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Bacillus thuringiensis – Based Biopesticides Against Agricultural Pests in Latin America

Ricardo Antonio Polanczyk, Sergio Antonio De Bortoli and Caroline Placidi De Bortoli
Universidade Estadual Paulista (UNESP)
Brazil

1. Introduction

Twenty years after its discovery, the first attempts to use the entomopathogenic bacterium *Bacillus thuringiensis* (*Bt*) to control pests were made in Europe. Due to the successes achieved in some laboratory trials, commercial production of this pathogen began in France, where commercial formulations entered the market in 1938 as the product Sporeine. In the U.S., the use of this pathogen increased after 1950, mainly for the control of lepidopteran pests, resulting in the production of a formulation called Thuricide (Beegle & Yamamoto, 1992; Peferoen & Lambert, 1992; Lord, 2005).

Bt-based biopesticides have stimulated pathology and microbial control studies, and *Bt* is now one of the main pathogens used in agricultural pest control (Lord, 2005; Brar et al., 2006; Bravo et al., 2011). More than 200 *Bt*-based biopesticides account for 53% of the worldwide market for biopesticides, generating annual revenues of 120–140 million dollars (CAB, 2010). The Americas are responsible for almost 50% of this consumption, particularly the US and Canada, with Latin America accounting for only 8–10% of the total consumption (Guerra et al., 2001).

It is noteworthy that the public's concern of pesticide residues in food and the effect of pesticides on the environment has encouraged the use of microbial products in the production of vegetables and fruits of high commercial value in particular. The production of Dipel (*Bt* kurstaki-based biopesticide) began in 1970. This product proved to be 20–200-fold more potent than other *Bt*-based biopesticides (Beegle & Yamamoto, 1992). Dipel is currently used to control more than 167 lepidopteran pests (Glare & O'Callagham, 2000).

The insecticidal action of *Bt* is mainly due to crystalline inclusions (crystals or "Cry") that contain proteins synthesized during the second phase of the growth cycle, coinciding with the formation of spores (Bravo et al., 2001; Copping & Menn, 2000). These crystals become protoxins when dissolved in alkaline medium in the digestive system of insects. In the presence of digestive enzymes, protoxines are converted into 4 or more toxic polypeptides (δ endotoxins). The hydrolyzed Cry toxins cross the peritrophic membrane and bind to specific receptors present in the apical microvilli membrane of the midgut columnar cells, forming pores that increase membrane permeability, thereby affecting ion gradients and osmotic balance in the apical membrane. The increase in water absorption leads to the lysis and eventual rupture and disintegration of the midgut cells, which represses feeding and results in death due to starvation. The insect may also die due to an environment favorable for spore germination after cell lysis in the midgut. In this new environment, because of the mixing of the hemolymph and midgut contents, the pH becomes acidic, spores multiply, and eventually septicemia and insect death occur (Hofte & Whiteley, 1989; Knowles, 1994; Copping & Menn, 2000).

Although the detection of parasporal inclusions (Cry) in *Bt* cells and speculation about their importance in pathogenicity date back to the 1950s, their formulations were based on the number of spores, with inconsistent results, until the 1970s. However, since then, new formulations of *Bt* have considered the presence of δ-endotoxins. This fact, together with the potential of rapid growth and sporulation in relatively cheap substrates, increased the potential of *Bt* to control insects and enhanced market success.

The purpose of this chapter is to report the use of *Bt* in biological control programs in several countries of Latin America as well as to discuss the ongoing research to emphasize the potential efficacy of these pathogens in pest management. Other issues such as characterization, toxins, isolation, production, formulation effects on natural enemies, plants expressing *Bt* genes, and the evolution of resistance are discussed using previous publications (Brar et al., 2006; Estruch et al., 1997; Glare & O'Callagham, 2000; Charles et al., 2000; Valicenti & Zanasi, 2005).

2. Argentina

Despite the restricted use of *Bt* to control pests in this country, a number of projects have been developed by research institutes (Botto, 1996). *Bt*-based biopesticides were first used in the early 1950s. The target insect was *Colias lesbia pyrrothea* (alfalfa caterpillar/clouded yellow), and the use of biopesticides increased crop yields by approximately 72%. However, efforts aimed at increasing the use of this pathogen were not resumed until 1984 (Sosa-Gómez & Moscardi, 1991).

The Centro de Investigaciones Biologicas, belonging to the Universidad Nacional de Mar del Plata, performs the isolation, selection, morphological and biochemical characterization, and genetic toxicologic investigation of new *Bt* strains. Studies conducted at this center are aimed at developing and characterizing the efficacy of *Bt*-based agents against *Anticarsia gemmatalis*, *Spodoptera frugiperda* (Lepidoptera), *Anthonomus grandis*, *Diabrotica speciosa*, and *Tenebrio molitor* (Coleoptera) (Beron et al., 1999; Beron & Salerno, 2000).

Currently, imported products based on *Bt* are used in Argentina to control *Rachiplusia nu* and *A. gemmatalis* (Bac-Tur, Dipel, and Gale BT-PM). However, their use is not extensive since the market is dominated by chemical pesticides, particularly pyrethroids, and research of these products is still hampered by the severe economic crisis faced in recent years. In view of this, in the short term, it is unlikely that the use of entomopathogens, including *Bt*, will increase. The lack of policies at the national level and the isolated or discontinuous nature of the developed projects are factors that restrict the advancement of biological control in this country (Botto, 1996).

3. Brazil

Early studies in Brazil regarding the use of *Bt*-based products were conducted by Figueiredo et al. (1960) and Pigatti et al. (1960). These authors highlighted the high potential of this pathogen to control many pests such as *Ascia monuste orseis*, *Sylepta silicalis*, *Dirphia sabina*, *Azochis gripusalis*, *Alabama argillacea*, *Mocis repanda*, *Xanthopastis timais*, *Musca domestica*, and *Erinnyis ello*. The first results in relation to the use of *Bt*-based products were considered promising and spurred further research on *Bt*. The first project that aimed to control an important agricultural pest in Brazil, the fall armyworm (*S. frugiperda*), with *Bt* was started in 1993 by Embrapa Milho and Sorgo (CNPMS in Sete Lagoas - MG).

Until the early 1990s, only 3 commercial products were available in the Brazilian market, all based on *Bt kurstaki* (Dipel, Thuricide, and Bactospeine) (Habib & Andrade, 1991). The introduction of *Bt*-based products has encountered problems related to use strategies, marketing, as well as negative opinions advertised by the manufacturers and retailers of chemical insecticides, which diffused the idea that the insects should be rapidly controlled (Alves et al., 1998). Despite these issues, the number of *Bt*-based products on the market has recently increased.

This increase occurred mainly due to changes in the marketing strategies of the companies that sell these products, which emphasized some advantages, such as the maintenance of populations of parasitoids, predators, and pollinators (Alves et al., 1998). However, despite the environmental and social advantages of microbial products, the area treated with *Bt*-based biopesticides is approximately 150,000 hectares (Souza, 2001). This low use is due to a number of factors such as competition with chemicals (higher cost), specificity (reduced action spectrum), and low persistence of most commercially available formulations in the field (inactivation by ultraviolet radiation). New products based on *Bt* should be available in the Brazilian market in the coming years, increasing the availability of biopesticides. The cost of treating 1 hectare ranges from US$7.5 to US$15.0, depending of the crop (Alves et al., 1998).

The incidence of *Ecdytolopha aurantiana* (citrus borer) parasitism has risen sharply in citrus orchards in Brazil since the late 1990s (Gallo et al., 2002). This is mainly due to the incidence of variegated chlorosis, the insect vector, and its chemical control with pyrethroid insecticides through the fog application of non-selective insecticides, which in most cases have high shock power or contact, causing high mortality among natural enemies. The citrus borer limits production in many citrus growing regions of São Paulo State, causing losses of up to 60%, which translates to losses of up to $50 million per year; moreover, citrus borer infestation creates export barriers for some countries. *Bt*-based biopesticides effectively control this pest (Alves et al., 2001b) when the first application is made with more than 6 females caught per trap; the second application occurs 20–30 days later. The bacterium has been used in over 50,000 hectares of citrus orchards in Brazil, mainly in São Paulo State. Among the most important restrictions in the use of this method is the difficulty in synchronizing the short period of larvae exposure (just 4 h on the surface before it penetrates the fruit) and product application and its low persistence in the field due to the action of ultraviolet radiation.

The persistence of the product in the field can be increased by adding vegetable or mineral oil (Gallo et al., 2002) or using formulations containing microencapsulated factors that protect against ultraviolet rays, as reported by Dunkle & Shasha (1989). These authors reported that spores and crystals microencapsulated with Congo red or folic acid exhibited persistent viability of at least 50% of its toxic activity for 12 days. To reduce the impact of this pest on citrus, the Group of Advisers on Citrus recommends, among others measures, the use of products based on *Bt* plus a half dose of insecticide (pyrethroid).

Lepidopteran larvae that cause major defoliation in soybean include *A. gemmatalis* (velvetbean caterpillar), which consumes approximately 90 cm^2 of leaves over the course of its development (Gallo et al., 2002). Despite the high efficiency of *Bt* against this pest (Habib & Amaral, 1985; Bobrovski et al., 2002), its use is restricted mainly due to the widespread use of *Baculovirus anticarsia* (nuclear polyhedrosis virus). This biopesticide has a lower cost, ranging from US$1.3 to US$2.0 per hectare (Alves et al., 1998) and high efficiency, controlling over 80% of the pest population (Moscardi, 1998). Its fast growth can be attributed to the simple strategies used, including the amenability of its multiplication in the field by producers themselves, and the private sector's interest in producing and marketing the product (Moscardi, 1998).

The "Associação Riograndense de Empreendimentos de Assistência Técnica e Extensão Rural" (EMATER-RS), the rural extension agency of the Rio Grande do Sul State, one of the main producers of soybean, led a program aimed at the biological control of *A. gemmatalis* that sprayed approximately 13,000 hectares with Dipel in 2001 and 2002. However, the total area treated with this biopesticide is possibly higher because the data refer only to cities where EMATER-RS works, and do not represent the entire state.

In Brazil, a major pest of pastures is *Mocis latipes* (striped grass looper). This pest occurs cyclically (of some years); when in large numbers, it can significantly reduce the amount of forage available by completely consuming plant leaves. *Bt*-based products are used against small caterpillars in initial outbreaks at concentrations of 0.6–1 kg/ha. An important aspect is that these products are selective, being harmless to humans, natural enemies, and other animals (Gallo et al., 2002), thus indicating its usefulness in pastures and forage crops.

An economic viability study of *Thyrinteina arnobia*, the most important pest of *Eucalyptus*, was performed using *Bt* in approximately 4,000 hectares of *Eucalyptus grandis* and *Eucalyptus urophylla* (White, 1995). The cost of control was US$12.50/ha (equivalent to 1% of the timber produced), and this remained uniform even after pest infestation. A return on investment occurred in the first year after the control was instituted, because the measures adopted led to an income of US$34.20/ha/year. By contrast, a lack of control measures can result in losses of US$460.70–878.91/ha, or 37–70 times the cost of control, depending of the infestation level. Although studies comparing *Bt*-based products with chemicals have not been conducted for this pest, the economic feasibility of using *Bt* to control *T. arnobia* is obvious.

The pickleworm moths *Diaphania nitidalis* and *Diaphania hyalinata* are pests of cultivated cucurbits, and their larvae feed on all plant parts, preferring the fruit . The new leaves and branches become dry after being attacked. In fruits, the larvae open galleries and destroy the pulp, causing decay. *D. nitidalis* attacks fruits of any age, whereas *D. hyalinata* attacks the leaves and fruit skin. When using Dipel to control *D. nitidalis*, it is essential to determine the age of larvae because Dipel must be applied to early-stage larvae, i.e., before the larvae enter the fruit, as it is ineffective once larvae enter the fruit.

Other pests controlled with *Bt*-based produced are the imperial moth (*Eacles imperialis magnifica*) and cassava hornworm (*E. ello*), which can completely defoliate coffee and cassava plants, respectively. For the tomato pinkworm (*Tuta absoluta*), applications should be made weekly, in the evening or at night, simultaneously with the use of the egg parasitoid *Trichogramma pretiosum*. This pest can attack all parts of the tomato plant at any stage of development, consuming the leaves, branches, and mainly the apical buds and destroying new shoots, resulting in depreciation of the commercial value of the fruit (Gallo et al., 2002).

The fall armyworm in Brazil can reduce corn yield by 20%, and under dry conditions, it cuts off the plants close the ground, causing serious crop damage (Gallo et al., 2002). Although Thuricide is cited as a product registered for *S. frugiperda* control (Compêndio de Defensivos Agrícolas, 2010), its use is restricted due to low efficacy in most regions. Preliminary results from laboratory experiments conducted in the Laboratory of Pathology and Microbial Control of Insects, Piracicaba, SP, Brazil (ESALQ-USP) indicate that populations of fall armyworm from different geographic regions have different levels of susceptibility to the same strain of *Bt* (Polanczyk et al., 2005). These initial results confirm the need for comprehensive monitoring to verify variation among populations, which would increase the effectiveness of this strategy.

Bt-based biopesticides can be used in combination with other substances such as molasses and corn bran (Gravena et al., 1980a) to reduce *Diatraea saccharalis* (sugarcane borer) infestation in sugarcane crops by more than 50%. Parasitoids such as *Trichogramma pretiosum* enhance the effectiveness of *Bt kurstaki* in the control of *T. absoluta* (Marques, 1993). Similarly, pesticides, including insecticides (Assunção et al., 1980; Gravena et al., 1980b; Gravena et al., 1983; Zanuncio et al., 1992), have the same effect on biopesticides in controlling certain pests.

Published research by EMBRAPA indicates the importance of this *Bt* in agricultural pest control (Batista et al., 2005; Praça et al., 2004). In addition to EMBRAPA reports, several other federal and state institutions have performed research related to *Bt*. Among them, the Laboratory of Microbiology and Genetic of the UNISINOS University investigated the selection of *Bt* strains against coleopteran and lepidopteran pests (Fiuza et al., 2002, Pinto & Fiuza, 2002; Schünemann et al., 2005). ESALQ-USP studied the production, formulation, and selection of *Bt* strains for insect pests such as *T. absoluta*, *S. frugiperda*, and *Sitophilus oryzae* (Marques & Alves, 1996; Alves et al., 1997; Alves et al., 1999; Alves et al., 2001; Giustolin et al., 2001; Polanczyk et al., 2005). The sublethal effects of this pathogen on target insects and its interaction with other biological control agents have been studied (Polanczyk & Ahmed, 2005a, b). In the Laboratory of Entomology and Plant Pathology, projects are underway to select *Bt* strains with efficacy against lepidopteran pests and investigate their effects on natural enemies (Grecco et al., 2006; Pratissoli et al., 2006).

However, according to IBGE (2006), the 19 major crops of economic importance (cotton, peanuts, rice, oats, potatoes, cocoa, coffee, sugarcane, onions, barley, beans, oranges, castor oil plant, cassava, corn, soybeans, sorghum, wheat, and triticale) occupy an area of 146.6 million hectares. However, only 150,000 hectares are treated with *Bt*-based biopesticides for pest control, although their potential use may be extended up to 6 million hectares.

4. Colombia

Despite the prevalence of insect parasitoids in biological control programs in Colombia, some *Bt*-based products, albeit on a small scale, are used in pest control (Botto, 1996). However, a few decades ago, Revelo (1965) emphasized the potential of this pathogen in the control of *S. frugiperda*, *Agrotis ipsilon*, and *D. saccharalis*, the 3 major pests of maize in Columbia. In 1990, US$5.8 million was spent to control *S. frugiperda* in cotton crops, and US$4.2 million was spent to control the pest in corn and sorghum. In area, these amounts represent 430,000 and 440,000 treated hectares, respectively (Bosa & Cotes, 1997).

Important pests of many vegetable crops such as *Leptophobia aripa*, *Ascia monuste*, and *Plutella xylostella* have been well controlled by *Bt*-based biopesticides; thus, the damage caused by these insects is economically insignificant (Ruiz, 1998). However, Ruiz also states that in most cases, control measures involving the use of biological control must be accompanied by other tactics to maintain the pest population level below the economic injury level.

In 1989, the Laverlam Company began research of biopesticides to protect the environment and integrate tactics of integrated pest management in Colombia. These efforts resulted in the production of Turilav (*Bt kurstaki*), which is used to control *Heliothis* spp., *A. argillacea*, *Agrotis* spp., and *Spodoptera* spp., particularly in cotton crops.

In Valle del Cauca, the use of *Bt* to control *T. absoluta* is part of an integrated pest management program that has successfully reduced the costs of control by more than 54% to approximately US$650 per hectare (Belotti et al., 1990; Garcia, 1992). In the same region, *Bt*-based biopesticides are used against *Caligo ilioneus*, an important sugarcane pest that reduces the weight of the plant by 26–56% and its sugar content by 8–18%. In cotton crops, *Bt* is applied to control occasional outbreaks of *A. argillaceae* when defoliation exceeds 30%.

Sánchez et al. (1999) verified the high efficacy of *Bt kurstaki* and *Bt aizawai* to control *D. hyalinata* and *D. nitidalis*. These pests are the most destructive parasites of melon crops, in which they defoliate the crop and feed on the branches and fruits, causing annual losses of up to 25% of the yield. The use of *Bt* control has reduced the amount of insecticides used, which were sprayed up to 24 times in just 1 season in some cases.

Bosa & Cotes (1997) reported the high efficacy of 2 *Bt* strains (*Bt*-127 and HD-137) against *S. frugiperda*. Arango et al. (2001) used 1,100 native *Bt* isolates to study their selectivity to control the fall armyworm. Among the tested material, 32 isolates exhibited activity against this pest. The most powerful strains expressed *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*, *cry1C*, and *cry1D*. Serotyping revealed the isolates to be *Bt kurstaki*, *Bt thuringiensis*, *Bt canadensis*, and *Bt indiana*.

Maduell et al. (2002) isolated *Bt* from 13 species of the *Piper* genus in Colombian forests, detecting *Bt* in 74% of the samples. Regarding the presence of *cry* genes, *cry1* was amplified from 70% of the material (usually toxic to lepidopterans), and 60% exhibited some toxicity (12–100% mortality) to *S. frugiperda* larvae. In similar research, Yaro et al. (1999) isolated and characterized this bacterium in different parts of the country.

Recent studies by several research institutes revealed that 3 native isolates (IBUN6.4, IBUN3.3, and IBUN2.6) exhibit promising efficacy against *Heliothis virescens*, and 2 others (IBUN4.0 and IBUN3.8) were selected for *S. frugiperda*. For *H. virescens*, Cry 1F is the most effective toxin, and Cry1B and Cry1E are effective against *S. frugiperda*.

δ-Endotoxin expression is directly associated with sigma factors responsible for different sporulation stages. Studies with mutagenic agents found a direct relationship between the mutation of σ^E and σ^K factors with changes in *cry1Aa*, *cry1Ba*, *cry2Aa*, and *cyt1Aa* expression and changes in the specificity of insecticidal toxins. Research undertaken by the IWC sought to develop asporogenic *Bt* strains via chemical agent-induced mutagenesis. A strain was obtained that is currently being characterized in bioassays against *S. frugiperda* to determine the effect of the mutations on the integrity of genes, proteins, and toxin efficiency. The great interest in asporogenic *Bt* strains is justified even though the generation of these strains is difficult. However, because they are mutants produced in the laboratory, these strains should be utilized only after careful assessment of their environmental impact.

Corporación Nacional para Investigaciones Biologicas, Corporación Colombiana de Investigación Agropecuária, and Microbiological Research Center da Universidad de los Andes e Biotechnology Institute da Universidad Nacional de Colombia have conducted *Bt* studies with the following objectives: pathogen isolation, δ-endotoxin toxic activity determination against lepidopterans, toxic protein characterization, and DNA segment amplification of *cry* genes.

5. Cuba

Since 1990, due to the drastic reduction in the assistance provided by the former Soviet Union, Cuba has instituted necessary reforms to meet the basic needs of its citizens. The country made several transformations in many areas, including agriculture, leading to the development of more sustainable technologies in integrated pest management programs (Rosset & Moore, 1999). In the 1960s, the importation of some products based on *Bt* to control *H. virescens* and *M. latipes* stimulated the search for native pathogen strains (Pérez & Vasquéz, 2001). The use of chemical insecticides, which were mainly provided by the former Soviet Union, prevailed in Cuban agriculture, with methyl parathion being widely used in pest control before 1990.

The Ministry of Agriculture of Cuba has accelerated and significantly expanded plans to increase the production biological control agents to replace imported chemical insecticides. Since 1990, there has been an 89% reduction in the import of chemical insecticides and fertilizers. At the end of 1991, approximately 56% of insecticides used in Cuban agriculture were organic, representing a savings of US$15.6 million per year. In 1994, with 222 labs, the Centros de Producción de Entomófagos y Entomopatógenos (CREE) began operations, producing insects, nematodes, and pathogens in 15 provinces of Cuba. Currently, CREE operates 280 labs (53 in cultivated sugarcane areas and 227 in fruit areas and other crops), and *Bt* is primarily used (Pérez & Vasquéz, 2001).

Due to the prevalence of these bacteria-based products in Cuba, researchers seek to discover new native strains with high potential for pest control. Several institutions such as Instituto de Investigaciones de Sanidad Vegetal, Instituto de Investigaciones Fundamentales de la Agricultura Tropical, Centro National de Sanidad Agropecuária, Instituto de Investigaciones de Cítricos y Frutales, Instituto Cubano de Investigaciones Derivados de la Caña de Azúcar, and Instituto de Ecología y Systemática have contributed to the development of biopesticides in several areas.

The first biopesticides produced in Cuba were based on *Bt*, and they have been used to control *H. virescens*, *M. latipes*, *P. xylostella*, *S. frugiperda*, *E. ello*, and *D. hyalinata* (Table 3).

Among the registered products, those belonging to the line Thurisav are used to control *M. latipes*, *P. xylostella*, *Heliothis* spp., *Trichoplusia ni*, *Spodoptera* spp., and mites.

M. latipes is one of the most important agricultural pests in Cuba, causing losses of approximately 86,000 tons per year (equivalent to 7,800 hectares). Pérez et al. (1991) tested several products based on different subspecies of *Bt* (*Bt thuringiensis*, *Bt kurstaki*, and *Bt dendrolimus*) against this pest in pastures, and *Bt dendrolimus* was the most effective, with mortality rates of 86–90%.

According to Blanco (2006), *Brassica oleracea capitata* is one of the most important vegetable crops in Cuban agriculture, and among the factors limiting its production is *P. xylostella* (diamondback moth), which reduces its yields by 75–95%. To avoid these losses, many governmental and nongovernmental organizations have worked together to develop control strategies focusing the context of integrated pest management (IPM). The tactics employed should include *Bt*-based biopesticides to manage this pest and others such as *Ascia monuste eubotea* and *T. ni* to significantly reduce the control costs. Due to the success of these products in Cuba, the area treated with *Bt* to control *P. xylostella* increased from 17,400 hectares in 1988–89 to 53,000 hectares in 1993–94. Initial entomopathogen studies to control pest mites (*Phyllocoptruta oleivora*, *Tetranychus tumidus*, *Polyphagotarsonemus latus*) began in 1980. For products based on *Bt*, the mortality varies from 70% to 100% depending on the species, preventing the unnecessary use of chemical insecticides (Pérez, 1996). Between 1994 and 1996, almost 1,000 hectares were sprayed with *Bt* (LBT-13) to control *P. latus* (broad mite) in potato crops, with an efficacy exceeding 85% at a dose of 3–5 L/ha. The same strain caused between 83% and 90% mortality in adults and 100% in nymphs of *P. oleivora* (citrus rust mite) in citrus crops 72 h after application (20 L of fermented solution/ha). Moreover, it is selective to natural enemies such as predatory *Cycloneda sanguinea* in all its stages of development (Rojas, 2006).

The LBT-24 strain is one of the most effective biological agents for *S. frugiperda* control, and the mortality observed in the field ranged from 70 to 90% (Montesbravo, 2006), resulting in an increase of 15% in crop yield. The use of *Bt* formulations to control *S. frugiperda* in Cuba is very important because this pest can reduce corn yield by up to 40%, and important results have been obtained using this tactic together with others, such as the nuclear polyhedrosis virus and parasitoids (*Telenomus* sp. and *Chelonus insularis*).

The efficacy of LBT and LBT-3-21 *Bt kurstaki* isolates has exceeded 84% in potato, tomato, and tobacco crops. In Cuba, more than 20 species of insects are potato pests, and some of them such as *P. latus*, *Spodoptera* spp. and *T. ni* are controlled by *Bt*. In potato and tomato, the use of *Bt* favors the preservation of the natural enemies of *Liriomyza trifolii*. The utilization of this pathogen allows the action of *Opius* spp. and *Heteroschema* sp., which induce greater than 70% mortality (Morales, 2006).

The lepidopteran *H. virescens* is the most important pest of cotton in Cuba, and the efficacy of *Bt*-based products is similar to that of chemicals (14% for chemicals vs. 15.13% for biopesticides) (Jiménez, 1996).

Forestry is an important component of the Cuban economy, and 18 species of harmful insects greatly limit production. The most important pest of pine forests is *Rhyacionia* *frustrana*, the larvae of which invade the sprouts and buds of pine. *Bt*-based insecticides are widely used to control this pest with satisfactory results (Vásquez et al., 1999).

6. México

The use of entomopathogenic agents to control pests in Mexico began in the 1950s. In 1968–69, tests demonstrated the effectiveness of products formulated with *Bt* (Thuricide 90T and Thuricide 90TS) in controlling *P. xylostella* (Carrillo, 1971). However, the use of these insecticides increased sharply after 1990 due to the great interest of the public and private institutions in the use of pathogens in pest control.

In 1999, the use of *Bt*-based insecticides in Mexico increased from 15% to 20% and became the most commonly used and accepted biopesticide in that country. It was employed in 100,000 hectares of corn, 174,000 acres of cotton, and 200,000 hectares of vegetables and other crops. In the Bajio region of Guanajuato, 100 tons of *Bt*-based biopesticides were applied in 2001. In the Aguascalientes State, products made from *Bt kurstaki* and *Bt aizawai* are used against pests of vegetables, spinach, and potatoes, treating approximately 1,000 hectares (Vallés, 1998). In the entire country, it was estimated that 4–10% of the insecticides used contain *Bt* bacterium as the active ingredient. The leading *Bt*-producing companies are multinational, and the cost of control is US$19/ha, which is competitive with the chemicals in the market (Guerra et al., 2001).

Among the pests reported in Table 5, some of them are very important in several crops. For example, *S. frugiperda* can cause losses of up to 58% in plants and reduce corn yield by 1,148 kg/ha at a plant density of 45,000/ha (Castro-Franco et al., 1995). This species, together with *Helicoverpa zea*, *Diatraea grandiosella*, and *D. saccharalis*, may be responsible for losses up to 30% in Mexican corn crops.

In addition to the aforementioned pests, Rodríguez et al. (1991) reported the effectiveness of *Bt* (DL_{100} 75 mg/kg diet) against *Galleria mellonella*, an apiculture pest that causes losses of honey, pollen, and wax production of up to 10%. In addition, Rodriguez & Trumble (1993) reported that this pathogen was useful in controlling tomato pests in the IPM context without harmful effect on natural enemies such as *Trichogramma* spp.

Edwards et al. (1999) obtained different CL50 estimates of the same isolate for several S. frugiperda populations collected in 5 regions of Mexico. The authors emphasized that variation may be due to geographical isolation, which results in reproductive isolation and physiologically different populations showing differential susceptibility to Bt.

The Centro de Investigación y de Estudios Avanzados, the International Maize and Wheat Improvement Center, and the Institute of Biotechnology, National Autonomous University of Mexico (UNAM) work together to locate new *Bt* toxins to control corn pests. Other research obtained promising results against *D. grandiosella*, *D. saccharalis*, *S. frugiperda*, *H. zea*, and *T. ni* (Bohorova et al. 1996; Bohorova et al. 1997; Del Rincón-Castro et al., 2006; Magdalena et al., 2001).

Researchers at the University of Guanajuato and Centro de Investigación de Estudios Avanzados del IPN investigated the characterization and selection of *Bt* against *Manduca sexta* and *T. ni* (Rosas et al., 1994; Corona et al., 1998; Rosales-Reyes et al., 2003).

At the Institute of Biotechnology UNAM, research has being conducted on the interaction between receptors and Cry1Ab toxins (lepidoptera-specific) to identify the regions responsible for toxicity. At the same institute, research for new insecticidal proteins that can potentially control insect pests has been performed using *Bt* (Bravo et al., 1998, Guerra et al., 2007). This project constructed a database composed of 500 *Bt* strains isolated from Mexican soil. Some of these were effective in controlling pests such as *Epilachna varivestis*, *Tapinoma melanocephalum*, *Phyllophaga* spp., *Anomala* spp., and *Bemisia tabaci*.

Another approach taken by researchers is to study the interaction of the toxins Cry1C and Cry1D with the intestinal membranes of insects such as *S. frugiperda*, *Rhopalosiphum maidis*, and *D. grandiosella*. More recently, work has been conducted to analyze pore formation in the insect midgut apical membrane and the subsequent effects on *Manduca sexta* gut cells.

7. Concluding remarks

The entomopathogenic bacterium *B. thuringiensis* is used against a wide range of pests in several crops in Latin America. Its widespread use against *P. xylostella* is noteworthy, even in countries not covered in this paper due to a lack of accurate information, as well as the use of *Bt*-based products produced in Cuba by small labs funded by the federal government.

Regional production is restricted to Cuba, and more recently, to Mexico, whereas imported products are used in other countries, which directly increases the cost of pest control and indirectly increases the final cost of production. The ability to formulate these products on a commercial scale should be considered by the governments of Latin American countries and/or private industries. The decrease in cost to the farmer would boost the use of these microbial products and increase the producers' profit, as the "products of biological origin," or those grown without pesticides, have better acceptance and price on the market in several countries.

The use of *Bt*-based products in Latin American countries depends more on political and economic aspects than technological approaches. Considering the basic economic differences in Brazil and Cuba, it would be logical to assume that the use of microbial products in Brazil, including those formulated with *Bt*, would be much greater than that in Cuba. However, the Cubans, under U.S. blockade, produced technology and developed strategies to use entomopathogens that exemplify how it is possible to make microbial control one of the pillars of sustainable agriculture. Brazil created a program that represents the most successful microbial control in the world—the control of *A. gemmatalis* with nuclear polyhedrosis virus. Brazil also undertook programs using bioinsecticides based on *Bt* frequent to control dipteran vectors. However, in Brazil, the availability of large numbers of pesticide formulations, supported by an intensive propaganda financed by multinational companies, together with the lack of a structure to implement advancements of alternative methods of control in production, has limited the use of entomopathogens. The solution to this impasse involves greater investment in technical assistance and research, principally in the production and formulation of general entomopathogens, aiming to convince the farmer that the biological method may in many cases be an important tactic of control that can minimize or even replace some pesticides to control certain pests.

Apparently, the use of plants expressing *Bt* genes has a negative impact on the use of products formulated with this bacterium, but this is negligible and unlikely to affect the national economy significantly. These crops, in addition to performing a vital role in economic development, have great potential to increase the use of bioinsecticides to control pests.

Other aspects such as knowledge of the biology and/or behavior of the target insects are very important, as they are directly related to their field efficiency. This is true for *E. aurantiana* and *T. absoluta*, 2 very important pests of citrus and tomato crops, respectively. Larvae of *E. aurantiana*, after emerging, spend little time on the fruit surface, and the larvae of *T. absoluta* remain inside the leaf for the most part of the larval stage. In both cases, the immature insects are exposed to *Bt* quickly. Therefore, although the efficacy in relation to some pests depends on the formulation, pathogenicity, and virulence of the pathogen, other aspects should be considered and studied to better enable the control of pests.

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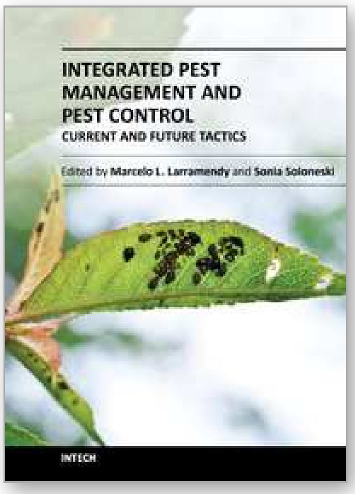

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821