ingestion

Feeding stimulants are known to greatly enhance *Bt* performance since most susceptible insects stop feeding after consuming food treated with δ-endotoxin. Detection and behavioral avoidance of food treated with Cry toxins have also been reported for many target species (Gould & Anderson 1991, Gould et al. 1991, Ramachandran et al. 1993). Formulation and application technology has improved the rate of toxin ingestion, increasing the probability that the target insect will consume a lethal dose after treatment.

Crystal Solubilization

Following ingestion, solubilization of crystalline δ-endotoxin is a prerequisite to all subsequent events in the intoxication pathway (Tojo & Aizawai 1983, Du et al. 1994).

High midgut pH (>9.5) was once thought to be essential to crystal solubility, but coleopteran-specific toxins were found to function at much lower pH (Koller et al. 1992). Midgut detergency and redox potential also affect solubilization. The rate and extent of crystal solubilization greatly influence toxicity levels in different hosts (Aronson et al. 1991, Bradley et al. 1995), as well as the rate of intoxication (Bauer & Pankratz 1992, Koller et al. 1992).

Enzymatic Processing

The Cry proteins are synthesized as protoxins that require processing by midgut enzymes to generate activated toxin (Ogiwara et al. 1992). The larger protoxins of about 130-140 kDa (e.g. CryI) are proteolytically cleaved, exposing the activated toxin which is a protease-resistant core of about 55-65 kDa (Höfte & Whiteley 1989). Many other toxins (e.g. CryII, CryIII, CryIVD) are synthesized as 70 kDa proteins and are similar to the N-terminal half of the larger protoxins. Enzymatic processing of these smaller 70 kDa toxins also occurs, with amino acids cleaved from the N terminus (Carroll et al. 1989).

Receptor Binding

The action of Cry δ-endotoxins on the midgut epithelium begins with binding of the activated toxin to receptors (Hoffman et al. 1988a, 1988b). Much of the host specificity of *Bt* toxins results from their ability to bind to specific receptors on the brush border membrane, although most toxins bind to more than one receptor (Van Rie et al. 1989, 1990a, Denolf et al. 1993, Estada & Ferré 1994). Amino acid sequence similarity in the receptor-binding domain of the toxin molecule is a useful predictor of overall host specificity (Van Rie et al. 1990, Cummings & Ellar 1994). The binding domain is also the most variable region of the toxin molecule (Li et al. 1991).

The function of these receptors in midgut physiology is an elusive research question. Recently, aminopeptidase N, a 120 kDa glycoprotein, was purified from the lepidopteran *Manduca sexta* and identified as the receptor for CryIA(c) (Knight et al. 1994, Sangadala et al. 1994). Aminopeptidase N is an abundant Zn^{+}-dependent ectoenzyme present in the brush border of membranes of the alimentary tracts of most animals (Ellar 1994, Garczinski & Adang in press).

Binding, while essential, is not sufficient to produce mortal damage, as shown by several studies that found specific binding of toxins to receptors on brush border preparations is not correlated with *in vivo* toxicity (Van Rie 1990a, Wolfersberger 1990, Ferré et al. 1991, Garczynski et al. 1991, Gould et al. 1992, Escriche et al. 1994, Sanchis et al. 1994, Masson et al. 1995). Other researchers showed that *in vivo* toxicity is, however, strongly correlated with measures of membrane disruption (Wolfersberger 1991) or membrane permeability (Carroll & Ellar 1993). They stressed that, although receptors play an essential role, post-binding factors are required for successful intoxication by *Bt* δ-endotoxins.

Intercalation, Pore Formation, and Cell Lysis

After binding to a receptor on the cell surface, the toxin then inserts or intercalates into the plasma membrane (English & Slatin 1992, Knowles & Dow 1993). Evidence from electrical conductance and ion leakage studies suggests that several toxin/receptor complexes aggregate to form lesions or leaky regions in the brush border membrane (Walters et al. 1993, 1994). Using a very different method, Masson et al. (1995)

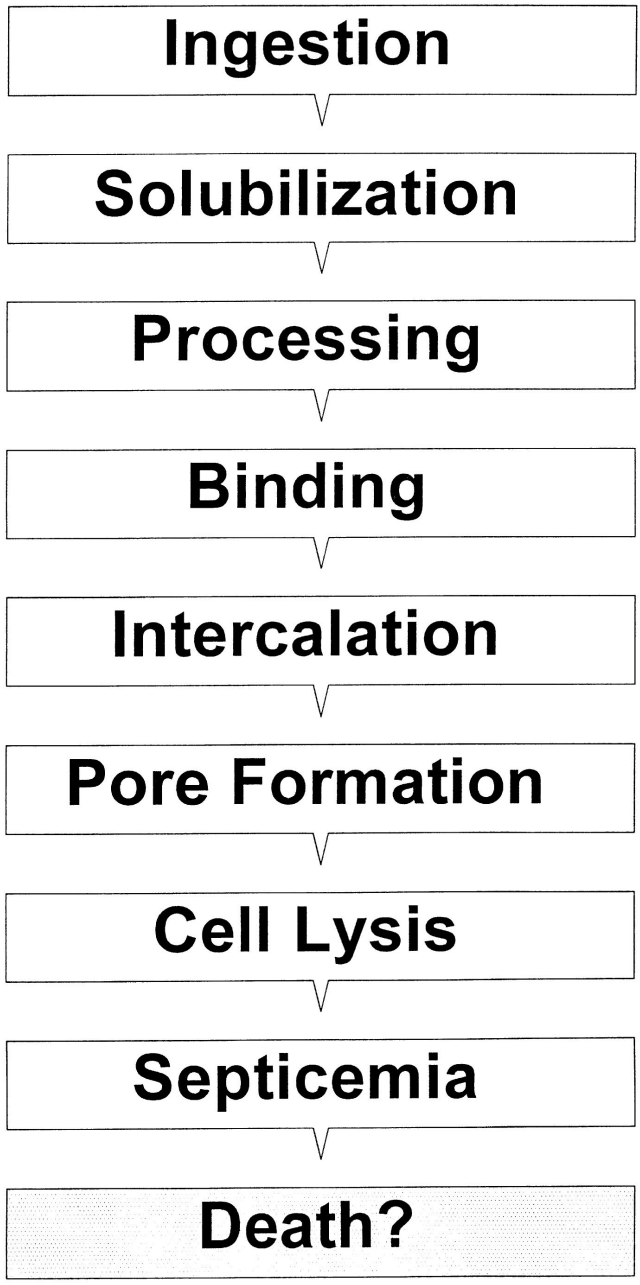

Figure 1. Generalized flow chart of events leading to *Bt* intoxication in a susceptible host.

also detected toxin-toxin aggregation, supporting evidence that the toxin acts as a multimer on the cell surface. Pores in the plasma membrane are estimated at 1-2 nm diam, disrupting the actively maintained osmotic balance, causing the cells to swell and burst by a process known as colloid-osmotic lysis (Knowles & Ellar 1987). Carroll & Ellar (1993) demonstrated that δ-endotoxin-induced changes in cell permeability is non-selective to cations, anions, neutral solutes, and water. A three-dimensional model of the CryIIIA protein structure supports the hypothesis that the toxin causes pores or channels to form in the lipid bilayer (Li et al. 1991). However, the role of midgut receptors in this toxin-induced leakage is still unclear (Knowles & Dow 1993, Parenti et al. 1995, Garczynski & Adang, in press).

Bacterial Septicemia and Death

The synergistic effect of *Bt* spores, in the presence of δ-endotoxin, on insect mortality leads to speculation that δ-endotoxins facilitate bacterial exploitation of the nutrients present in the insect hemolymph (Ali et al. 1985, Wilson & Benoit 1990, Borgonie 1995). Death occurs when lysis of midgut cells causes irreparable breakdown of the midgut integrity, allowing *Bt* and other bacteria present in the lumen to gain access to the body cavity. The insect hemolymph provides an excellent medium for bacterial growth. Death caused by bacterial septicemia usually occurs within 2-3 days post-ingestion. However, the immediate cessation of feeding observed in most insects after ingestion of *Bt* (Angus 1954), as well as the rapid regenerative capability of midgut epithelial cells, can allow damaged regions of the midgut to heal. The actual recovery of treated insects is dependent on many intrinsic and extrinsic factors including host genetics, age, and vigor; dosage and potency of toxin ingested; various environmental factors including host plant species (Meade & Hare 1994, Moldenke et al. 1994); and the presence of *Bt* spores and other bacteria in the insect gut (Miyasono et al. 1994).

Resistance

Over several decades of commercial use, the continued efficacy of *Bt*-based insecticides led to considerable skepticism that resistance to *Bt* was possible (Burges 1971, Krieg & Langenbruch 1981). However, recent field and laboratory evidence suggest otherwise. The slow development of field resistance in the past may have resulted from low selection pressure exerted by early formulations and usage patterns (Stone et al. 1991). Other researchers believed that the complex mode of action of *Bt*, often involving multiple toxins and *Bt* spores, provided protection against resistance because a single mutation in the insect would be unlikely to affect susceptibility (Boman 1981, Briese 1981, de Barjac 1987). McGaughey & Whalon (1992), however, suggested that at high levels of selection, the multicomponent-toxicity pathway merely expands behavioral and/or physiological opportunities for adaptation to *Bt*. Technological advancements in *Bt* toxicity, host range, stability, formulation, application, and ultimately the expression in transgenic plants, are greatly improving biopesticide potency and efficacy (Feitelson et al. 1992, Stone & Sims 1993, Carlton & Gawron-Burke 1993). Unfortunately, while providing high levels of pest suppression, improved efficacy will rapidly help select for the segment of the population that is capable of withstanding *Bt* intoxication.

Possible shifts in susceptibility to *Bt* were first reported by Kinsinger & McGaughey (1979), with a 42-fold difference among "natural" populations of *Plodia interpunctella* (Hübner), the Indianmeal moth, and up to a 15-fold difference in *Cadra cautella* (Walker), the almond moth. However, the underlying cause of this variation

is unknown because the history of *Bt* applications in these grain storage facilities was not reported. In a subsequent study over a five-state area, the mean median lethal concentration (LC_{50}) for populations of *P. interpunctella* from grain bins treated with *Bt* was 1.2-fold higher than the mean LC_{50} for populations from untreated bins (McGaughey 1985). When these *Bt*-exposed populations were selected further in the laboratory, 30-fold resistance developed in 2 generations, and 100-fold resistance in 15 generations. Initially, resistance to *Bt* in the Indianmeal moth was considered somewhat unique because exposure to natural infestations of *Bt* in stored grains may increase the genetic variability in *Bt* susceptibility (Kinsinger & McGaughey 1979). In addition, the dark, stable and closed environment of grain bins favored selection for resistance by long toxin residual times because of no UV exposure and by minimizing the potential of outbreeding with susceptibles from other populations.

It was not until Tabashnik et al. (1990) first reported field resistance to *Bt* in Hawaiian populations of *Plutella xylostella* (L.), the diamondback moth, that the potential for widespread resistance to *Bt* was generally acknowledged. Resistant populations of *P. xylostella* have also been documented in field populations from Florida (Jansson & Lecrone 1990), New York (Shelton et al. 1993), the Philippines (Kirsch & Schmutterer 1988, Ferré et al. 1991), Japan (Hama et al. 1992), Thailand, and Malaysia (Georghiou 1994). These recent reports of resistance to *Bt* in the field have provided credibility to the results of laboratory selections for *Bt* resistance (for reviews, see Briese 1981, Georghiou 1990, Stone et al. 1991, McGaughey & Whalon 1992, Tabashnik 1994a). As laboratory and field data accumulate, concern is growing that these unique bacterial toxins may be rendered useless as pest management tools, particularly with the imminent commercialization of transgenic plants expressing single activated toxic fragments (May 1993, Whalon & McGaughey 1993).

Laboratory Selections

The increased effort researchers are now devoting to *Bt* resistance is reflected in the many experiments designed to select for *Bt* resistance in the laboratory (Table 2). The results summarized in Table 2 represent selection experiments, performed and ongoing during the last decade, which achieved significant levels of resistance to *Bt* preparations containing either a mixture of spores and native δ-endotoxin crystals, or individual Cry toxins in various forms. These results represent only a few of the more than 50 laboratory selection experiments performed with at least 16 insect species (Tabashnik 1994a). Significant levels of resistance have been documented in nine species of Lepidoptera and two species each of Diptera and Coleoptera, with resistance ratios ranging from 1.1 to >1000.

Concern about rapid adaptation of insects to δ-endotoxin-based transgenic plants has led to a steady increase in the number of researchers performing laboratory selection experiments with single toxins and in some cases with the specific gene products that insects will ingest when consuming the foliage from bioengineered plants (Estada & Ferré 1994). Researchers found that resistance ratios were consistently higher, and resistance developed more rapidly, in insect populations selected with individual toxins than in populations selected with *Bt*-insecticides that contain live spores and multi-toxin crystals, such as *Bt* subsp. *kurstaki* (*Btk*) (Tables 1 and 2). The results of these experiments suggest that deployment of the high dose, single toxin strategy in the design of transgenic plants will quickly generate resistant populations of the target pest.

The selection experiment reported by Moar et al. (1994) best illustrates the differential response of *Spodoptera exigua* populations subjected to selection pressure from either *Btk* spore/crystal preparations or purified CryIC toxin (Table 2). After 20 gen-

erations, larvae selected with *Btk* spore/crystal preparations were only 3- to 4-fold resistant. However, the cohort selected with pure CryIC protoxin was 1000-fold resistant after 21 generations. This confirms that the complex mode of action, present in most commercially *Bt*-based insecticides available today, is important in the low prevalence of field resistance for pest populations treated with *Bt*. It may also explain the lack of *Bt*-resistance generated in laboratory experiments which used crude or formulated Bt preparations containing both δ-endotoxins and spores for selections (Tabashnik 1994a). Before 1985, large quantities of a single, pure Cry toxin, made possible through advances in molecular biology, were simply not available.

Although laboratory-generated resistance within a susceptible species may not reflect the rates and mechanisms generated in field populations, laboratory populations are invaluable tools for the study of potential risk of resistance, physiological and behavioral mechanisms of resistance, cross-resistance, genetics, stability, fitness costs, and, ultimately, for the development of methods for monitoring, managing, and delaying resistance.

Heritability

It is apparent that the genetic capacity to evolve resistance to *Bt* δ-endotoxin is widespread in insects. Although some efforts to select for resistance have failed, this may reflect either insufficient selection pressure, a genetic bottleneck due to lack or loss of genetic diversity in the laboratory colony, or both (McGaughey & Whalon 1992, Whalon & McGaughey 1993). Selection experiments often provide the necessary information to estimate heritability (h^2), the proportion of the observed variability that is caused by additive genetic variation (Falconer 1989). Estimated heritability was used by Tabashnik (1992, 1994a) to estimate the ability of populations to develop resistance in 27 selection experiments. He showed that *P. interpunctella* has a relatively high h^2 compared to other moths. This reflects low phenotypic variation, perhaps resulting from its stable environment, and high additive genetic variation for the resistance trait, perhaps resulting from exposure to *Bt* in its environment.

Tabashnik (1994a) also discussed the potential usefulness and limitation of the heritability estimates in assessing resistance risk, i.e., predicting the rate at which a pest will evolve resistance (Tabashnik 1992, Keiding 1986, Tabashnik & McGaughey 1994). *Plodia interpunctella* has a high estimated h^2 and adapts readily to *Bt* in the laboratory, but high levels of field resistance are unknown. McGaughey (1985) suggested that the infrequent applications of *Bt* in grain bins, as well as *Bt*'s limited efficacy, helped preserve the susceptible individuals within the treated population, thereby generating only low levels of resistance.

In contrast, susceptible populations of *P. xylostella* with a comparatively low h^2 do not achieve significant levels of resistance to *Bt* in laboratory selections (Devriendt & Martouret 1976, Krieg & Langenbruch 1981). However, moderately resistant field populations of *P. xylostella* quickly reach high levels of resistance during laboratory selections (Tabashnik et al. 1991). This shows that selection is occurring in pest populations that are being intensively managed with *Bt*-based insecticides. Similar results of rapid laboratory adaptation to *Bt* after intensive field exposure were found in populations of mosquitoes (Gill et al. 1992), *P. interpunctella* (McGaughey & Johnson 1992), and *Leptinotarsa decemlineata* (Say) (Whalon et al. 1993) (Table 2).

Intraspecific Susceptibility

In most species, phenotypic variations in *Bt* tolerance have a strong genetic basis, and species with high variability in this trait will develop resistance more quickly un-

TABLE 2. SUMMARY OF SIGNIFICANT LABORATORY SELECTION EXPERIMENTS WITH *BT* δ-ENDOTOXINS[1].

Species	*Bt* Strain or δ-Endotoxin[2]	Populations Selected	Generations Selected	Resistance Ratios[3]	Reference(s)
LEPIDOPTERA					
Plodia interpunctella	*Btk*	6	12-22	8-113	McGaughey & Beeman 1988
	Btk HD-1	1	na	305	McGaughey & Johnson
	Bta HD-112	1	na	28	1992, 1994
	Bta HD-133	1	na	94	
	Bte HD-198	1	na	32	
	Btk + *Bta*	1	16	15	
Cadra cautella	*Btk*	3	24	3-8	McGaughey & Beeman 1988
Homoeosoma electellum	*Btk*	1	11	1.7	Brewer 1991
Plutella xylostella	*Btk*	3	5-9	15-66	Tabashnik et al. 1991
Heliothis virescens	CryIA(b)	1	18	65	Stone et al. 1989, Sims & Stone 1991
	CryIA(c)	1	17	50	Gould et al. 1992
	CryIA(c)	1	>19 episodes[4]	10,000	Gould et al. in press
Spodoptera exigua	*Btk*	1	20	3	Moar et al. 1994
	CryIC	1	32	>1000	
Spodoptera littoralis	*Btk*	1	8	1.4	Salama & Matter 1991
	CryIC[5]	2	14	10-50	Müller et al. 1994

[1]Summarized, in part, from Tabashnik (1994a).
[2]*Bt* preparations may contain spores and one or more toxins; Cry preparations usually do not contain *Bt* spores, and many were cloned and expressed in acrystalliferous strains of *Bt* or other species of bacteria.
[3]Resistance ratio = LC_{50} of resistant colony/LC_{50} of the susceptible colony. Range reflects combined results of more than one resistant population.
[4]Selections were not performed every generation.
[5]Larvae were selected with CryIC crystalline protoxin through generation 14. Continued selections were done with CryIC toxin (requiring activation with trypsin).
[6]Activated toxin.
[7]Laboratory population exposed for 98 generations to *Bti* prior to selection with CryIVD.
[8]Field population with no prior exposure to *Bti*.
[9]Laboratory population included individuals surviving *Btt* field applications for 1 to 2 years.
[10]Includes unpublished data.

TABLE 2. SUMMARY OF SIGNIFICANT LABORATORY SELECTION EXPERIMENTS WITH *BT* δ-ENDOTOXINS[1].

Species	*Bt* Strain or δ-Endotoxin[2]	Populations Selected	Generations Selected	Resistance Ratios[3]	Reference(s)
Trichoplusia ni	CryIA(b)[6]	1	7	31	Estada & Ferré 1994
Choristoneura fumiferana	*Bts*	1	8	3.8	van Frankenhuyzen et al. 1995
DIPTERA					
Aedes aegypti	*Bti*	3	14	1.1	Goldman et al. 1986
		1	8	1.8	Saleh 1987
Culex quinquefasciatus	CryIVD	1[7]	3	70	Gill et al. 1992
		1[8]	22	15	
COLEOPTERA					
Leptinotarsa decemlineata	CryIIIA	8[9]	32	>900	Whalon et al. 1993[10]
Chrysomela scripta	CryIIIA	1	35	>3000	Bauer et al. 1994[10]

[1]Summarized, in part, from Tabashnik (1994a).
[2]*Bt* preparations may contain spores and one or more toxins; Cry preparations usually do not contain *Bt* spores, and many were cloned and expressed in acrystalliferous strains of *Bt* or other species of bacteria.
[3]Resistance ratio = LC_{50} of resistant colony/LC_{50} of the susceptible colony. Range reflects combined results of more than one resistant population.
[4]Selections were not performed every generation.
[5]Larvae were selected with CryIC crystalline protoxin through generation 14. Continued selections were done with CryIC toxin (requiring activation with trypsin).
[6]Activated toxin.
[7]Laboratory population exposed for 98 generations to *Bti* prior to selection with CryIVD.
[8]Field population with no prior exposure to *Bti*.
[9]Laboratory population included individuals surviving *Btt* field applications for 1 to 2 years.
[10]Includes unpublished data.

der intensive selection pressure (Tabashnik 1994a). Baseline data on target pest sensitivity to *Bt* are essential in assessing the risk of resistance. Tabashnik (1994a) estimated variations in *Bt*-susceptibility among populations of 15 insect species within three orders and found minimal variation except in three species of moths. Variation in two stored product moths (up to 42-fold) was not associated with *Bt* treatments, but presumably resulted from natural exposure to *Bt* in grain bins (Kinsinger & McGaughey 1979). Variation in the third moth, *P. xylostella*, however, was attributable to repeated exposure to *Bt* foliar sprays. Tabashnik et al. (1990) determined that the susceptibility of intensively treated populations varied up to 40-fold, whereas the susceptibility for populations receiving minimal *Bt* exposure in the field and in laboratory colonies varied no more than 7-fold.

Increasing reliance on *Btk*-based products for the suppression of spruce budworm, *Choristoneura fumiferana* (Clemens), an important defoliator of coniferous forests in North America, stimulated interest in assessing the risk of field resistance (van Frankenhuyzen et al. 1995). Studies on the variation in *Bt* tolerance within and among nine spruce budworm field populations from Ontario with no previous *Bt* exposure reveal substantial familial differences in sensitivity to *Bt*, whereas differences between populations are minimal. This finding suggests that *Bt* tolerance is genetically based, and that spruce budworm has the potential to adapt to *Bt*. One laboratory selection experiment (Table 2) illustrates an increase in frequency of the resistance trait (van Frankenhuyzen et al. 1995).

To gain baseline data on the *Bt* susceptibility of two pests targeted by transgenic cotton, *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie), Stone & Sims (1993) bioassayed populations from 14 states with activated CryIA(c) toxin and a commercial *Btk*-based insecticide. Although previous exposure to *Bt* was not identified, significant differences within populations of both species were detected. Moreover, the variability in *Bt* tolerance was consistently higher for the activated toxin in both species. This again suggests that an individual toxin as expressed in transgenic cotton would stimulate adaptation more rapidly than the commercial preparations that contain a mixture of spores and crystals. Stone & Sims (1993) acknowledged that monitoring and managing for resistance will be an exciting challenge when insect-resistant cotton, expressing *Bt* δ-endotoxin, is deployed.

Mechanisms

An understanding of the mechanisms of resistance to *Bt* toxins will prove essential in the future design and management of transgenic plants containing *Bt* toxin genes. At present, the primary mechanism of resistance reported for *P. interpunctella* (Van Rie et al. 1990b) and *P. xylostella* (Ferré et al. 1991) is a reduction in the binding of toxin to receptors on the midgut brush border membrane. This is the same mechanism known to account for much of the host specificity to various δ-endotoxins in Lepidoptera (Hoffman et al. 1988b).

Plodia interpunctella, selected for resistance to *Btk* (see Table 1), shows a 50-fold reduction in midgut brushborder membrane receptor-binding affinity to CryIA(b) (Van Rie et al. 1990b). However, *in vivo* toxicity of CryIC (not present in *Btk*) increased when bioassayed in the *Btk*-resistant *P. interpunctella*. This increase in toxicity resulted from an increase in the CryIC binding sites. These results show that at least two distinct molecular changes occurred in the midgut receptor population in response to *Bt* selection. Although the role of these receptor molecules in the insect midgut are, as yet, poorly characterized, Van Rie et al. (1990b) suggest the increase in one receptor population may compensate for diminished function in the other. In a strain

of *P. interpunctella* selected for resistance to *Bt* subsp. *entomocidus* (*Bte*), Oppert et al. (1994) reported reduced proteolytic activation of CryI(A)c.

Using similar methodologies, resistance mechanisms studies of *Btk*-resistant field populations of *P. xylostella* demonstrated greatly reduced or lack of toxin binding to midgut receptors, suggesting a change or complete loss of the receptor (Ferré et al. 1991, Tabashnik et al. 1994). Using a different method for quantifying receptor binding, Masson et al. (1995) reported a loss of receptors in a strain of resistant *P. xylostella*, although not adequate to explain the high level of resistance.

The results from studies of other resistant insect species suggest that factors other than receptor binding can contribute to resistance. For example, two separate studies of resistance in *H. virescens* found no relation between resistance to CryIA(b) or CryIA(c) and toxin-receptor binding (MacIntosh et al. 1991, Gould et al. 1992). In addition, studies of *Trichoplusia ni* (Hübner) determined that CryIA(b) and CryIA(c) share the same receptor, but a strain of *T. ni* selected for resistance to CryIA(b) showed cross-resistance to CryIA(c) (Estada and Ferré 1994). Resistance mechanisms in these insects may involve changes in post-binding events such as channel formation, leakage, and repair rate.

Cross-resistance

The apparent specificity, diversity, and genetic versatility of *Bt* δ-endotoxins suggest that resistance might be managed by deploying toxins in mixtures or sequences (Georghiou 1990, Stone et al. 1991, Van Rie 1991). Although at least 12 lepidopteran-active δ-endotoxins are available (Adang 1991), evidence is mounting that selection for resistance to one or more δ-endotoxins causes resistance to others (Tables 3 and 4). This phenomenon, known as cross-resistance, typically occurs when mechanisms of toxicity are similar.

Many of the resistant insect populations generated in the laboratory and the field were selected with *Bt*-based insecticides containing multiple toxins; the most studied products are formulated with *Btk* strain HD-1, which is comprised of live spores and a mixture of five toxins (Table 1). In many studies, cross-resistance is referred to as an increase in tolerance of a population, selected with one *Bt* isolate, to an isolate containing a different mixture of toxins. For example, efforts to find other *Bt* isolates to control *P. interpunctella* resistant to *Btk* (140-fold) in grain bins showed that these insects were also resistant to 32 of the 57 *Bt* isolates assayed (McGaughey & Johnson 1987). Resistance was highest among various *Btk* isolates, suggesting some degree of specificity. Many *Bt* isolates overlap considerably in their δ-endotoxin composition. In the context of the following discussion, cross-resistance is defined as an increase in the tolerance of a population to a toxin absent in the preparation used for selection. Resistance, on the other hand, refers to increasing tolerance to a toxin that is present in the *Bt* isolate used in selection.

Strains of *P. interpunctella*, selected for resistance to *Btk*, *Bte*, *Bt* subsp. *aizawai* (*Bta*) strain HD-112, *Bta* strain HD-133, or a mixture of *Btk* and *Bta* HD-133, showed some level of resistance and cross-resistance to six δ-endotoxins tested (McGaughey & Johnson 1994) (Table 3). *Btk* tended to select for high levels of resistance to the entire complex of CryIA toxins which are 82 to 90% homologous in their amino acid sequences (Höfte & Whitley 1989). Resistance was highest to CryIA(b) and CryIA(c). Evidence suggesting that they share the same binding site (Wolfersberger 1990) is also supported by the high levels of cross-resistance to CryIA(c) in populations selected with *Bta* HD133 and *Bte*, which is likely derived from the presence of CryIA(b). The specificity of the target receptor appears to be greater for CryIA(c) than for CryIA(b) because cross-resistance is significantly greater than the selected resis-

TABLE 3. PATTERNS OF RESISTANCE AND CROSS-RESISTANCE TO INDIVIDUAL δ-ENDOTOXINS IN *P. INTERPUNCTELLA* AND *P. XYLOSTELLA* SELECTED FOR RESISTANCE TO *BT* ISOLATES THAT CONTAIN MULTIPLE TOXINS.

Bt Strain Used in Selection (Resistance Ratio[2])	Resistance Ratios for Individual δ-Endotoxins[1,2]						
	CryIA(a)	CryIA(b)	CryIA(c)	CryIB	CryIC	CryIF	CryIIA
Plodia interpunctella[3]							
Btk HD-1 (70)	6	263	2,816	**13**	**2**	na[4]	5
Bta HD-112 (28)	4	24	**27**	**7**	14	na	5
Bta HD-133 (94)	17	226	**789**	**44**	19	na	**24**
Bte HD-198 (32)	10	27	**150**	**9**	5	na	**20**
Btk HD-1 + *Bta* HD-133 (164) and (100)	4	253	2,267	**17**	32	na	10
Plutella xylostella[5]							
Btk HD-1 (3,300)	y[6]	>750	>6,800	**5**	**1**	**>240**	y

[1]Resistance ratios printed in bold type represent cross-resistance (i.e. toxins are not present in the *Bt* isolate used in selection (see Table 1).
[2]Resistance ratio = LC_{50} of resistant colony/LC_{50} of the susceptible colony.
[3]Results from McGaughey & Johnson (1994).
[4]na, data not available.
[5]Results compiled from Tabashnik et al. (1993, 1994b).
[6]y, significant level of resistance, but ratio was not reported.

tance. Cross-resistance to CryIB and CryIC was significant, although very low, which is consistent with their relatively low amino acid sequence similarity to the CryIA toxins (ranging from 55 to 67%) (Tabashnik et al. 1994b). As expected, *Bt* isolates containing more dissimilar toxins, such as found in *Bta* and *Bte*, selected for a broader spectrum of cross-resistance in the treated insects than did isolates producing similar Cry toxins such as *Btk* HD-1. The complexity in the pattern of resistance results, in part, from differential selective pressure exerted by different levels of toxicity and the amounts of each toxin in each *Bt* strain. Overall, the results of this selection experiment clearly contradict the claim that toxin mixtures will prevent or retard the development of resistance (Tabashnik & McGaughey 1994).

Field-resistant *P. xylostella* from Hawaii, further selected with *Btk* in the laboratory, show a similar pattern of broad, but highly variable, resistance and cross-resistance among the Cry toxins tested (Tabashnik et al. 1993, 1994b) (Table 3). Again, resistance was highest to the CryIA toxins, and cross-resistance to CryIB and CryIC was low. Intermediate cross-resistance to CryIF is supported by its amino acid sequence homology (70 to 72% to the CryIA toxins).

A different field population of *P. xylostella* from the Philippines, also resistant to CryIA(b) (resistance ratio 236), showed no resistance to *Btk*, CryIA(a), CryIA(c) and no cross-resistance to CryIB, and CryIC (Ferré et al. 1991, Ballester et al., in press). These researchers determined that CryIA(b) has a single binding site that is also recognized by CryIA(a) and CryIA(c). Loss or modification of the CryIA(b) binding site results in the loss of its toxicity. Ballester et al. (in press) noted that this narrow spectrum of resistance is somewhat unique and suggested that this population may represent a biotype present in the Phillipines, not a case of field selection by *Btk*.

To understand the complexity of these emerging cross-resistance patterns, we must select insect populations with individual toxins. In the case of most *Bt* isolates, which produce several toxins, this requires cloning and transferring the *cry* gene into an acrystalliferous strain of *Bt*, or into another species of bacteria, such as *Escherichia coli* or *Pseudomonas fluorescens*. In some studies, the gene is modified to simulate the specific Cry products being expressed in transgenic plants or microorganisms.

The first such study that also included cross-resistance data was the selection of *H. virescens* with purified CryIA(c) (Gould et al. 1992) (Table 4). After 17 generations, the population was 50-fold resistant to CryIA(c) and, as reported for other lepidopterans, was 13-fold cross-resistant to CryIA(b). This population, however, was also cross-resistant to CryIIA, CryIB, and CryIC. In a subsequent paper, Gould et al. (in press) also reported high levels of cross-resistance to CryIF. No measurable differences in the concentration of CryIA(c), or CryIA(b) binding sites or binding affinities, were detected between selected and unselected *H. virescens*, suggesting other mechanisms of resistance are involved. Similar broad-spectrum cross-resistance was also reported for two species of *Spodoptera* and two coleopterans, *Chrysomela scripta* F. (Table 4) and *L. decemlineata* (Whalon, unpublished results).

In contrast, cross-resistance in *Trichoplusia ni* (Hübner) selected with CryIA(b) had a higher degree of specificity within the CryIA group of toxins (Estada & Ferre 1994). This specificity was similar to that reported for *P. xylostella* from the Phillipines (Ballester et al. in press), although no data were presented on unrelated toxins for *T. ni*. As determined for several other lepidopterans, receptor-binding assays reveal that CryIA(b) and CryIA(c) share the same high-affinity binding sites, and resistance to one would imply cross-resistance to the other. However, no cross-resistance to CryIA(c) was detected, suggesting CryIA(c) toxicity results from other binding sites or alternative mechanisms.

Overall, cross-resistance patterns and their underlying physiological mechanism are very complex and somewhat unpredictable, even within a closely related group of

TABLE 4. SUMMARY OF STUDIES ON CROSS-RESISTANCE TO δ-ENDOTOXINS IN INSECTS SELECTED WITH ONE PURIFIED δ-ENDOTOXIN.

Species[1]	Cross-Resistance Ratios for Specific δ-Endotoxins[2,3]									
δ-Endotoxin[2] Used in Selection (Resistance Ratio[3])	CryIA(a)	CryIA(b)	CryIA(c)	CryIB	CryIC	CryID	CryIE	CryIF	CryIH	CryIIA
Heliothis virescens										
CryIA(c) (50)	y[4]	13	—	y	y	na[5]	na	na	na	53
CryIA(c) (558-10,000)[6]	na	2,364	—	y	2.5	na	na	3,678	na	15
Spodoptera exigua[7]										
CryIC (>1000)	na	>93	na	na	—	na	80[8]	na	12	73
Spodoptera littoralis										
CryIC (50)	na	na	na	na	—	y	y	na	na	na
Trichoplusia ni										
CryIA(b) (31)	0	—	0	na	na	na	na	na	na	na
Chrysomela scripta[9]										
CryIIIA (>3000)	ns[10]	ns	ns	328	na	na	na	na	na	ns

[1]See references listed in Table 2.
[2]δ-endotoxins do not contain *Bt* spores, and many were cloned and produced in acrystalliferous strains of *Bt* or other species of bacteria.
[3]Resistance ratio = LC_{50} of resistant colony/LC_{50} of the susceptible colony.
[4]y, significant level of resistance, but ratio was not reported.
[5]na, data not available.
[6]Cross-resistance bioassays performed over time as selections continued and resistance to CryIA(c) was increasing (Gould et al. in press).
[7]W. J. Moar, personal communication.
[8]Trypsinized CryIE/CryIC fusion protein.
[9]Bauer, unpublished.
[10]ns, not susceptible.

toxins and susceptible insects. A more complete understanding of how each toxin interacts within a particular target species at the molecular level is critical to selecting δ-endotoxins for design of transgenic plants that do not favor broad-spectrum cross-resistance.

Adaptation to δ-Endotoxin

The recent appearance of resistance to conventional *Bt*-based insecticides was correlated with improved formulation and more intensive usage patterns (Tabashnik et al. 1990). In addition to operational uses of the *Bt* toxins, pest genetics, behavior, physiology, and ecology are critical factors in predicting resistance risk.

The best studied examples of *Bt*-resistance, *P. interpunctella* and *P. xylostella*, share many attributes that leave their populations particularly vulnerable to rapid selection (Table 5). These attributes include multivoltinism, short generation time, and populations that tend to be isolated and stable. These attributes are typical of many agricultural and medical pests, and they temper enthusiasm for bioengineered plants which produce continuous and high levels of δ-endotoxin. It is apparent, however, that strategies are needed to delay or avoid resistance in pests that are intensively managed with *Bt*, regardless of the deployment method.

Inheritance

The development of strategies to manage resistance requires some understanding of the inheritance of a resistance trait in the pest population (Gould 1986). This was demonstrated by Tabashnik (1994b) using a population genetics model to simulate the response of a pest population to different resistance management strategies. The best strategy, i.e., one that delays resistance the longest, must be customized to the number of alleles and inheritance of the trait within the population.

Studies of the genetics of resistance typically involve determining the susceptibility of progeny from crosses between individuals from the selected and unselected pop-

TABLE 5. FACTORS ASSOCIATED WITH DEVELOPMENT OF RESISTANCE TO *BT* IN TWO LEPIDOPTERANS.

Factors	*P. interpunctella*	*P. xylostella*
Pest Attributes and Management		
Short generation	yes	yes
Generations/year	5-6	8-10
Population isolation	yes	yes
Realized heritability	high	low
Crop rotation	no	no
Operational Use of *Bt*		
Multiple toxins	yes	yes
Applications/generation	5-6	5-8
Frequency/generation	2-4 days	1.5-2 days
Selection pressure	high	high

ulations. In both *P. interpunctella* and *P. xylostella*, resistance is autosomally inherited (no maternal effects or sex linkage), partially recessive (progeny susceptibility more similar to unselected parent), and apparently due to one or a few major loci (McGaughey 1985, McGaughey & Beeman 1988, Hama et al. 1992, Tabashnik et al. 1992). The genetic basis of resistance to the CryIA toxin-complex in *H. virescens* is partially recessive and due to a single locus or set of tightly linked loci (Gould et al. in press). In *L. decemlineata*, resistance to CryIIIA is also autosomally inherited, and conferred by one incompletely dominant gene (Rahardja & Whalon 1995). Knowledge of the genetic basis of resistance is critical to our understanding of the stability of the trait within the selected population.

Stability

Perhaps one of the simplest resistance management strategies involves providing the pest population with intermittent time periods in which *Bt* is not used for control. The success of such temporal refuges is dependent on the stability of the resistance trait after *Bt* exposure ceases. The rate of reversion is dependent on the inheritance of the resistance trait and the fitness costs associated with resistance.

Different populations of resistant *P. xylostella* revert at different rates when selection with *Bt* is relaxed. Typically, the decline is slow and incomplete; for example, one *Btk*-resistant population declined from 29-fold to 1-fold after 32 generations without selection (Tabashnik et al. 1991). Similar results were reported in other *Btk*-resistant populations of *P. xylostella* (Tabashnik et al. 1991, 1994, Hamma et al. 1992) and in other resistant species, including *P. interpunctella* (McGaughey & Beeman 1988), *H. virescens* (Sims & Stone 1991), and *L. decemlineata* (Rahardja & Whalon 1995). In a recent study, rapid and complete reversal of resistance occurred after 13 generations without selection in a population of *P. xylostella* with 2800-fold resistance to *Btk* (Tabashnik et al. 1994a). This result suggests that resistance is achieved with significant loss in fitness, perhaps related to the alteration in the midgut binding sites documented in the study. Groeters et al. (1993, 1994) quantified reduced egg hatch, survival to the adult stage, fecundity, and mating success in male moths as significant fitness costs of resistance. Despite the rapid reversion to the susceptible genotype, the population responded rapidly to reselection (Tabashnik et al. 1994a). Rapid resurgence of resistance in relaxed populations is typical, indicating the persistence of a low number of highly resistant individuals. If the alleles for resistance become fixed in the population, or other alleles compensate for losses in fitness, resistance becomes stable and reversion to susceptibility is unlikely.

Resistance Management

In an effort to preserve the utility of these unique insecticidal proteins, knowledge of resistance management to conventional pesticides is useful (Gould 1988a, 1988b, Stone et al. 1991, McGaughey & Whalon 1992, Whalon & McGaughey 1993, McGaughey 1994, Tabashnik 1994a). Unfortunately, selection for the resistance trait in a pest population is probably the inevitable consequence of insecticide use (Denholm & Rowland 1992). The goal then becomes how to design and manipulate operational strategies that best conserve susceptibility, thereby delaying resistance. The implementation of integrated pest management (IPM) strategies that optimize the goals of resistance management involves 1) diversifying the sources of mortality to avoid selection for a single mechanism, 2) reducing selection pressure for the major mortality factors, 3) maintaining susceptible individuals by providing refuges and encouraging immigration, 4) monitoring for increasing resistance to any one of the mortality agents, and 5) responding to resistance through management strategies designed to

reduce the frequency of the resistance trait (Whalon & McGaughey 1993). Unfortunately, IPM is rarely implemented before a resistance crisis occurs, and generally another insecticide is available to replace the old one.

Conventionally applied *Bt*-based insecticides are more amenable to such IPM strategies, because the short residual time and host specificity help reduce selection pressure associated with the *Bt* toxins. Insects surviving *Bt* exposure are generally in a weakened condition, facilitating their exploitation by other mortality factors such as beneficial insects (Tabashnik 1986, 1994a) and pathogens (Krieg 1971, Jacques & Morris 1981). Other stressing agents, such as adverse weather conditions and low plant nutritional quality, will also cause higher mortality in insects recovering from *Bt* exposure. The cumulative suppression exerted by these factors also reduces selection pressure by reducing the frequency of pesticide applications. However, recent improvements in *Bt* formulations involve increasing toxicity, increasing residual times, and in some cases broadening host range, thereby bringing selection pressure for resistance more in line with conventional insecticides (Tabashnik 1994a).

The expression of δ-endotoxins in transgenic plants is considered analogous, in some respects, to intrinsic host plant defenses selected for by classical plant breeders (Gould 1988a). Unfortunately, pests adapt to resistant cultivars, and without appropriate deployment strategies, they are rendered ineffective (Gould 1986, Cox & Hatchett 1986). At present, bioengineering insect-resistant plants involves the incorporation of *Bt* δ-endotoxin genes into plants. There was considerable optimism several years ago that these plants would remain durable (Gould 1986,Gould 1988b, Rousch 1989).

Early optimism and excitement over genetically-engineered plants expressing δ-endotoxin, perhaps bolstered by the presumption of an unlimited variety of these proteins, have given way to serious concern over the durability of these plants, and with it, conventional uses of *Bt*. Resistance management strategies in the context of *Bt* were recently reviewed in some detail by McGaughey & Whalon (1992), Whalon & McGaughey (1993), Robison et al. (1994), and Tabashnik (1994a). These strategies include 1) **mixtures** of toxins with different mechanisms, either within the same plant or in different plants, or expressed serially over time (Gould 1986); 2) **synergists** to increase toxicity (MacIntosh et al. 1990); 3) **rotations** to alternative toxins temporally to reduce the frequency of resistant individuals (Tabashnik 1989); 4) **refuges**, temporal and spatial, to facilitate survival of susceptible individuals (Gould & Anderson 1991); 5) **low doses** of toxin that produce sublethal effects, such as reduced fecundity and slowed development, favoring other mortality factors; 6) **ultrahigh doses** of toxin that kill resistant heterozygotes and homozygotes (Denholm & Rowland 1992, Tabashnik 1994a); and 7) **gene regulation** of toxin titre, location, and induction (Whalon & McGaughey 1993).

Tabashnik (1994b) used a population genetics model (Mallet & Porter 1992) to simulate the effect of several resistance management strategies on resistance (single locus with two alleles) in a pest such as *Heliothis*. Transgenic plants expressing δ-endotoxin were planted as 1) pure stands of toxic plants, 2) seed mixtures with varying proportions of toxic and toxin-free plants, 3) toxin-free plants in refugia, and 4) seed mixtures + refugia. Across a range of conditions, seed mixtures always delayed the onset of insect resistance to the toxin, when compared to pure stands of toxic plants. Refugia will delay resistance as long, or in some cases longer, than seed mixtures because refugia reduce selection without altering dominance (Mallet & Porter 1992). Refugia will delay resistance longer than mixtures + refugia only under specific conditions. However, the implementation of pest control within refugia limits their ability to delay resistance in proportion to the efficacy of the controls.

The distinction between seed mixtures and refugia is the spatial distribution of toxin-free plants relative to the dispersal capability of pest larvae. Due to close proximity, larvae may move easily between toxic and toxin-free plants in fields planted with a seed mixture, whereas movement from toxic stands to toxic-free refugia is less likely. The tissue-specific expression of toxin through gene regulation is analogous to seed mixtures, because larvae can move freely between toxic and toxin-free tissue (Gould 1988a). Tabashnik (1994b) recommends maximizing spatial refuges, as well as temporal refuges, such as alternative crops and controls. The only method known to prolong the efficacy of any insecticide is to minimize the exposure of the target pests to the toxin in space and time (Denholm & Rowland 1992). Resistance management tactics must be validated in the field, under the inevitable practical, economic, and political constraints imposed by agriculture today (May 1993).

CONCLUSIONS

The genetic capacity of insect populations to evolve resistance to *Bt* δ-endotoxins is now well documented in many species within eight different insect orders. Although high-level field resistance is known only in *P. xylostella*, much has been learned from studying the many insect populations selected for resistance to *Bt* in the laboratory. We now know that 1) *Bt* resistance alleles are present at varying levels in different insect species and populations, 2) within a single species, the genetics, mechanisms, level, and stability of resistance vary between selected populations, 3) selection with a blend of toxins can select for resistance to each toxin in the blend, 4) resistance occurs more rapidly with purified toxins than with spore/crystal preparations, 5) cross-resistance to δ-endotoxins is almost ubiquitous and often unpredictable, and 6) reselection of revertant populations is rapid.

Today, *Bt*-based insecticides are frequently used in intensive agriculture, either in conjunction with conventional insecticides as a backup for control failure, or, as a last resort once resistance to other registered insecticides has occurred. Many insect pests, therefore, are already adapted to mixtures of δ-endotoxins. It is probable that the deployment of transgenic plants will precede the development of resistance management strategies, because advances in resistance management technology have not kept pace with those made in biotechnology. Developing and validating realistic resistance management plans that preserve the durability of δ-endotoxins deployed in transgenic plants may prove far more complex than the theory and techniques that actually generated the plants. Unfortunately, experimentally developed tactics to delay resistance have often been too naive or unrealistic for large-scale field implementation (Hoy 1995).

Fortunately, many researchers with experience and knowledge of resistance management with conventional insecticides have shifted the emphasis of their research into the development of strategies designed to prolong the durability of these unique bacterial toxins in transgenic plants. In general, resistance management seeks to minimize the exposure of the target pest to a toxin in time and space. This can be accomplished by developing IPM plans for these crops that include synergists, seed mixtures and refugia, tissue-specific and inducible toxin expression, and alternating crops or control measures (Hoy 1995). Substantial benefits to the environment will be gained if insects can be successfully managed through the careful deployment of genetically-engineered insect-resistant plants. However, only few *Bt* δ-endotoxins, all with similar modes of action, are now available to plant molecular biologists. Deployment of these plants before the management tactics are validated will result in the loss of *Bt*-based insecticides for many of the pests they are targeted to control.

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REFERENCES CITED

ADANG, M. J. 1991. *Bacillus thuringiensis* insecticidal crystal proteins: gene structure, action, and utilization, pp. 3-24 *in* K. Maramorosch [ed], Biotechnology for biological control of pests and vectors. Boca Raton, Florida:CRC Press.

ALY, C., M. S. MULLA, AND B. A. FEDERICI. 1985. Sporulation and toxin production by *Bacillus thuringiensis* var. *israelensis* in cadavers of mosquito larvae (Diptera: Culicidae). J. Invertebr. Pathol. 46: 251-258.

ANGUS, T. A. 1954. A bacterial toxin paralyzing silkworm larvae. Nature (London) 173: 545-546.

ARONSON, A. I. 1993. Insecticidal toxins, pp. 953-963 *in* A. L. Sonenshein, J. A. Hoch, and R. Losick [eds], *Bacillus subtilus* and other gram-positive bacteria: biochemistry, physiology, and molecular biology. Washington, DC:American Society for Microbiology.

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.

BALLESTER, V., B. ESCRICHE, J. L. MÉNSUA, G. W. RIETHMACHER, AND JUAN FERRÉ. Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to CryIA(b). Biocontrol Sci. Technol. In Press.

BAUER, L. S., AND H. S. PANKRATZ. 1992. Ultrastructural effects of *Bacillus thuringiensis* var *san diego* on midgut cells of the cottonwood leaf beetle. J. Invertebr. Pathol. 60: 15-25.

BAUER, L. S., C. N. KOLLER, D. L. MILLER, AND R. M. HOLLINGWORTH. 1994. Laboratory selection of the cottonwood leaf beetles, *Chrysomela scripta*, for resistance to *Bacillus thuringiensis* var. *tenebrionis* δ-endotoxin. p. 68 *in* Abstracts VIth Intern. Coll. Invertebr. Pathol.

BECKER, N., AND J. MARGOLIT. 1993. Use of *Bacillus thuringiensis israeliensis* against mosquitoes, pp. 147-170 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley.

BERLINER, E. 1911. Über die Schlaffsucht der Mehlmottenraupe (*Ephestia kuehniella* Zell), und ihren Erreger *Bacillus thuringiensis* n. sp. Zeitschrift für angewandtes. Entomol. 2: 29-56.

BOMAN, H. G. 1981. Insect Responses to Microbial Infections, pp. 769-784 *in* H. D. Burges and N. W. Hussey [eds], Microbial control of pests and plant diseases 1970-1980. New York:Academic Press.

BORGONIE, G., R. VAN DRIESSCHE, F. LEYNS, G. ARNAUT, D. DE WAELE, AND A. COOMANS. 1995. Germination of *Bacillus thuringiensis* spores in bacteriophagous nematodes (Nematoda: Rhabditida). J. Invertebr. Pathol. 65: 61-67.

BRADLEY, D., M. A. HARKEY, M. K. KIM, K. D. BIEVER, AND L. S. BAUER. 1995. Insecticidal CryIB crystal protein of *Bacillus thuringiensis* has dual specificity to coleopteran and lepidopteran larvae. J. Invertebr. Pathol. 65: 162-173.

BRAVO, A., S. JANSENS, AND M. PEFEROEN. 1992. Immunocytochemical localization of *Bacillus thuringiensis* insecticidal crystal proteins in intoxicated insects. J. Invertebr. Pathol. 60: 237-246.

BREWER, G. J. 1991. Resistance to *Bacillus thuringiensis* subsp. *kurstaki* in the sunflower moth (Lepidoptera: Pyralidae). Environ. Entomol. 20:316-322.

BRIESE, G. J. 1981. Resistance of insect species to microbial pathogens, pp. 511-545 *in* E. W. Davidson [ed], Pathogenesis of Invertebrate Microbial Diseases. Totowa, New Jersey:Allanheld Osmun.

BURGES, H. D. 1971. Possibilities for pest resistance to microbial control agents, pp. 445-457 *in* H. D. Burges and N. W. Hussey [eds], Microbial Control of Insects and Mites. New York:Academic Press.

BURGES, H. D., AND J. A. HURST. 1977. Ecology of *Bacillus thuringiensis* in storage moths. J. Invertebr. Pathol. 30: 131-139.

CARLTON, C. C., AND C. GAWRON-BURKE. 1993. Genetic improvement of *Bacillus thuringiensis*, pp. 43-61 *in* L. Kim [ed], Advanced Engineered Pesticides. New York:Marcel Dekker.

CARROLL, J., AND D. J. ELLAR. 1993. Analysis of *Bacillus thuringiensis* δ-endotoxin action on insect-membrane permeability using a light-scattering assay. Eur. J. Biochem. 214: 771-778.

CARROLL, J., J. LI, AND D. J. ELLAR. 1989. Proteolytic processing of a coleopteran-specific delta-endotoxin produced by *Bacillus thuringiensis* var. *tenebrionis*. Biochem. J. 261:99-105.

CHILCOTT, C. N., B. H. KNOWLES, D. J. ELLAR, AND F. A. DROBNIEWSKI. 1990. Mechanism of action of *Bacillus thuringiensis* parasporal body, pp. 44-65 *in* H. de Barjac and D. Sutherland [eds], Bacterial Control of Mosquitoes and Blackflies: Biochemistry, Genetics, and Applications of *Bacillus thuringiensis* and *Bacillus sphaericus*. New Brunswick:Rutgers University Press.

COX, T. S., AND J. H. HATCHETT. 1986. Genetic model for wheat/Hessian fly (Diptera: Cecidomyiidae) interaction: strategies for deployment of resistance genes in wheat cultivars. Environ. Entomol. 15: 24-31.

CUMMINGS, C. E., AND D. J. ELLAR. 1994. Chemical modification of *Bacillus thuringiensis* activated δ-endotoxin and its effect on toxicity and binding to *Manduca sexta* midgut membranes. Microbiol. 140: 2737-2747.

DE BARJAC, H. 1987. Operational bacterial insecticides and their potential for future improvements, pp. 63-73 *in* K. Maramorosch [ed], Biotechnology in Invertebrate Pathology and Cell Culture. San Diego, California:Academic Press.

DENHOLM, I., AND M. W. ROWLAND. 1992. Tactics for managing pesticide resistance in arthropods: theory and practice. Annu. Rev. Entomol. 37: 91-112.

DENOLF, P., S, JANSENS, M. PEFEROEN, D. DEGHEELE, AND J. VAN RIE. 1993. Two different *Bacillus thuringiensis* delta-endotoxin receptors in the midgut brush border membrane of the european corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). Appl. Environ. Microbiol. 59: 1828-1837.

DEVRIENDT, M., AND D. MARTOURET. 1976. Absence de résistance à *Bacillus thuringiensis* chez la teigne des crucifères, *Plutella maculipennis*. Entomophaga 21: 189-199.

DU, C., P. A. MARTIN, AND K. W. NICKERSON. 1994. Comparison of disulfide contents and solubility at alkaline pH of insecticidal and noninsecticidal *Bacillus thuringiensis* protein crystals. Appl. Environ. Microbiol. 60: 3847-3853.

ELLAR, D. J. 1994. Structure and mechanism of action of *Bacillus thuringiensis* endotoxins and their receptors. Biocon. Sci. Technol. 4: 445-447.

ELY, S. 1993. The engineering of plants to express *Bacillus thuringiensis* δ-endotoxins, pp. 105-124 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley, 311 pp.

ENGLISH, L., AND S. L. SLATIN. 1992. Mode of action of delta-endotoxin from *Bacillus thuringiensis:* a comparison with other bacterial toxins. Insect Biochem. Molec. Biol. 22: 1-7.

ESCRICHE, B., A. C. MARTINEZ-RAMIREZ, M. D. REAL, F. J. SILVA, AND J. FERRÉ. 1994. Occurrence of three different binding sites for *Bacillus thuringiensis* δ-endotoxins in the midgut brush border membrane of potato tuber moth, *Phthorimaea operculella* (Zeller). Arch. Insect Biochem. Physiol. 26: 315-327.

ESTADA, U., AND J. FERRÉ. 1994. Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and selection for resistance to one of the crystal proteins. Appl. Environ. Microbiol. 60: 3840-3846.

FALCONER, D. S. 1989. Introduction to Quantitative Genetics. New York:Longman.

FEDERICI, B. A. 1993. Insecticidal bacterial proteins identify the midgut epithelium as a source of novel target sites for insect control. Arch. Insect Biochem. Physiol. 22: 357-371.

FERRÉ, J., M. D. REAL, J. VAN RIE, S. JANSENS, AND M. PERFEROEN. 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.

FEITELSON, J. S. 1993. The *Bacillis thuringiensis* family tree, pp. 63-71 *in* L. Kim [ed.], Advanced Engeered Pesticides, New York: Marcel Dekker.

FEITELSON, J. S., J. PAYNE, AND L. KIM. 1992. *Bacillus thuringiensis*: insects and beyond. Bio/technology 10: 271-276.

GARCZYNSKI, S. F., AND M. J. ADANG. *Bacillus thuringiensis* CryIA(c) δ-endotoxin binding aminopeptidase in the *Manduca sexta* midgut has a glycosyl-phosphatidylinositol anchor. Insect Biochem. Molec. Biol. In Press.

GARCZYNSKI, S. F., J. W. CRIM, AND M. J. ADANG. 1991. Identification of putative insect brush border membrane-binding molecules specific to *Bacillus thuringiensis* δ-endotoxin by protein blot analysis. Appl. Environ. Microbiol. 57: 2816-2820.

GASSER, C. S., AND R. T. FRALEY. 1989. Genetically engineering plants for crop improvement. Science 244: 1293-1299.

GELERNTER, W., AND G. E. SCHWAB. 1993. Transgenic bacteria, viruses, algae and other microorganisms as *Bacillus thuringiensis* delivery systems, pp. 89-104 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis,* an environmental biopesticide: theory and practice. New York:Wiley, 311 pp.

GEORGHIOU, G. P. 1990. Resistance potential to biopesticides and consideration of countermeasures, pp. 409-420 *in* J. E. Casida [ed], Pesticides and Alternatives. New York:Elsevier.

GEORGHIOU, G. P. 1994. Mechanisms and microbial characteristics of invertebrate resistance to bacterial toxins, pp. 48-50. *in* Proc. VIth Intern. Coll. Invertebr. Pathol., Montpellier, France:Soc. Invertebr. Pathol.

GEORGHIOU, G. P., AND A. LAGUNES. 1988. The occurrence of resistance to pesticides: cases of resistance reported worldwide through 1988. Food and Agricultural Organization, Rome, 325 pp.

GILL, S. S., E. A. COWLES, AND P. V. PIETRANTONIO. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37: 615-36.

GOLDBERG, L. J., AND J. MARGALIT. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles serengetii, Uranotaenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens.* Mosq. News. 37: 355-358.

GOLDMAN, I. F., J. ARNOLD, AND B. C. CARLTON. 1986. Selection for resistance to *Bacillus thuringiensis* subspecies *israelensis* in field and laboratory populations of the mosquito *Aedes aegypti.* J. Invertebr. Pathol. 47: 317-324.

GOULD, F. 1986. Simulation models for predicting durability of insect-resistant germplasm: Hessian fly (Diptera: Cecidomyiidae) resistant winter wheat. Environ. Entomol. 15: 1-10.

GOULD, F. 1988a. Evolutionary biology and genetically engineered crops: Consideration of evolutionary theory can aid in crop design. BioScience 38: 26-33.

GOULD, F. 1988b. Genetic engineering, integrated pest management and the evolution of pests, pp. 15-18 *in* Planned Release of Genetically Engineered Organisms. Cambridge (UK):Elsevier.

GOULD, F., AND A. ANDERSON. 1991. Effects of *Bacillus thuringiensis* and HD-73 delta-endotoxin on growth, behavior, and fitness of susceptible and toxin-adapted strains *Heliothis virescens* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 30-38.

GOULD, F., A. ANDERSON, D. LANDIS, AND H. VAN MELLAERT. 1991. Feeding behavior and growth of *Heliothis virescens* larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins. Entomol. Exp. Appl. 58: 199-210.

GOULD, F., A. ANDERSON, A. REYNOLDS, L. BUMGARNER, AND W. MOAR. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to some *Bacillus thuringiensis* toxins. J. Econ. Entomol. In Press.

GOULD, F., A. MARTINEZ-RAMIREZ, A. ANDERSON, J. FERRE, F. J. SILVA, AND W. J. MOAR. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. Proc. Natl. Acad. Sci. 88: 7986-7990.

GROETERS, F. R., B. E. TABASHNIK, N. FINSON, AND M. W. JOHNSON. 1993. Resistance to *Bacillus thuringiensis* affects mating success of the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 86: 1035-1039.

GROETERS, F. R., B. E. TABASHNIK, N. FINSON, AND M. W. JOHNSON. 1994. Fitness costs of resistance to *Bacillus thuringiensis* in the diamondback moth (*Plutella xylostella*). Evol. 48: 197-201.

HAMA, H., K. SUZUKI, AND H. TANAKA. 1992. Inheritance and stability of resistance to *Bacillus thuringiensis* formulations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). Appl. Entomol. Zool. 27: 355-362.

HEIMPEL, A. M., AND T. A. ANGUS. 1959. The site of action of crystalliferous bacteria in Lepidoptera larvae. J. Insect Pathol. 1: 152-170.

HOFFMAN, C., P. LUTHY, R. HUTTER, AND V. PLESKA. 1988a. Binding of the delta-endotoxin from *Bacillus thuringiensis* to brush border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). Eur. J. Biochem. 173:85-91.

HOFFMAN, C., H. VANDERBRUGGEN, H. HÖFTE, J. VAN RIE, AND S. JANSENS. 1988b. Specificity of *Bacillus thuringiensis* delta-endotoxin is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. Proc. Natl. Acad. Sci. USA 85: 7844-7848.

HÖFTE, H., AND H. R. WHITELEY. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev. 53: 242-255.

HONÉE, G., AND B. VISSER. 1993. The mode of action of *Bacillus thuringiensis* crystal protein. Entomol. exp. appl. 69: 145-155.

HOY, M. A. 1995. Multitactic resistance management: an approach that is long overdue? Florida Entomol. 78:443-451.

ISHIWATA, S. 1901. On a kind of severe flasherie (sotto disease). Dainihan Sanbshi Kaiho 9: 1-5.

JAQUES, R. P., AND O. N. MORRIS. 1981. Compatibility of pathogens with other methods of pest control and with different crops, pp. 695-715 *in* H. D. Burges [ed], Microbial control of pests and plant diseases 1970-1980. New York:Academic Press.

JANSSON, R. K., AND S. H. LECRONE. 1990. Management of diamondback moth, *Plutella xylostella*, with nonconventional chemical and biological insecticides. Proc. Florida State Hortic. Soc. 103: 122-126.

KEIDING, J. 1986. Prediction of resistance risk assessment, pp. 279-297 *in* National Research Council. Pesticide Resistance: Strategies and Tactics for Management. Washington, DC:National Academy.

KELLER, B., AND G. LANGENBRUCH. 1993. Control of coleopteran pests by *Bacillus thuringiensis* and resistance management, pp. 171-191 *in* P. F. Entwistle, J. S.

Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley.

KINSINGER, R. A., AND W. H. MCGAUGHEY. 1979. Susceptibility of populations of Indianmeal moth and almond moth to *Bacillus thuringiensis* isolates (Lepidoptera: Pyralidae). J. Econ. Entomol. 72: 346-349.

KIRSCH, K., AND H. SCHMUTTERER. 1988. Low efficacy of a *Bacillus thuringiensis* (Berl.) formulation in controlling the diamondback moth, *Plutella xylostella* (L.), in the Philippines. J. Appl. Entomol. 105: 249-255.

KNIGHT, P. J. K., N. CRICKMORE, AND D. J. ELLAR. 1994. The receptor for *Bacillus thuringiensis* CryIA(c) delta-endotoxin in the brush border membrane of the lepidopteran *Manduca sexta* is aminopeptidase N. Mol. Microbiol. 11: 429-436.

KNOWLES, B. H., AND J. A. T. DOW. 1993. The crystal delta-endotoxins of *Bacillus thuringiensis*: models for their mechanism of action on the insect gut. Bioessays 15: 469-475.

KNOWLES, B. H., AND D. J. ELLAR. 1987. Colloid-osmotic lysis is a general feature of the mechanism of action of *Bacillus thuringiensis* delta-endotoxin with different insect specificity. Biochem. Biophys. Acta 924: 509-518.

KOLLER, C. N., L. S. BAUER, AND R. M. HOLLINGWORTH. 1992. Characterization of the pH-mediated solubility of *Bacillus thuringiensis* var. *san diego* native delta-endotoxin crystals. Biochem. Biophys. Res. Comm. 184:692-699.

KONI, P. A., AND D. J. ELLAR. 1994. Biochemical characterization of *Bacillus thuringiensis* cytolytic δ-endotoxin. Microbiol. 140: 1869-1880.

KRIEG, A. 1971. Interactions between pathogens, pp. 459-468 *in* H. D. Burges and N. W. Hussey [eds], Microbial control of insects and mites. New York:Academic Press.

KRIEG, A., AND G. A. LANGENBRUCH. 1981. Susceptibility of arthropod species to *Bacillus thuringiensis*, pp. 837-898 *in* H. D. Burges and N. W. Hussey [eds], Microbial Control of Pests and Plant Diseases 1970-1980. New York: Academic Press.

KRIEG, A., A. M. HUGER, G. A. LANGENBROOK, AND W. SCHNETTER. 1983. *Bacillus thuringiensis* var. *tenebrionis*: ein neuer gegenuber larven von Coleopteren wirksamer. Pathotyp. Z. Ang. Entomol. 96: 500-508.

LAMBERT, B., AND M. PEFEROEN. 1992. Insecticidal promise of *Bacillus thuringiensis*. Bioscience 42: 112-122.

LI. J., J. CARROLL, AND D. J. ELLAR. 1991. Crystal structure of insecticidal delta-endotoxin from *Bacillus thuringiensis* at 2.5 Å resolution. Nature 353: 815-821.

MACINTOSH, S. C., G. M. KISHORE, F. J. PERLAK, P. G. MARRONE, AND T. B. STONE. 1990. Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. J. Agric. Food Chem. 38: 1145-1152.

MACINTOSH, S. C., T. B. STONE, R. S. JOKERST, AND R. L. FUCHS. 1991. Binding of *Bacillus thuringiensis* proteins to a laboratory-selected line of *Heliothis virescens*. Proc. Natl. Acad. Sci. USA 88: 8930-8933.

MALLET, J., AND P. PORTER. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. London B 250: 165-169.

MARRONE, P. G., AND S. C. MACINTOSH. 1993. Resistance to *Bacillus thuringiensis* and resistance management, pp. 221-235 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley.

MARTIN, P. A. W., AND R. S. TRAVERS. 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl. Environ. Microbiol. 55: 2437-2442.

MASSON, L., A. MAZZA, R. BROUSSEAU, AND B. TABASHNIK. 1995. Kinetics of *Bacillus thuringiensis* toxin binding with brush border membrane vesicles from susceptible and resistant larvae of *Plutella xylostella*. J. Biol. Chem. 270: 1-10.

MAY, R. M. 1993. Resisting resistance. Nature 361: 593-594.

MCGAUGHEY, W. H. 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis.* Science 229:193-195.

MCGAUGHEY, W. H. 1994. Problems of insect resistance to *Bacillus thuringiensis*. Agr. Ecosystem. Environ. 49: 95-102.

MCGAUGHEY, W. H., AND R. W. BEEMAN. 1988. Resistance to *Bacillus thuringiensis* in colonies of Indianmeal moth and almond moth (Lepidoptera:Pyralidae). J. Econ. Entomol. 81: 28-33.

MCGAUGHEY, W. H., AND D. E. JOHNSON. 1987. Toxicity of different serotypes and toxins of *Bacillus thuringiensis* to resistant and susceptible Indianmeal moths. J. Econ. Entomol. 80: 1122-1126.

MCGAUGHEY, W. H., AND D. E. JOHNSON. 1992. Indianmeal moth (Lepidoptera: Pyralidae) resistance to different strains and mixtures of *Bacillus thuringiensis*. J. Econ. Entomol. 85: 1594-1600.

MCGAUGHEY, W. H., AND D. E. JOHNSON. 1994. Influence of crystal protein composition of *Bacillus thuringiensis* strains on cross-resistance in Indianmeal moths (Lepidoptera: Pyralidae). J. Econ. Entomol. 87: 535-540.

MCGAUGHEY, W. H., AND M. E. WHALON. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. Science 258: 1451-1455.

MEADE, T., AND J. D. HARE. 1994. Effects of genetic and environmental host plant variation on the susceptibility of two noctuids to *Bacillus thuringiensis*. Entomol. exp. appl. 70: 165-178.

MEADOWS, M. P. 1993. *Bacillus thuringiensis* in the environment: ecology and risk assessment, pp. 193-220 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley.

MIYASONO, M, M. YAMAMOTO, S. INAGAKI, K. OHBA, T. ISHIGURO, Y. HAYASHI, AND R. TAKEDA. 1994. Effects of several microorganisms on the insecticidal activity of δ-endotoxin of *Bacillus thuringiensis* serovar *kurstaki* HD-1. Jpn. J. Appl. Entomol. Zool. 38: 101-108.

MOAR, W., M. PUSZTAI-CAREU, H. VAN FAASSEN, R. MCCOLLUM, AND S. CLEMENT. 1994. Resistance to *Bacillus thuringiensis* CryIC in *Spodoptera exigua*. p. 65 *in* Abstracts VIth Intern. Coll. Invertebr. Pathol.

MOLDENKE, A. F., R. E. BERRY, J. C. MILLER, J. G. WERNZ, X. H. LI. 1994. Toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to gypsy moth, *Lymantria dispar*, fed with alder or Douglas fir. J. Invertebr. Pathol. 64: 143-145.

MÜLLER-COHN, J., J. CHAUFAUX, C. BUISSON, N. GILOIS, V. SANCHIS, AND D. LERECLUS. 1994. *Spodoptera littoralis* resistance to the *Bacillus thuringiensis* cryIC toxin and cross-resistance to other toxins. p. 66 *in* Abstracts VIth Intern. Coll. Invertebr. Pathol.

NATIONAL RESEARCH COUNCIL. 1986. Pesticide Resistance: strategies and tactics for management. National Academy, Washington, DC. 471 pp.

NAVON, A. 1993. Control of lepidopteran pests with *Bacillus thuringiensis*, pp. 125-146 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley.

OGIWARA, K., L. S. INDRASITH, S. ASANO, AND H. HORI. 1992. Processing of delta-endotoxin from *Bacillus thuringiensis* subsp. *kurstaki* HD-1 and HD-73 by gut juices of various insect larvae. J. Invertebr. Pathol. 60: 121-126.

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.

PARENTI, P, V. MANUELA, G. M. HANOZET, M. TASCA, AND B. GIORDANA. 1995. Interaction of the insecticidal crystal protein CryIA from *Bacillus thurigiensis* with amino acid transport into brush border membranes from *Bombyx mori* larval midgut. J. Invertebr. Pathol. 65: 35-42.

PEFEROEN, M. 1992. Engineering of insect-resistant plants with *Bacillus thuringiensis* crystal protein genes, pp. 135-153 *in* A. M. R. Gatehouse, V. A. Hilder, and D. Boulter [eds], Plant genetic manipulation for crop protection. Wallingford, United Kingdom:CAB International.

RAHARDJA, U., AND M. E. WHALON. 1995. Inheritance of resistance to *Bacillus thuringiensis* subsp. *tenebrionis* δ-endotoxin in the Colorado potato beetle (Coleoptera: Chrysomelidae) J. Econ. Entomol. 88: 21-26.

RAMACHANDRIN R., K. F. RAFFA, M. J. MILLER, D. D. ELLIS, AND B. H. MCCOWN. 1993. Behavioral responses and sublethal effects of spruce budworm (Lepidoptera: Tortricidae) and fall webworm (Lepidoptera: Arctiidae) to *Bacillus thuringiensis* CryIA(s) toxin in diet. Environ. Entomol. 22: 197-211.

ROBISON, D. J., B. H. MCCOWN, AND K. F. RAFFA. 1994. Responses of gypsy moth (Lepidoptera: Lymanitriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) to transgenic poplar, *Populus* spp., containing a *Bacillus thuringiensis* δ-endotoxin gene. Environ. Entomol. 23: 1030-1041.

ROUSCH, R. T. 1989. Designing resistance management programs: how can you choose? Pestic. Sci. 26: 423-441.

SALAMA, H. S., AND M. M. MATTER. 1991. Tolerance level to *Bacillus thuringiensis* Berliner in the cotton leafworm *Spodoptera littoralis* Biosduval (Lepidoptera: Noctuidae). J. Appl. Entomol. 111: 225-230.

SALEH, M. S. 1987. Effect of larval selection with two bioinsecticides on susceptibility levels and reproductive capacity of *Aedes aegypti*. Anz. Schadingskd. Pflanz. Umweltschutz 60: 55-57.

SANCHIS, V., J. CHAUFAUX, AND D. PAURON. 1994. A comparison and analysis of the toxicity and receptor binding properties of *Bacillus thuringiensis* CryIC δ-endotoxin on *Spodoptera littoralis* and *Bombyx mori*. FEBS Letters 353: 259-263.

SANGADALA, S., F. W. WALTERS, L. H. ENGLISH, AND M. J. ADANG. 1994. A mixture of *Manduca sexta* aminopeptidase and phosphatase enhances *Bacillus thuringiensis* insecticidal CryIA(c) toxin binding and $^{86}Rb^{+}$-K^{+} efflux *in vitro.* J. Biol. Chem. 269: 10088-10092.

SCHWARTZ, J. M., B. E. TABASHNIK, AND M. W. JOHNSON. 1991. Behavioral and physiological responses of susceptible and resistant diamondback moth larvae to *Bacillus thuringiensis*. Entomol. Exp. Appl. 61: 179-187.

SHELTON, A. M., J. L. ROBERTSON, J. D. TANG, C. PEREZ, AND S. E. EIGENBRODE. 1993. Resistance of diamondback moth to *Bacillus thuringiensis* subspecies in the field. J. Econ. Entomol. 86: 697-705.

SIMS, S. R., AND T. B. STONE. 1991. Genetic basis of tobacco budworm resistance to an engineered *Pseudomonas fluorescens* expressing the δ-endotoxin of *Bacillus thuringiensis* var. *kurstaki*. J. Invertebr. Pathol. 57: 206-210.

SMITH, R. A., AND G. A. COUCHE. 1991. The phylloplane as a source of *Bacillus thuringiensis* variants. Appl. Environ. Microbiol. 57: 311-315.

STONE, T. B., AND S. R. SIMS. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 989-994.

STONE, T. B., S. R. SIMS, S. C. MACINTOSH, R. L. FUCHS, AND P. G. MARRONE. 1991. Insect resistance to *Bacillus thuringiensis*, pp. 53-66 *in* K. Maramorosch [ed], Biotechnology for biological control of pests and vectors. Boca Raton, Florida:CRC Press.

STONE, T. B., S. R. SIMS, AND P. G. MARRONE. 1989. Selection of tobacco budworm for resistance to a genetically engineered *Pseudomonas fluorescens* containing the delta-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*. J. Invertebr. Pathol. 53: 228-234.

TABASHNIK, B. E. 1986. Evolution of pesticide resistance in predator-prey systems. Bull. Entomol. Soc. America 32: 156-161.

TABASHNIK, B. E. 1989. Managing resistance with multiple pesticide tactics: theory, evidence and recommendations. J. Econ. Entomol. 82: 1263-1269.

TABASHNIK, B. E. 1992. Resistance risk management: realized heritability of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidopteran: Plutellidae), tobacco budworm (Lepidopteran: Noctuidae), and Colorado potato beetle (Coloeptera: Chrysomelidae). J. Econ. Entomol. 85: 1551-1559.

TABASHNIK, B. E. 1994a. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39: 47-79.

TABASHNIK, B. E. 1994b. Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered. Proc. R. Soc. London B 255: 7-12.

TABASHNIK, B. E., AND W. H. MCGAUGHEY. 1994. Resistance risk assessment for single and multiple insecticides: responses of Indianmeal moth (Lepidoptera: Pyralidae) to *Bacillus thuringiensis*. J. Econ. Entomol. 87:834-841.

TABASHNIK, B. E., N. L. CUSHING, N. FINSON, AND M. W. JOHNSON. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83: 1671-1676.

TABASHNIK, B. E., N. FINSON, C. F. CHILCUTT, N. L. CUSHING, AND M. W. JOHNSON. 1993. Increasing efficiency of bioassays: evaluation of resistance to *Bacillus thuringiensis* in the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 86: 635-644.

TABASHNIK, B. E., N. FINSON, F. R. GROETERS, W. J. MOAR, M. W. JOHNSON, K. LUO, AND M. J. ADANG. 1994a. Reversal of resistance of *Bacillus thuringiensis* in *Plutella xylostella*. Proc. Natl. Acad. Sci. USA 91: 4120-4124.

TABASHNIK, B. E., N. FINSON, AND M. W. JOHNSON. 1991. Managing resistance to *Bacillus thuringiensis*: lessons from the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 84: 49-55.

TABASHNIK, B. E., N. FINSON, M. W. JOHNSON, AND D. G. HECKEL. 1994b. Cross-resistance to *Bacillus thuringiensis* toxin CryIF in the diamondback moth (*Plutella xylostella*). Appl. Environ. Microbiol. 60: 4627-4629.

TABASHNIK, B. E., J. M. SCHWARTZ, N. FINSON, AND M. W. JOHNSON. 1992. Inheritance of resistance to *Bacillus thuringiensis* in the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 85: 1046-1055.

TOJO, A., AND K. AIZAWA. 1983. Dissolution and degradation of *Bacillus thuringiensis* delta-endotoxin by gut juice protease of the silkworm, *Bombyx mori*. Environ. Microbiol. 45: 576-580.

VAN FRANKENHUYZEN. 1993. The challenge of *Bacillus thuringiensis*, pp. 1-35 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley.

VAN FRANKENHUYZEN, K., C. W. NYSTROM, AND B. E. TABASHNIK. 1995. Variation in tolerance to *Bacillus thuringiensis* among and within populations of the spruce budworm (Lepidoptera: Tortricidae) in Ontario. J. Econ. Entomol. 88: 97-105.

VAN RIE, J. S. 1991. Insect control with transgenic plants: resistance proof ? Trends Biotechnol. 9: 177-179.

VAN RIE, J., S. JANSENS, H. HÖFTE, D. DEGHEELE, AND H. VAN MELLAERT. 1989. Specificity of *Bacillus thuringiensis* delta-endotoxins. Eur. J. Biochem. 186: 239-247.

VAN RIE, J., S. JANSENS, H. HÖFTE, D. DEGHEELE, AND H. VAN MELLAERT. 1990a. Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Appl. Environ. Microbiol. 56: 1378-1385.

VAN RIE, J., W. H. MCGAUGHEY, D. E. JOHNSON, B. D. BARNETT, AND H. VAN MELLAERT. 1990b. Mechanisms of insect resistance to the microbial insecticide *Bacillus thuringiensis*. Science 247: 72-74.

VISSER, B., D. BOSCH, AND G. HONÉE. 1993. Domain-function studies of *Bacillus thuringiensis* crystal protein, pp. 71-88 *in* P. F. Entwistle, J. S. Cory, M. J. Bailey, and S. Higgs [eds], *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. New York:Wiley.

WALTERS, F. S., C. A. KULESZE, A. T. PHILLIPS, AND L. H. ENGLISH. 1994. A stable oligomer of *Bacillus thuringiensis* delta-endotoxin, CryIIIA. Insect Biochem. Molec. Biol. 24: 963-968.

WALTERS, F. S., S. L. SLATIN, C. A. KULESZE, AND L. H. ENGLISH. 1993. Ion channel activity of n-terminal fragments from cryIA(c) delta-endotoxin. Biochem. Biophys. Res. Comm. 196: 921-926.

WATKINSON, I. 1994. Global view of present and future markets for *Bt* products. Agri. Ecosys. Environ. 49: 3-7.

WHALON, M. E., AND W. H. MCGAUGHEY. 1993. Insect resistance to *Bacillus thuringiensis*, pp. 215-232 *in* L. Kim [ed], Advanced Engineered Pesticides, New York:Marcel Dekker.

WHALON, M. E., D. L. MILLER, R. M. HOLLINGWORTH. E. J. GRAFIUS, AND J. R. MILLER. 1993. Selection of a Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 226-233.

WILSON, G. R., AND T. G. BENOIT. 1990. Activation and germination of *Bacillus thuringiensis* spores in *Manduca sexta* larval gut fluid. J. Invertebr. Pathol. 56: 233-236.

WOLFERSBERGER, M. G. 1990. The toxicity of two *Bacillus thuringiensis* δ-endotoxins to gypsy moth larvae is inversely related to the affinity of binding sites on the midgut brush border membranes for the toxins. Experientia 46: 475-477.

WOLFERSBERGER, M. G. 1991. Inhibition of potassium-gradient-driven phenylalanine uptake in larval *Lymantria dispar* midgut by two *Bacillus thuringiensis* δ-endotoxins correlates with the activity of the toxins as gypsy moth larvicides. J. Exp. Biol. 161: 519-525.

WU, D., J. J. JOHNSON, AND B. A. FEDERICI. 1994. Synergism of mosquitocidal toxicity between CytA and CryIVD proteins using inclusions produced from cloned genes of *Bacillus thuringiensis*. Mol. Microbiol. 13: 965-972.

YAMAMOTO, T., AND G. K. POWELL. 1993. *Bacillus thuringiensis* crystal proteins: recent advances in understanding insecticidal activity, pp. 215-232 *in* L. Kim [ed], Advanced Engineered Pesticides, New York:Marcel Dekker.

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