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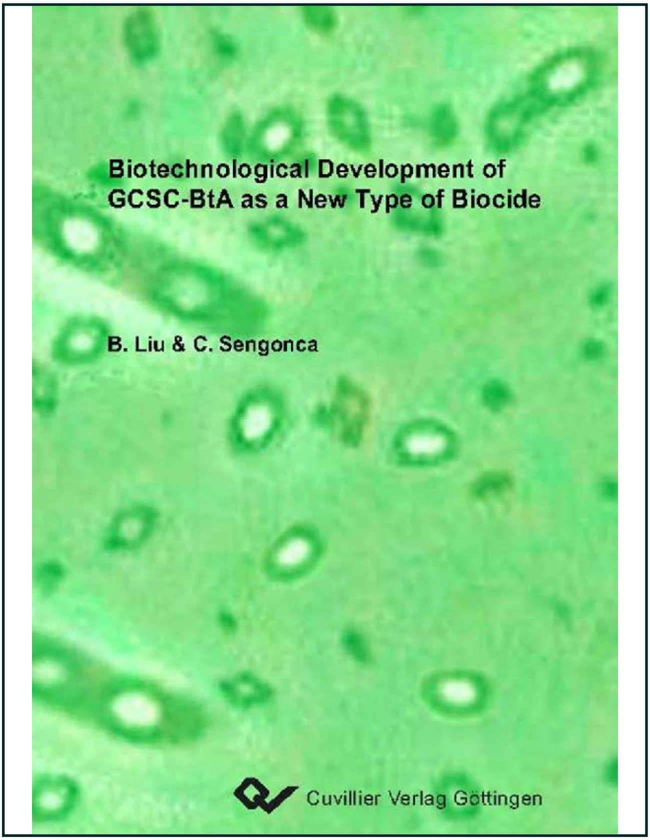
# Biotechnological Development of GCSC-BtA as a New Type of biocide



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# CHAPTER 1

# Importance of Biocides in Integrated Pest Management System

## 1.1 IPM system for crop protection

IPM is the development of a set of practices that maintain pest populations (arthropod, pathogens, weeds) at levels below those which cause economically significant losses (Biggs et al., 2000); it emphasizes minimal intervention - particularly with synthetic biocides - and husbandry of natural regulating mechanisms be they biological or cultural, manipulation of predators, parasitoids and pathogens, exploitation of host resistance, the use of behaviour modifying chemicals and a search for crop configurations/livestock systems less susceptible to pest attack (Ciglar & Baric, 1994).

In the case of crops, IPM involves all aspects of the crop cycle from site selection and land preparation to harvest. Similar concerns apply to livestock systems (Delgado et al., 1999). It is generally accepted that IPM is an approach that takes account of interactions between all biological agents that cause loss as well as an appropriate mix of control technologies (Biever et al., 1994). IPM demands an understanding of systems and the parameters that govern their dynamics far beyond that required to kill pests. Recognizing the importance of policy-makers, researchers, extension workers and farmers within any system, it must successfully involve each group in the development and implementation of integrated technologies for crop and animal protection.

If the pace of IPM implementation is to be increased, more resources must be committed over a longer time-frame and there must be much improved coordination accompanied by functional specialization amongst both donors and research institutions (Dlott et al., 1993). IPM is firmly rooted in biology and ecology. It seeks to harness indigenous regulatory mechanisms for pest population control and exploit physiological and behavioural characteristics of pest species in their management (Marrone & Persley, 1996). It also emphasizes the adaptation of indigenous 'well-adapted' or 'robust' cropping/livestock systems in the development of more productive agriculture. Such an approach demands a detailed understanding of characteristics of agro-ecosystems and their dynamics in response to intervention (Menn, 1996).

## 1.2 Biocide in IPM system

Increasing concern is now being expressed about our ability to characterize pest organisms (Prischepa et al., 2002). As methods of control become more specific and sophisticated, it becomes more important to distinguish variability in order to ensure that the appropriate method is applied (Waage, 1998). The rationale is clear for management of host resistance and pesticide resistance. The adaptation of research outputs from molecular biology to provide accurate, simple field diagnostics for plant diseases is a high priority (Tommasini et al., 2001).

Biological control is an important area of IPM (Veire & Van-de-Veire, 1991). The term is used here to cover the use of all biological or biologically derived agents (Waage, 1998). Classical biocontrol based on the release of exotic or supplementation of indigenous natural enemies has had striking successes but is heavily constrained as an IPM component by problems of rearing and supply, particularly for parasitoids and predators of insect pests (Sastrosiswojo, 1996). Bacteria, viruses and their toxins have presented similar difficulties compounded by problems of formulation, application, persistence and potential side effects. One exception is those products based on the soil bacterium *Bacillus thuringiensis*, e.g. Dipel® (Abdel Megeed et al., 1997).

The use of behaviour-modifying chemicals has found wide application, particularly with insect pheromones, as a tool of great importance in a number of pest management systems (Lundholm & Stackerud, 1980). Farmers need to be able to make reactive decisions in order to respond to and solve a particular problem. The good practices of one farmer can be undone by a neighbour employing more traditional pest control practices (e.g. preventative application of pesticides) (Agnihothrudu et al., 1997).

IPM is inherently an inter-disciplinary, multi-functional approach to solving pest problems. Current institutional structures in both developed and developing countries do little to simplify the task of the farmer practioner (Chin et al., 1992). Components of the problem, in both disciplinary and operational terms, are commonly abstracted to form the principal axis for the organization of public sector institutions (Dlott et al., 1993). The management of research, extension and technical support services are frequently operated independently of one another, centered in different institutions and often with conflicting goals and interests. These activities are almost always under-resourced and unable to compete with the commercial sector (Talekar, 1988).

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# CHAPTER 2
# Study on *Bacillus thuringiensis*

## 2.1 Achievements and Approach to *Bacillus thuringiensis*

### 2.1.1 About *Bacillus thuringiensis*

B.t. has been known as an insect pathogen for almost a century (Tomlin, 1994). The major insecticidal principle is the protein delta- endotoxin. During sporulation B.t. forms crystalline parasporal inclusion bodies which contain delta-endotoxin either free or as part of a larger protein. After uptake by feeding, the parasporal bodies dissolve in the insect gut and delta-endotoxin is liberated. It then docks onto epithelial cells and causes them to swell and burst which leads to the death of the insect. Today several different strain types are known with activity either against lepidoptera and diptera, against diptera alone, or against coleoptera. The size of the active delta-endotoxin is in the range of 60-90 kDa, depending on the bacterial strain and its spectrum of activity (Adams et al., 1996; Carroll et al., 1989). Due to its highly insect specific mode of action delta-endotoxin is not toxic to other living organisms. Its first use as an insecticide was reported in 1938 (Jacobs, 1989), and commercialization started in 1957 (Cannon, 1993).

Today insecticidal products based on B.t. delta-endotoxin are being produced and marketed by Abbott, Caffaro, Ecogen, Mycogen and Thermo Trilogy. In 1996 the market volume of B.t products worldwide was 160 million US$ (Fernandez Larrea Vega, 2002). Recently the gene for B.t. delta-endotoxin has been used to create transgenic crop plants which express delta-endotoxin and thus become insect resistant Such insect resistant maize was introduced to the market by Novartis (Koziel et al., 1993), and Monsanto is commercializing insect resistant transgenic cotton (Gasser & Fraley, 1992).

The classification B.t. toxin is shown in Table 2-1. B.t. strains produce two types of toxin. The main types are the Cry (crystal) toxins, encoded by different cry genes, and this is how different types of B.t. are classified. The second types are the Cyt (cytolytic) toxins, which can augment the Cry toxins, enhancing the effectiveness of insect control. Over 50 of the genes that encode the Cry toxins have now been sequenced and enable the toxins to be assigned to more than 15 groups on the basis of sequence similarities. The table below shows the state of such a classification in 1995, but an alternative classification has recently been proposed.

**Table 2-1.** ***Bacillus thuringiensis*** **toxins and their classification**

| Gene | Crystal shape | Protein size(kDa) | Insect activity |
|---|---|---|---|
| cry I [several subgroups: A(a), A(b), A(c), B, C, D, E, F, G] | bipyramidal | 130-138 | lepidoptera larvae |
| cry II [subgroups A, B, C] | cuboidal | 69-71 | lepidoptera and diptera |
| cry III [subgroups A, B, C] | flat/irregular | 73-74 | coleoptera |
| cry IV [subgroups A, B, C, D] | bipyramidal | 73-134 | diptera |
| cry V-IX | various | 35-129 | various |

## 2.1.2 Importance of *Bacillus thuringiensis*

B.t. is a species of bacteria that has insecticidal properties affecting a selective range of insect orders. There are at least 53 subspecies of B.t., also called serotypes or varieties (Barjac et al., 1990), and probably over 800 strain isolates (Ramachandran et al., 1993). B.t. was first isolated in 1901 in Japan from the diseased silk-worm larvae (Krieg, 1986). It was later isolated from Mediterranean flour moths and named B.t. in 1911 (Lambert & Peferoen, 1992). It was not until 1958 that B.t. was used commercially in the United States (Jenkins & McCarty, 1994). By 1989, B.t. products had captured 90-95 percent of the biopesticide market (Feitelson et al., 1992).

B.t. products available in the United States with extent to the whole world are comprised of one of five varieties of B.t.: *B.t.* var. *kurstaki* and var. *morrisoni*, which cause disease in moth and butterfly caterpillars. *B.t.* var. *israelensis* which causes disease in mosquito and blackfly larvae. *B.t.* var. *aizawai* which causes disease in wax moth caterpillars, and *B.t.* var. *tenebrionis*, also called var. *sandiego*, which causes disease in beetle larvae (Entwistle et al., 1993). Other strains of B.t. have been discovered that exhibit pesticidal activity against nematodes, mites, flatworms, and protozoa (Feitelson et al., 1992).

B.t. is a live microorganism that kills certain insects and is used to kill unwanted insects in forests, agriculture, and urban areas. In a purified form, some of the proteins produced by B.t. are acutely toxic to mammals. However, in their natural form, acute toxicity of commonly-used B.t. varieties is limited to caterpillars, mosquito larvae, and beetle larvae (Lee & Scott, 1989). B.t. is less toxic to mammals and shows fewer environmental effects than many synthetic insecticides. However, this is no reason to use it indiscriminately (Moraes et al., 2000). Its environmental and health effects as well as those of all other alternatives must be thoroughly considered before use. B.t. should be used only when necessary, and in the smallest quantities possible. It should always be used as part of a sustainable management program (Saito et al., 2000). As hazards of conventional, broad acting pesticides are documented, researchers look for pesticides that are toxic only to the target pest, have less impact on other species, and have fewer environmental hazards. B.t. biocide, however, there is evidence suggesting that B.t. is not as benign as the manufacturers would like us to believe, and that care is warranted in its use (Milam et al., 2000).

### 2.1.3 Application of *Bacillus thuringiensis*

B.t. products are used to control moth pests in fruits, vegetables, and beehives; blackfly and mosquito pests in ponds and lakes; and several beetle pests in vegetables and shade trees. Common brand names include Dipel (Zaschita, 1981), Foray (Cadogan & Scharbach, 1993), Thuricide (all *B.t. kurstaki*) (Niemczyk & Bakowski, 1970), Vectobac (Ramoska et al., 1982), Mosquito Attack (all *B.t. israelensis*) (Snarski, 1990), and *B.t. tenebrionis*.

B.t. products have been used to control the agricultural pests in many crops i.e. artichokes, apples, nectarines, plums, broccoli, cantaloupe, peppers, almonds, alfalfa, oranges, cotton, cherries, melons, strawberries, public health, tomatoes, lettuce, grapes, and others, with a different proportion in USA, similar using range to that in China. In California, where pesticide use reporting is more comprehensive than in other states, almost 52,000 pounds of B.t. were used on diverse crops in 1991. Grapes, lettuce, and tomatoes account for almost half the B.t. used in California. B.t. is also widely used in cotton production in Texas, Mississippi, and Louisiana as well as in the production of fruits and vegetables in California, Arizona, and Florida. B.t. is extensively used national-wide in the production of certain fruit and vegetable crops. The percent of crops treated with B.t. has raised up to a range of 30%-60% in USA. The most frequent use was in spinach.