WHAT IS BACILLUS THURINGIENSIS[1]

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Abstract. *Bacillus thuringiensis* (Bt) is a bacterium that belongs to the genus *Bacillus*. It is pathogenic to insect larvae, mainly *Lepidoptera* species. Several insecticidal toxins can be produced by the different strains of *Bt*. The most important one is the crystal (delta-endotoxin) which, alone or in conjunction with the spore, will kill the insects. Parasites and predators of *Lepidoptera* and beneficial insects are generally unaffected by *Bt*. In the U.S. commercial products of *Bt* are produced with the HD-1 strain and contain only the spore and crystal as their entomopathogenic ingredients. To be effective these must be ingested. Therefore timing of the application and thorough coverage of the treated foliage are important. *Bt* can be applied by conventional means. Highly alkaline water should not be used for mixing and excessive heat during storage should be avoided. A brief listing of *Bt* formulations for gypsy moth control is included.

The literature contains numerous examples of microorganisms pathogenic to a variety of insect species. Known entomopathogens include some species of fungi, protozoa, microsporidia, nematodes, viruses, and bacteria. As early as 1879 a fungus, *Metarrhizium anisoplia* (Burges and Hussey 1970) was applied for field control of an agricultural insect pest (*Anisoplia austriaca*). Since the early 1900's the *Bacillus thuringiensis* group of bacteria has received the most attention and for a number of years commercial formulations of *Bt* have been available for use in both agricultural and forest insect pest control. As the name indicates, and in common with other members of the genus *Bacillus*, it is a spore-forming, rod-shaped bacterium, motile by peritrichous flagella. At the time of sporulation, *Bt* forms a protein parasporal body termed the delta-endotoxin, commonly referred to as the crystal. When sporulation is complete, both the spore and crystal are lysed from the sporangium and released into the surrounding growth medium (Figure 1).

In 1905, Ishiwata first isolated this type of microorganism from dying silkworn larvae and named it *Bacillus sotto*. Approximately 10 years later, it was found that only old sporulated cultures of the bacteria could induce disease in the silkworm. In 1911, a German entomologist named Berliner isolated a similar spore-forming bacterium from diseased Mediterranean flour moths (*Anagasta (Ephestia) kuhniella*) in Thuringia, hence the name *Bacillus thuringiensis*. This original culture was lost, but in 1927 Mattes reisolated this microorganism and it soon became the source of extensive research and early commercial development of *Bt*. In the mid 1950's it was determined that the *Bacillus sotto* of Ishiwata and *Bacillus thuringiensis* of Berliner were varieties of the same species (Fast 1974). Now *Bacillus thuringiensis* Berliner is regarded as the type species of these bacilli collectively called the "crystalliferous bacteria".

The potential for development of *Bt* as a biological insecticide was demonstrated shortly after its reisolation. Indeed, in 1929 Metalnikov and Chorine (1929) reported on the control of gypsy moth with Bt. These early reports were verified in 1960 by Cantwell and his colleagues (1961). Even before 1938 the first commercial product of *Bt*, *Sporeine*, was already available. After the second world war several U.S. firms produced *Bt* commercially. Until the early 1960's formulations of *Bt* were prepared from the Berliner strain. In the mid 1960's several other strains were investigated. The HD-1 strain became available in the late 1960's and is now used for commercial production of *Bt* in the United States.

Toxins

Commercial formulations of *Bt* contain the spore and crystal (or delta-endotoxin) as their entomopathogenic ingredients. However, other agents produced by some strains are toxic to insects. Briefly the major toxins of *Bt* are:

Alpha-exotoxin: this toxin was identified in the supernatants of fermentations by Toumanoff in 1953 as the enzyme lecithinase C. It is water-soluble and heat-labile and is also produced by bacteria other than *Bt*. A toxin considered iden-

[1] Presented at the annual conference of the International Society of Arboriculture in Hartford, Connecticut in August 1980.

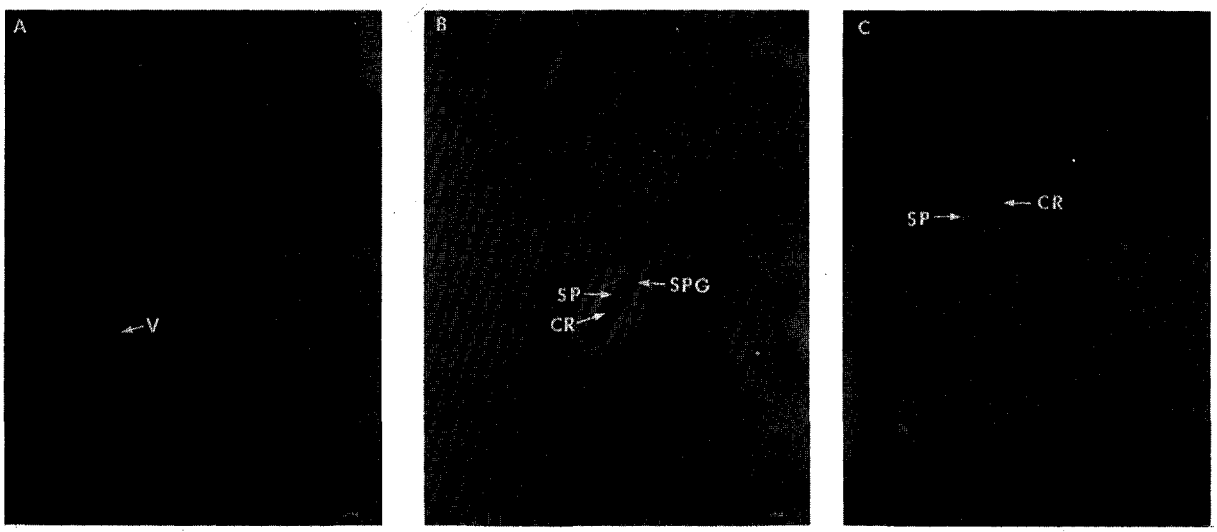

Figure 1. Development stages of *Bacillus thuringiensis:* A) Vegetative growth stage; v = vegetative cells. B) Sporulating stage; SPG = sporangium, SP = spore, CR = crystal (delta-endotoxin). C) Released spores and crystals. Bar equals 1 μm.

tical to this but not lecithinase C was recently reported by Krieg (1971) as being toxic, per os, to mice and diamondback moth (*Plutella maculipennis*). He named it the mouse factor or the thermosensitive toxin.

Beta-exotoxin: other than the spore and crystal, this was one of the first toxins found. It is produced and released in the fermentation medium during vegetative growth of some strains of *Bt*. It is water-soluble, heat-stable and highly toxic to flies. It has been identified as an adenine nucleotide-like compounds. Synonyms for this toxin are the McConnell-Richards factor, the fly knock-down factor and recently it has been renamed thuringiensin. This toxin is not absorbed or affected by passage through the gut of cattle and at one time it was proposed as a cattle feed additive for the control of flies in the feces. However, it can be toxic to vertebrates when introduced by parenteral injection and can have teratogenic effects in insects. These observations have led regulatory agencies to restrict preparations that contain this toxin from being used in the United States.

Louse factor: this exotoxin was first reported in powder preparations of some strains of *Bt* in 1974 (Gingrich et al. 1974); it is water-soluble and heat-stable and is produced by strains that do not produce the beta-exotoxin. It is very toxic to mammal-biting lice and has been suggested as a new toxin.

Delta-endotoxin: the crystal, as it is also called, is a broad spectrum toxin; as far as we know, its activity is limited to larvae of Lepidoptera, mosquitos, chrionomids, and blackflies (Lacey et al. 1978). All the safety data collected within the last twenty years have shown that the crystal has no adverse effect on nontarget invertebrates or on vertebrates. The term "delta-endotoxin" used to describe this crystal is in reality a misnomer, for the crystal itself is not toxic to insects until it is dissolved either *in vitro* under specific conditions or in the midgut of the larva. The dissolution releases from the insoluble protein matrix a small protein (50-100,000 daltons) which is the true toxin. Therefore, susceptibility of an insect to this toxin may in part, or perhaps entirely, depend on the insect's ability to digest the crystal into its toxic subunits. The observed potency may actually reflect the rate at which the insect's digestive system brings about this dissolution. The delta-endotoxins from different strains of *Bt* can differ quantitatively and qualitatively in their insecticidal activities.

Spore: originally *Bt* was considered only as an infective agent but with the realization that the

delta-endotoxin was one of the principal factors in the insecticidal activity, interest in the spore diminished. Recently proteins have been found on the spore coat that are homologous to the delta-endotoxin. The spore has also been found to be toxic to some Lepidoptera larvae, and so interest in the spore has been revived. The spore is formed at the termination of growth at the same time the crystal is produced. In some insects death can come very quickly after ingestion of the spores and crystals. This is due to the action of the crystal alone. In other insect species both the spore and crystal are necessary for optimal potency.

There is no question that as research continues and new strains of *Bt* are investigated, other toxins will be found. Possibly, strains that produce specific toxins that fulfill a particular economic need may eventually be developed commercially. Presently, strains that exhibit a high insecticidal potency and have a broad spectrum of activity are of interest for commercial development.

Mode of Action

The chronological development of the intoxication process of *Bt* in a susceptible host can be described briefly as follows: first the spores and crystals must be ingested. Shortly after ingestion the alkaline midgut digestive system of the insect dissolves the crystal releasing the toxic protein fraction(s). Once this small protein is released and activated it affects the permeability of the cell membranes in the gut, causing the cells to swell and burst. At this point gut paralysis occurs and feeding ceases. Some gut content spills into the hemolymph and in highly susceptible insects, death follows in a few hours. In less susceptible insects the ingested spores invade the hemolymph, germinate and multiply; the larvae die of apparent septicemia in 24 to 48 hours or longer. Consequently, the effectiveness of *Bt* depends on: 1) the quality and type of proteins present in the crystal, 2) the insect's ability to digest the crystal and release the active toxic fraction while it is in the midgut, and 3), in less susceptible species, the ability of the ingested spores to invade the hemolymph, germinate, multiply, and cause a lethal septicemia. Generally, susceptibility to a given dose of *Bt* is related to the age and biomass of the insect; younger larvae are more susceptible than older ones.

Spectrum of Activity

The spectrum of activity of *Bt* preparations tends to be limited to larvae of *Lepidoptera.* This is not to say that a particular strain (and some are being uncovered now) will not be pathogenic to other groups of insects. For example, *Bt* var. *israelensis* is highly toxic to mosquitos and black flies but not to Lepidoptera. However, most predators and parasites of susceptible Lepidoptera, as well as beneficial insects, are unaffected by *Bt*. A listing of those susceptible insects that are of agricultural and forest interest will be found on the labels of commercial products.

Taxonomy

Presently there are 20 varieties of *Bt* grouped into 16 serological groups called serotypes. (Table 1; Faust 1975, deBarjac 1978, deBarjac et al. 1977). The serotype is based on the composition of the flagella antigen of the vegetative cells. Some insect species such as the gypsy moth are susceptible to representative strains of more than one serotype (Dubois and Squires 1970). There are however, unique characteristics that should be mentioned: within the serotype 1 variety *thuringiensis* group, some strains, including the type species, *B. thuringiensis* Berliner, produce the beta-exotoxin, whereas others (also known as *Bt* variety *amuscatoxicus* (Faust 1975)) do not. Another serotype group of interest is serotype 2 variety *finitimus*; the crystal of the strains of this variety is atoxic to Lepidoptera. The HD-1 strain of *B. thuringiensis*, which is presently used for commercial production, belongs to the serotype 3ab, variety *kurstaki.* Strains of this group do not produce the beta-exotoxin, and the delta-endotoxin (crystal) has a fairly broad spectrum of activity against a large number of Lepidoptera species. Other varieties that tend to be unique are *galleriae* and *israelensis*. Variety *galleriae* belongs to group serotype 5ab and is unique in that representative strains are very potent against the greater wax moth (*Galleria mellonella*), an insect that is relatively insensitive

to the HD-1 strain of variety *kurstaki*. Finally, the strains of variety *israelensis*, which belong to the recently formed serotype 14 group, are also unique in that they are very potent against mosquito and black fly larvae.

Table 1. Taxonomic groups of *Bacillus thuringiensis*.

Serotype	*Variety*	*Beta-exotoxin*
1	thuringiensis	+,−
2	finitimus	−
3a	alesti	−
3ab	kurstaki	−
4ab	sotto	−
	dendrolimus	−
4ac	kenyae	−
5ab	galleriae	−
5ac	canadensis	+
6	entomocidus	−
	subtoxicus	−
7	aizawa	+
	pacificus	−
8	anagasiae (morrisoni)	+
9	tolworth	+
10	darmstadiensis	+
11	toumanoffi	+
12	thompsoni	−
13	pakistani	−
14	israelensis	−

Standardization

The potency listed on the labels of *Bt* product containers is in International Units of Potency. This system of standardizing *Bt* came from the realization that the spore count was unreliable as a measure of the potency of *Bt* products. Different insect species, and even insects of the same species but from different geographical locales, may differ significantly in their susceptibility to a *Bt* preparation. Adding to this confusion, the potency of a given strain produced under different fermentation conditions may differ against the same insect species. These variables made it very difficult to compare the efficacy of the different products. To alleviate this difficulty an international standard was devised and accepted in 1965. This standard, called E-61 and produced from a strain of variety *thuringiensis*, contains only the spores and crystal as active ingredients. It is arbitrarily defined to contain 1,000 International Units of Activity per milligram of dry powder. With the development and use of the HD-1 strain, a second international standard, HD-1-S-1971, was developed and accepted as the standard for *Bt* products derived from the HD-1 strain. The HD-1-S-1971, by virtue of its increased effectiveness compared to E-61, is defined to contain 18,000 International Units of Potency per milligram (Dulmage et al. 1971,, Burgerjon and Dulmage 1977).

Now all *Bt* products have a calculated International Unit of Potency on the label. This figure is derived from parallel bioassays between the product and the standard. The relationship is as follows:

$$\frac{LC_{50}\ (\text{HD-1-S-1971})}{LC_{50}\ (\text{product})} \times 18{,}000\ \text{IU/mg} = \text{Potency of the product in IU/mg.}$$

The LC_{50} (that concentration in terms of milligrams per milliliter of artificial diet that effects 50% mortality of the standard) is divided by the LC_{50} of the test product when both are compared by parallel bioassay. This ratio is then multiplied by the potency of the standard in International units per milligram. The computed figure is the potency of the product. The assumption is that whatever variation is due to the insect itself, either in terms of age or geographical locale, is equally measured in both the standard and the product. The difference in LC_{50} between the standard and the product will reflect the potency of the product. In this way different preparations can be compared, regardless of the insect's variation at the time the different assays are conducted. Also it provides a means of comparing two preparations where one may be formulated as a powder and the other as a liquid concentrate. For labelling purposes the potencies of commercial products in the United States are determined with HD-1-S-1971, and the test insect used is the cabbage looper (*Trichoplusia ni*).

The development and acceptance of these standards has facilitated the comparison of different preparations of *Bt*. However, the standards are by no means a solution in themselves. For instance, they are of little benefit when dealing with the greater wax moth and mosquitos, insects that are minimally susceptible to HD-1. Undoubtedly, as specific preparations of *Bt* effective against these insect species are produced commercially, new standards will be developed.

Formulation Development

In the late 1950's and early 1960's several companies in the United States were involved in the commercial production and distribution of *Bt*. Their products were crude powders of spores, crystals, and inert ingredients and were tank-mixed in water and oil. These early formulations had inherent suspendability problems, often clogged spray systems, and gave uneven spray distribution. At that time the major thrust was to improve the performance of *Bt* by increasing the potency of the material rather than by improving its formulation.

With the commercial development of the HD-1 strain a significant improvement in potency was achieved. Today essentially all commercial formulations of *Bt* are based on the HD-1 strain. Presently, three companies in the United States produce *Bt*[1]. Sandoz Inc. produces Thuricide 16B® , Thuricide 24B® , and Thuricide 32B® which are formulated as aqueous emulsifiable suspensions. Abbott Laboratories produces Dipel® , which is a wettable powder, and Dipel 4L® which is a nonaqueous emulsifiable suspension; and Biochem Products produces a wettable powder called Bug Time® . *Bt* is also commercially produced in France, Russia, and Japan.

Today *Bt* formulations are largely spore and crystal concentrates, prepared for use primarily as water suspensions. Only recently have manufacturers attempted to improve other factors that affect the successful use of *Bt*. These factors include the sticking quality on the foliage (rainfastness), coverage (drop density per unit of foliage surface area), reduction of evaporation during aerial application, palatability, resistance to solar ultraviolet degradation, and ease of mixing. Present formulations are somewhat improved in these areas, but further improvements are still needed. Table 2 lists the changes that have been made in the formulation of *Bt* used aerially against the gypsy moth.

The potency of these *Bt* products is calculated on the basis of the International Unit of Potency per unit weight or volume of concentrate. The applied volume of a formulation containing the specified International Unit per acre is dictated by

Table 2. Chronological development of *BT* formulations used in aerial application against the gypsy moth.

Year	*Finished spray* gal/acre	*Ingredients*
	BERLINER STRAIN	
1961	2	Tung oil, 9D-207 H_2O Thuricide W.P. (.25, .50, 1, & 2 lb/acre)
1962	2 & 4	Thuricide 65 .W.P. (lb/gal) in #2 fuel oil and H_2O + Lovo 192 - 6 oz
1963	2	Thuricide 90T .50 gal + 1.50 gal H_2O 1 gal + 1 gal
1965	2	Thuricide 90TS 1 pt & 2 pt to 2 gal H_2O
1966	2	2 pt Thuricide 90TS 6 oz Pinolene 1674
		1 lb Biotrol BtB 6 oz Pinolene 1674
	HD-1 STRAIN	
1971	80 (Ground)	Thuricide HPC NuFilm-Bt .20 pt/gal
1972	2	Dipel W.P. 50 lb CIB-1 gal, Propionic Acid Santoquin, Maywood
		Thuricide EC 1 qt 1 qt CIB, Chevron Spray sticker 4 oz
1973	2	Dipel W.P. 2 lb .50 gal CIB, 6 oz Chevron
		Thuricide 16B 2 qt 2 qt H_2O
1974	2/3	Dipel L.C. — 2 qt. H_2O 1 qt
	1/2	Thuricide 16B
	1	Thuricide 16B - 2 qt, H_2O - 2 qt
	2	Dipel W.P. - 1 lb .50 gal CIB, 6 oz Chevron

[1]**The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.**

the intended use and equipment. Aerially, *Bt* is applied in a final volume of 1 to 2 gallons per acre, whereas with ground equipment the applied volume can range from 10 to 100 gallons per acre. The quantity of the material applied per acre is dictated by the susceptibility of the target insect. For highly susceptible insect species such as the cabbage looper, 4 billion International Units per acre are recommended; against the gypsy moth, a less susceptible insect, 8 billion International Units per acre are recommended per treatment. Recommended doses are found on the labels of commercial products.

There are a number of cautions to be observed in mixing and preparing tank mixes of *Bt* (Lewis, 1978). These are: 1) the crystal of *Bt* is very sensitive to alkali. Water with a high pH should not be used to mix a *Bt* concentrate; the resulting alkaline suspension (e.g. pH of 8) will destroy the toxic effect of the crystal and dramatically reduce the effectiveness of the *Bt*. 2) *Bt* should be tank-mixed fresh; it should not be used if the tank mix has been prepared more than 24 hours earlier. 3) *Bt* should not be frozen or subjected to extremely high temperatures (above 43°C) for any length of time. This limits the storage conditions of concentrates before mixing.

Field Use

The major use of *Bt* products in the United States is against agricultural pests, primarily of cole crops, alfalfa, and cotton. Only 3% of the *Bt* is used against forest insect pests; of this more than 90% is used east of the Mississippi River. Harper (1974) summarizes the forestry uses of *Bt* in the United States through 1974.

Any *Bt* product can be used with aerial or ground application equipment without special adaptation. Generally, application with ground equipment is more successful than aerial application, because the volume used results in better coverage of the foliage and the loss of material through evaporation — a major problem of aerial application — is reduced. Timing of application of *Bt* is very important. For agricultural use, *Bt* is applied repeatedly at 5-to-7 day intervals until the crop is harvested. However, for forestry use two factors determine the timing: the degree of foliage expansion and the size of the larvae and their stage of development. Since *Bt* must be ingested to be effective, foliage should be sprayed when its expansion is maximum. Also, since susceptibility to a particular dose is in part related inversely to larval size, *Bt* should be applied when the larvae are still small. Timing of the application requires a subjective judgment weighing both the extent of foliage development and the size of the larvae; prevention of foliage damage being of overriding concern. Additionally, a large enough area should be treated to forestall reinvasion by insects from surrounding untreated areas.

The number of treatments and the volume used both have a direct bearing on the cost of using *Bt*. To protect agricultural cash crops, repeated weekly use, even at high gallonage per acre, can still be cost-effective. For residential or forestry use, weekly or multiple applications become costly, and different values must be considered: the long-term harvest of the woodland and esthetic value of the trees themselves. However, the costs of residential and forestry application can be reduced by judicious use of *Bt* as a biological agent rather than as a chemical insecticide by developing better formulations, improving the residual properties of *Bt*, and developing and using Integrated Pest Management (IMP) concepts.

Evaluation

Bt is designed to kill insects, primarily caterpillars, so insect mortality is a very important criterion of its effectiveness. Another criterion is the degree of foliage protection or damage reduction. When applied properly, either with ground equipment (Figure 2) or aerially (Figure 3), excellent foliage protection can be achieved. However, different user groups may have different objectives; some want complete elimination of the pest, others will accept a few residual caterpillars with reduced damage to their trees. It is possible to reduce foliage consumption without killing all the insects. *Bt* will never eliminate all the insects in a treated area, but it can achieve acceptable population reduction if timed and applied properly. Like chemical insecticides, *Bt* should not be expected to have any carry-over effects the following year.

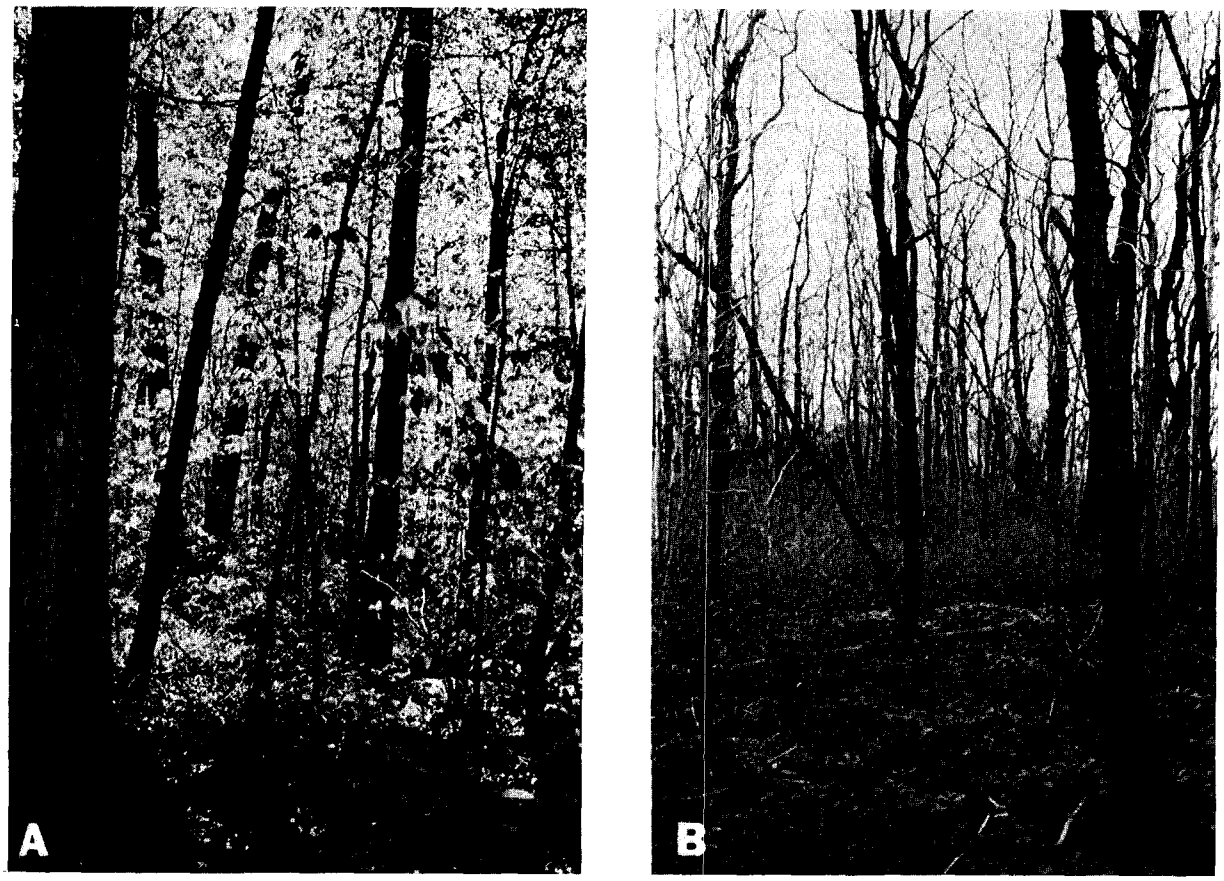

Figure 2. (A) Ground view of a gypsy moth infested area treated with *Bt*. (B) Adjacent untreated area.

Bt is environmentally and ecologically less destructive than chemical insecticides. With food crops, *Bt* can be used up to the day of harvest and has no entry restrictions. *Bt* does not have any effects on honeybees. Parasites and predators, which may well influence the population density of the pest in following years, are not affected. These are important considerations where residues must be minimized, as around reservoirs and in watershed areas.

Cost and Benefit

Bt costs between $12 and $15 per gallon of material or about $5 per pound. Using these rough figures and a dose of 4 billion International Units per acre, *Bt* costs between $2 and $4 per acre per application, a cost comparable to other insecticides. However, since *Bt* is not used in ultralow volumes the higher volume used adds to the application cost. So does the frequent need for multiple applications to deal with an extended eclosion period. Further, *Bt* is relatively slow acting, has no knock-down effect, must be eaten, requires good coverage, preferably on both surfaces of the leaves, and is limited in insecticidal activity to the larval stages of the insect pest. Finally, results of aerial use of *Bt* have been inconsistent, largely because of improper application and attempts to control less susceptible insects.

With continued development and increasing use of *Bt*, many of these undesirable cost factors can be reduced or eliminated. New strains of *Bt* that are significantly more potent than HD-1 are presently being uncovered and may be considered for commercial development. Their use will reduce the amount of active ingredient applied

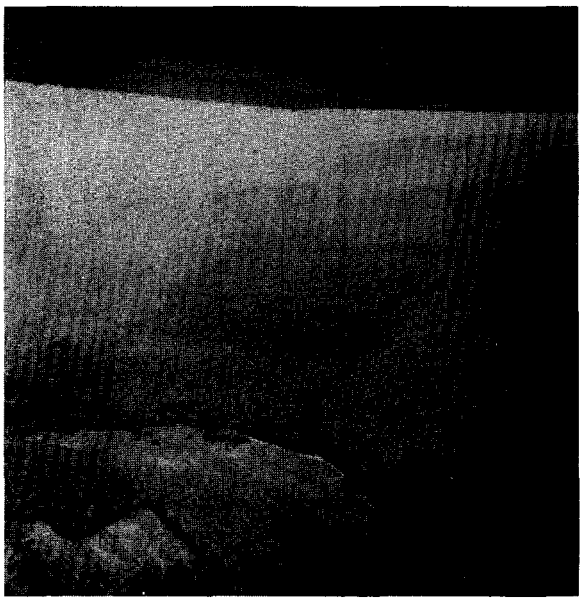

Figure 3. Aerial view of a square block (dark area) treated with *Bt* in a larger area infested by gypsy moth. Surrounding light area is totally defoliated by gypsy moth.

without reducing potency. Formulations with adjuvants to reduce the degradative effects of solar radiation and increase the residual activity are being investigated to reduce the need for multiple applications and increase the effective coverage. *Bt* has no effect on beneficial life forms and preserves natural enemies that aid in suppressing the pest population. *Bt* is readily available; increased production would reduce its cost; it does not need special application equipment; and it has no tolerance limit. When applied properly, *Bt* is as effective against susceptible pests as most chemical pesticides. It should be evaluated in the larger context of environmental and total ecosystem cost, as an integral part of the Integrated Pest Management concept.

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