Development and Current Status of *Bacillus thuringiensis* for Control of Defoliating Forest Insects

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Abstract

Bacillus thuringiensis (Bt) is a naturally occurring bacterium that is now widely used for control of spruce budworm and several other defoliating forest insects in Canada. It took more than 25 years of research and development to bring Bt from first experimental use in the early 1960s into full operational use in the mid 1980s. Critical to this process was the adoption of HD-1 for commercial use, the standardization of formulations based on international units, and the development of higher potency products. An increase in product potency from 4 BIU/L in the early 1970s to 16.9 BIU/L in the mid 1980s, concomitant with improvements in formulation and application technology, reduced treatment costs while at the same time improving reliability of efficacy. A shift in political climate pushed Bt into operational use by 1985. Since then, Bt has been used in more than half of all spruce budworm control programs. The use of high-potency formulations (12.7 to 16.9 BIU/L) applied undiluted at 30 BIU in 2.4 L/ha or less has significantly reduced but not eliminated initial constraints of high treatment costs and inconsistent efficacy. Current research is focussed on further reducing these constraints. In the short term, registration of formulations containing 25 BIU/L or more is expected to permit application of reduced dosages in less than 1 L/ha and to clear the way for adoption of a split application scheme to increase reliability of efficacy. Selection and commercialization of more effective strains and the enhancement of natural strains by genetic or genetic engineering techniques offer exciting prospects for improvements in the longer term.

Résumé

Bacillus thuringiensis (B.t.) est une bactérie naturelle, aujourd'hui largement utilisée contre la tordeuse des bourgeons de l'épinette et plusieurs autres défoliateurs des forêts du Canada. Depuis son premier emploi expérimental, au début des années 60, jusqu'à son utilisation en grand, dans le milieu des années 80, il s'est passé plus de 25 années de recherche-développement. Une étape critique de cette évolution a été l'adoption du HD-1 pour emploi industriel, la normalisation des préparations grâce à l'emploi des unités internationales ainsi que la mise au point de produits plus puissants. Ainsi, l'accroissement de la puissance, de 4 unités biologiques internationales au litre (UBI/L), au début des années 70, à 16,9, au milieu des années 80, en même temps que les améliorations des techniques de préparation et d'application, ont permis de réduire les coûts du traitement tout en améliorant la fiabilité de l'efficacité. Un nouveau climat politique a favorisé l'emploi en grand de B.t. dès 1985. Depuis, B.t. a servi dans plus de la moitié de tous les programmes antitordeuse. Le recours à des préparations très puissantes (de 12,7 à 16,9 UBI/L) appliquées sans dilution à raison de 2,4 L/ha a permis de réduire considérablement, sans les éliminer complètement, les contraintes initiales posées par les coûts élevés du traitement et l'efficacité inconsistante. La recherche actuelle se propose de réduire ces contraintes. À court terme, l'homologation des préparations renfermant 25 UBI/L ou plus est prévue afin de permettre l'application de doses réduites à raison de moins de 1 L/ha et de préparer la voie à un plan d'application répétée, pour une efficacité plus fiable. La sélection et la commercialisation de souches plus efficaces ainsi que l'amélioration des souches naturelles par des techniques génétiques ou du génie génétique recèlent des promesses excitantes d'amélioration à la longue.

Introduction

The past decade of large-scale spraying against defoliating forest Lepidoptera in Canada is characterized by a gradual increase in the use of microbial insecticides based on the bacterium *Bacillus thuringiensis* Berliner (commonly abbreviated as Bt). Aerial application of Bt is now considered a viable and effective strategy for providing foliage protection. It took more than 25 years of concerted collaboration between researchers, forest managers, and industry in both Canada and the United States to reduce the major obstacles of inconsistent efficacy and high cost. Many field tests were conducted between initial availability of commercial products in the early 1960s and operational acceptance in the mid 1980s. Most of those tests have been reviewed extensively (Morris *et al.* 1975; Smirnoff and Morris 1982; Morris 1982b; Cunningham 1985). This review describes events that made a critical contribution to the operational use of Bt in forestry, with emphasis on achievements in the last eight years. An overview of its current status (1989) is presented and prospects for further optimization are discussed.

Background

Bacillus thuringiensis is a naturally occurring bacterium that is pathogenic to larvae of many insect species. It was first recognized as a disease agent in silkworm in Japan and flour moths in Germany (Berliner 1915) and has since then been isolated from various species around the world (Krieg and Langenbruch 1981), including many forest insects (Morris 1982b). It now appears to be a worldwide and ubiquitous component of soil microbiota (DeLucca *et al.* 1981; Martin and Travers 1989).

Insecticidal activity of the bacterium is mainly associated with a parasporal protein body, often referred to as the crystal, which is formed during sporulation. After ingestion by a susceptible insect, the crystal is converted by digestive juices to a toxic protein that destroys the cells lining the gut (Percy and Fast 1983). The larva stops feeding and dies within a few days if a lethal dose is ingested, but recovers and resumes feeding if the dose is sublethal (Fast and Regniere 1984; Retnakaran *et al.* 1983; van Frankenhuyzen and Nystrom 1987). Germination of ingested spores and subsequent septicemia by multiplication of vegetative cells contribute to larval death in many species (Heimpel and Agnus 1959; Smirnoff 1974).

Bacillus thuringiensis is a collective name for a complex of more than 30 currently known subspecies, each with

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unique insecticidal properties (Dulmage 1981). Toxicity is exhibited specifically against Lepidoptera (e.g. subsp. *kurstaki*), Diptera (e.g. subsp. *israelensis*), or Coleoptera (e.g. subsp. *tenebrionis*). There is a high level of specificity within each subspecies. For example, only 200 species of predominantly foliage-feeding Lepidoptera are known to be susceptible to the *kurstaki* subsp., which is used in commercial formulations available for defoliator control. Because most other insect orders are unaffected (Krieg and Langenbruch 1981), Bt is a narrow-spectrum insecticide that is safe to natural enemies and other beneficial and nontarget organisms (Morris 1982b).

Commercial production is achieved by fermentation (Dulmage and Rhodes 1971). When cultured in an appropriate nutrient broth, vegetative cells sporulate and eventually lyse, releasing spores and crystals into the medium. The spore-crystal complex forms the active ingredient of commercial formulations. Bt was first available commercially in the late 1930s as a product called Sporeine in France. Commercial production in North America did not begin until the early 1950s (Hall 1963) and was primarily directed against agricultural pests.

Early Use

The first experimental aerial applications of Bt in forestry were conducted in 1960 against spruce budworm, *Choristoneura fumiferana*, in New Brunswick (Mott *et al.* 1961) and black headed budworm, *Acleris gloverana*, in British Columbia (Kingshorn *et al.* 1961). Further tests in 1962 and 1969 yielded results that were encouraging but far from adequate (Morris *et al.* 1975). Two events in the 1960s were of particular significance to the development of Bt. First was the discovery of HD-1 (Dulmage 1970), a *kurstaki* isolate that was more toxic to lepidopterans than the one that was initially used in commercial products. HD-1 was quickly adopted in North America for commercial production. Second was the establishment of an international system for standardizing the potency of formulations. Early formulations were standardized on the basis of spore counts, which did not relate directly to insecticidal activity. Suggestions by Bonnefoi *et al.* (1958) and Burgerjon (1959) to express potency in biological units based on insect bioassays resulted in the adoption of a European Bt standard in 1966 (Burgess 1967), which was later replaced by HD-1 as the North American standard (Dulmage *et al.* 1971). Insecticidal activity is now routinely expressed as the number of international units per unit weight or volume, as determined in bioassays against the cabbage looper, *Trichoplusia ni*, in parallel with the HD-1 standard.

Availability of formulations based on HD-1 and with standardized potency started a period of intensive field testing between 1971 and 1973 in Ontario and Quebec. Formulations containing about 4 billion international units per liter (BIU/L) were tested at 10–20 BIU/ha in 4.7–18.9 L. Although results were highly inconsistent, the good showing in some trials encouraged the expectation that Bt could indeed provide adequate foliage protection and provided the basis for continued and intensified testing in the remainder of the decade (Morris *et al.* 1975).

Potential and Constraints

Variable results of trials in the early 1970s (Smirnov *et al.* 1970; Morris 1982; Morris 1982b; Dorais 1985) were attributed to

inconsistent spray deposition, and consequently efforts were primarily directed at increasing the delivery of droplets to the target by improving both formulation and application technology. Formulations were altered to improve spray deposition by using spray additives (Morris *et al.* 1980) and to improve effectiveness by adding small amounts of the enzyme chitinase (Smirnov *et al.* 1973; Smirnov 1974; Morris 1976) or low concentrations of chemical insecticides (Morris and Armstrong 1974; Morris 1977). Various aircraft and delivery systems were tested, ranging from helicopters and single-engine biplanes equipped with rotary atomizers to four-engine aircraft fitted with boom and nozzles (Smirnov and Morris 1982). Although effectiveness of Bt showed marked improvement during those trials, results remained inconsistent with cost of treatment up to four times higher than chemical insecticide treatment (Blais 1976).

Improvements in cost effectiveness were achieved in the late 1970s when commercial formulations became more concentrated, that is, when potencies increased from 4.2 BIU/L in early products (e.g. Thuricide 16B) to 8.4 BIU/L (e.g. Thuricide 32LV, Dipel 88, Novabac 3) (Table 1). More concentrated products reduced shipping costs (bulk transport), permitted application of lower volumes, and improved spray plane productivity (Dorais 1985). By the end of the decade, performance of Bt for spruce budworm control had improved to the point that it was recommended for use in environmentally sensitive areas (Canada-US guidelines, Morris 1980), and was considered an operational alternative to chemical insecticides in Quebec (Dorais 1985). As a result, Bt was used operationally on about 100,000 ha in 1979 and 1980 (Table 2). However, variable efficacy and a three- to four-fold higher cost of treatment (Table 3) remained major obstacles to wider acceptance. Other constraints that put severe restrictions on spray program managers and undermined their confidence in Bt included reportedly reduced efficacy against populations exceeding 25 larvae per 45-cm branch and a window of application that was restricted to about 10 days after onset of bud flush (Dorais 1985).

Reducing the Constraints

Use of Bt in forestry accelerated rapidly in the early 1980s. By 1985, Bt had become a fully operational insecticide that was widely used for control of spruce budworm (Table 2) as well as other defoliators (Table 4). This surge in progress can be attributed to a confluence of several critical developments. The use of higher dosages and lower volumes, operational use before cost parity was achieved, better formulations, new knowledge on effective droplet sizes and dosage requirements, and corresponding improvements in application technology all contributed to reducing the obstacles of inconsistent efficacy and high costs, while a concurrent shift in political climate favouring the use of biologicals pushed Bt into operational use before uncertainties were completely resolved.

Application Rate

Inconsistent efficacy arose partially from the use of marginal dosages and from the difficulty of obtaining adequate spray coverage. The dosage of 20 BIU in 4.7 L/ha, recommended in the 1978 Canada-US guidelines (Morris 1980), was essentially a compromise between available product potency, the minimum emitted volume considered

Table 1. Bt products currently registered or pending registration¹ for aerial application in forestry.

Year	Trade name	Type of ² formulation	Potency (BIU/L) ³	Registrant (supplier)
1973	Thuricide 16B	FC	4.2	Zoecon
1978	Novabac-3	FC	8.6	Biochem
1980	Dipel 88	S	8.4	Abbott
1981	Thuricide 32B	FC	8.4	Sandoz
1984	Thuricide 32LV	FC	8.4	Sandoz
	Thuricide 32F	FC	8.3	Sandoz
	Bactospeine	FC	9.7	Duphar
	Futura	FC	14.4	Duphar (Chemagro)
	Thuricide 48LV	FC	12.7	Sandoz
1985	Dipel 132	S	12.7	Abbott
	Envirobac-ES	FC	8.4	Pfizer
	Dipel 176 (8L)	S	16.9	Abbott
1988	Futura XLV	FC	14.8	Duphar (Chemagro)
	Dipel 48AF	FC	12.7	Abbott
1989	Foray 48B	FC	16.9	Abbott
		FC	12.7	Novo
		S	25.9	Abbott
1990	Futura XLV-HP	FC	33.0	Duphar (Chemagro)
	Biodart	FC	16.9	ICI-Canada
	Dipel 64AF	FC, S	NA ⁴	Ecogen
	Dipel 264			
	Condor			

¹Pending registration.²Flowable concentrate (FC); emulsifiable suspension (S).³Billion International Units per litre (BIU/L).⁴Expressed as concentration of toxin protein.

Source: Agriculture Canada, Pesticides Directorate, Ottawa.

Table 2. Operational use of Bt for control of spruce budworm, *Choristoneura fumiferana*, 1979-1988: number of hectares sprayed (ha) with Bt and percentage of total area treated (%).

Year	Ontario		Quebec		New Brunswick		Newfoundland		Nova Scotia		Total	
	ha	%	ha	%	ha	%	ha	%	ha	%	ha	%
1979	3,454	17	17,030	3	205	1	5,870	100	556	100	27,115	1
1980	4,374	44	22,180	12	10,500	1	11,761	100	25,670	100	74,485	4
1981	6,576	67	15,001	2	0	0	1,900	1	31,194	100	54,671	2
1982	3,068	90	31,877	2	4,000	1	4,725	10	19,153	100	62,823	2
1983	2,763	87	45,627	4	10,300	1	0	0	20,726	100	79,416	3
1984	2,688	82	296,568	42	37,300	4	3,110	12	20,537	100	360,660	20
1985	29,370	100	512,155	73	81,000	12	3,450	100	49,719	100	675,363	45
1986	150,663	100	32,789	50	111,500	22	0	0	56,155	100	351,107	45
1987	76,689	100	197,992	100	91,300	19	0	0	31,080	100	397,191	51
1988	14,023	100	208,064	100	210,500	49	0	0	0	0	432,587	63
1989	30,516	100	165,034	100	104,900	18	0	0	0	0	300,450	39
Total	324,184		1,544,317		661,505		30,816		254,790		2,815,612	

Sources: Carrow (1983), Annual Forest Pest Control Forum Reports, Canadian Forestry Service, Ottawa.

Table 3. Relative cost¹ of treatment with Bt and chemical insecticides in New Brunswick and Quebec, 1980-1988.

Year	Quebec			New Brunswick ²		
	Bt	Chem.	Ratio	Bt	Chem.	Ratio
1980	31.31	8.59	3.6			
1981	27.94	6.24	4.5			
1982	18.16	5.79	3.1			
1983	23.54	7.67	3.0			
1984	18.63	10.16	1.8	16.38	11.52	1.4
1985	22.52	12.99	1.7	18.71	11.82	1.6
1986	17.37			16.60	11.50	1.4
1987	23.24			21.54	16.42	1.3
1988	23.02			21.30	16.98	1.2

¹Cost of operation excluding assessment and surveys, administrative overhead and research costs in \$/ha.²Small spray planes only, not including TBM.

Source: N. Carter, NB Dept. Natural Resources; L. Dorais, Quebec Department of Energy and Resources.

necessary to obtain adequate coverage, and the desire to reduce treatment costs. Most of the experimentation throughout the 1970s was conducted within the recommended rate of 20 BIU/ha, even though the minimum effective dosage rate had never been determined. Inadequacy of this application rate was first suspected when Fast (1976) compared expected dose on the target foliage with estimated dose requirements for spruce budworm larvae, and was confirmed between 1979 and 1981 when the guidelines were tested in extensive field trials under the auspices of CANUSA (Morris 1980, 1981; Cunningham 1985). It was not until 1981 and 1982 that field tests were designed specifically to determine optimum dosage and volume application rates (Morris 1984; Lewis *et al.* 1984). The general conclusion was that 20 BIU/ha was effective only under optimum conditions and against low populations, whereas 30 BIU/ha produced much more consistent results, even against populations exceeding 30 larvae per 45-cm branch (Morris 1982a; Carrow 1983; Dorais 1985).

Table 4. Operational use of Bt against defoliating forest insects, 1985-1989.

Target species	Number of hectares sprayed in:					Total
	1985	1986	1987	1988	1989	
Spruce budworm	675,694	351,107	397,061	432,587	304,948	2,161,196
Jack pine budworm	248,676	482,032	105,463	0	4,763	840,934
Hemlock looper	2,365	5,420	4,183	23,788	5,362	41,118
Gypsy moth	170	103,094	40,249	13,784	12,951	170,248
Total	926,905	941,653	546,956	470,159	328,024	3,213,496

Source: Annual Forest Pest Control Forum Reports, Forestry Canada, Ottawa.

Availability of concentrated formulations permitted application of the higher dosage in a lower volume. The initial call for concentrated products was based on the desire to reduce shipping costs and volume application rates (Blais 1976). Pioneering work in this area was done by Smirnoff, who developed a concentrated formulation (Futura) that could be applied at 20 BIU/ha in a final volume of 2.5 L. Trials at that rate were conducted as early as 1978 (Smirnoff 1980). Consistently good results between 1980 and 1983 (Smirnoff and Valero 1983; Smirnoff 1985) resulted in the registration of Futura in 1984 (Table 1). Commercial formulations containing 12.7 BIU/L became available for experimental use in the early 1980s. Application of such formulations at 30 BIU/ha in 2.4 L provided excellent results (Dimond 1982) and was as effective as application in 4.7 or 9.4 L/ha (Morris 1984). These findings were reflected in updated guidelines for operational use (Morris *et al.* 1984; Dimond and Morris 1984).

Operational Experience

Operational use before cost and efficacy were competitive with chemical insecticides catalysed significant cost reductions and provided the experience needed to improve efficacy. From 1979 to 1983, Bt was applied as a small but consistent proportion of the spruce budworm control program (Table 2) at 20–30 BIU in 2.4–7.0 L/ha (Carrow 1983). Increased use and competitive bidding by suppliers forced the price down. The average cost per BIU dropped from \$0.68 in 1980 to \$0.32 in 1983 and the costs per hectare fell from \$13.18 to \$6.96 in spite of a general increase in dosage from 20 to 30 BIU/ha (Carrow 1983). By 1983 the differential in application costs between Bt and chemical insecticides had narrowed to a two- to three-fold difference, depending on the jurisdiction. Practical experience gained from operational use between 1979 and 1983 also reduced the problem of reliability; users agreed that foliage protection at 30 BIU/ha was generally as good as that obtained with chemical insecticides (Carrow 1983). Early operational experience thus precipitated essential cost reductions while increasing confidence in the product, which opened the door for much wider use in subsequent years.

Undiluted Application

The trend of the late 1970s to increase product potency continued in the 1980s. Initial problems with handling of the highly viscous formulations were eliminated with the introduction of low-viscosity formulations. Potency of registered products increased from 8.4 BIU/L in 1980 to 12.7 BIU/L in 1984 and 16.9 BIU/L in 1988 (Table 1). The major benefit of the high-potency formulations was that they could be applied undiluted because they were designed for optimum

atomization and reduced droplet evaporation. Application of undiluted formulations had yielded promising results in trials in 1981 and 1982 (Dimond 1982; Morris 1984) and was increasingly seen as the ultimate way to reduce treatment costs (Carrow 1983; Dimond and Morris 1984). Application of undiluted product was first used operationally in 1982 in New Brunswick on 3,200 ha (Kettela 1983, 1985), and became the standard for spruce budworm control after 1984, when formulations containing 12.7 BIU/L were approved for operational use. From then on, Bt was routinely applied at 20 or 30 BIU/ha in 1.6 or 2.4 L. Successful application of such low volumes hinged on concurrent improvements in application technology that were based on a better understanding of effective droplet sizes and dose requirements (see next section).

Application of undiluted high-potency products undoubtedly made the most important contribution to reducing the constraints of high cost and unreliable efficacy. The use of low spray volumes reduced application costs by increasing spray plane productivity. Elimination of on-site mixing further increased plane productivity by allowing more loads per spray period. For example, productivity of four-engine sprayplanes in the Quebec spray program increased from 42,740 ha per year for application of 20 BIU in 4.7 L/ha (diluted) to 125,320 ha for undiluted application of the same dose in 1.6 L/ha (Dorais 1985). Furthermore, handling time and costs were reduced and problems associated with instability of mixed formulations were avoided, thus facilitating overall program management. The net effect of those factors was a further narrowing of the cost differential between Bt and chemicals (Table 3). In the state of Maine, total treatment cost showed a three-fold reduction between 1980 and 1985 (Irland and Rumpf 1987). The use of high-potency products also increased efficacy and reliability. In the 1984–1986 spray programs in Quebec, when both chemical insecticides and Bt were used on a large scale, single applications of Bt at 30 BIU/ha in 2.4 L performed generally as well as double applications of chemicals (Table 5). However, this was not the case in New Brunswick in 1988 (Carter 1988).

Effective Droplet Size and Dose Requirements

Implementation of undiluted application depended critically on improvements in the application of chemical insecticides, in particular the use of small droplets. In early Bt applications, spray systems were calibrated to deliver droplets in the 100–300 μm size range in an effort to optimize spray deposition as measured on Kromekote cards (Morris 1980; Morris *et al.* 1984). Data generated by the New Brunswick Spray Efficacy Research Group in the early 1980s (Picot *et al.* 1985, 1986) and work on the pine beauty moth,

Table 5. Comparative efficacy of Bt and chemical insecticides for operational control of spruce budworm in Quebec, 1984-1986.

Year	Product	Dosage/ha	Volume (L/ha)	Total area (ha)	No. larvae /45-cm tip	% Mortality	% Reduction in defoliation
1984	Matacil	2×52 g ai ¹	1.4	141,521	7.4	77.0	43
	Thu 48LV	1×30 BIU ²	2.4	182,346	7.4	73.0	35
1985	Fenitro	2×210 g ai	1.4	162,676	8.0	81.2	40
	Thu 48LV	1×30 BIU	2.4	456,639	8.8	83.0	46
1986	Fenitro	2×210 g ai	1.4	33,038	13.8	93.5	33
	Thu 48LV	1×30 BIU	2.4	18,160	14.4	86.1	31

¹Grams active ingredient (g ai).²Billion International Units (BIU).

Source: L. Dorais, M. Auger, Quebec Dept. of Energy and Resources.

Panolis flammea, in Scotland (Holden and Bevan 1978) provided overwhelming evidence that droplets of < 100 µm impinge more effectively on coniferous foliage than large droplets. The use of such small droplets requires efficient atomization of the spray formulation. For aerial application this is best achieved with rotary atomizers. Availability of non-volatile, low-viscosity formulations permitted direct translation of this concept to the application of Bt. Rotary atomizers are capable of generating a high proportion of droplets below 100 µm from small volumes of undiluted high-potency formulations (Yates and Cowden 1986) and are the key to success of undiluted applications.

Because Bt has to be ingested to be effective, success of a spray treatment is determined by the interaction between insect feeding activity, the number of spray droplets per unit feeding area and the dose in those droplets. The importance of droplet density has long been recognized. Spray volumes were chosen and aircraft were calibrated to obtain a deposit of at least 25 droplets per square centimeter on Kromekote cards placed on the ground (Smirnov and Morris 1982; Morris 1980) because lower deposits were associated with poor efficacy (Grimble and Morris 1983). The dose delivered to the target foliage was not assessed as a critical determinant of efficacy until 1981 when Fast and Sundaram (unpublished data) explored the interaction between size, density and toxicant concentration of spray droplets in determining spruce budworm mortality. Using a droplet size of 30-50 µm, they demonstrated that increasing product concentration from 2.1 to 12.7 BIU/L increased mortality from 20 to >90%. The number of droplets required to obtain 50% mortality decreased from 10 droplets per needle for a 2.1 BIU/L concentration to < 1 droplet for 8.4 BIU/L. In a subsequent field trial in 1983, a deposit of 1 droplet per needle of a 6.3 BIU/L spray mixture resulted in a 50% reduction in larval survival (Fast *et al.* 1985a). Work in Quebec in 1984 established that 0.5 droplets per needle of a 12.7 BIU/L formulation was required to obtain 50% foliage protection or more (Lambert 1987).

The pronounced effect of concentration on the density of spray droplets required for acceptable efficacy was further demonstrated in field trials in 1984 and 1985. Fast *et al.* (1985b, 1986) related estimates of larval survival and defoliation to density of spray droplets (mostly in the 25-75 µm size range) on balsam fir foliage for formulations containing 8.4-16.9 BIU/L after undiluted application at 30 BIU/ha. Higher potency products provided better efficacy, particularly

at lower deposits. Similar relationships were established in subsequent years for various high-potency products (e.g. Wiesner and Kettela 1987). In general, formulations with 12.7 BIU/L or more provided at least 50% reduction in defoliation at a deposit of 0.5 droplets per needle, while higher droplet densities were required for less concentrated formulations. A high concentration of Bt in the spray droplets thus played a key role in the increase in efficacy associated with undiluted application.

Volume versus Concentration

Work by Fast *et al.* (1985, 1986) was significant because it demonstrated the dominant role of product potency in determining efficacy. Their findings suggest that the more concentrated the formulation is, the fewer droplets are required to deliver an efficacious dose to the target and the more consistent efficacy will be. Thus, optimum dosage requirements should be considered in terms of BIU/L rather than BIU/ha. If the potency is high enough, acceptable efficacy should be obtainable at lower application rates as long as the applied volume can be atomized into a sufficient number of droplets to deliver an efficacious dose to the target. That thinking stimulated the development of products with even higher potencies, currently up to 33.0 BIU/L (Table 1), which can be applied in volumes as low as 0.6 or 0.9 L/ha, and provided the basis for the development of the so-called enhanced atomization application technique.

Enhanced Atomization Application

Droplets 15-55 µm in diameter appear optimal for spruce budworm-insecticide contact on coniferous foliage (Picot *et al.* 1985, 1986). They also impinge effectively on silk strands of budworm feeding shelters, which may be an important dose transfer mechanism (Nigam 1987). Droplet volume in that size range generated by rotary atomizers can be enhanced by reducing the flow rate. Van Vliet and Picot (1987) demonstrated in wind tunnel studies that the Micronair AU4000 atomizer produced >80% of the emitted volume in the 15-55 µm size range at flow rates below 2 L/min. Such flow rates allow maximum rotational speed and enhance atomization of the spray liquid compared with the standard flow rates of 5-7 L/min, thus producing more droplets in the effective size range. Enhanced atomization trials in New Brunswick in 1987 demonstrated that undiluted application of a 12.7 BIU/L formulation at 15 BIU/ha using a flow rate of 2 L/min per atomizer was as effective against spruce

Figure 1. Use of various Bt formulations in operational spraying against forest defoliators in all jurisdictions, 1985-1988.

budworm as the standard application of 30 BIU/ha using 6.7 L/min. Availability of products containing 33 BIU/L in 1988 enabled application of 15 BIU/ha in 0.5 L. Droplet deposition and efficacy were comparable with conventional application of 30 BIU/ha using 5.0 L/min (E. G. Kettela, unpublished data). Work is continuing to further test this promising technique.

Operational Use

The proportion of the area treated with Bt increased from 1-4% between 1979 and 1983 to 20% in 1984 and 45-50% in subsequent years (Table 2). This was primarily due to increasing use in Quebec, and in Ontario following the provincial government's decisions in 1985 to stop aerial use of chemical insecticides in public forests. Chemical insecticides remained the main tool in New Brunswick until 1988 when Bt was used in almost half of the program. Although increasing public pressure against the use of chemical insecticides in forests played a key role in the switch to Bt, the switch was possible because improvements in efficacy and cost effectiveness achieved in the early 1980s gave politicians a viable, albeit more expensive, alternative.

Operational use after 1984 involved predominantly undiluted applications of formulations with 12.7 or 14.4 BIU/L at 20 or 30 BIU in 1.6-2.4 L/ha. The main products used were Dipel 132, Thuricide 48LV, and Futura (Fig. 1.). First operational use of a 16.9 BIU/L product (Dipel 176) at 30 BIU in 1.8 L/ha occurred in 1988 in Newfoundland against hemlock looper, *Lambdina fiscellaria*. Formulations containing 25-33 BIU/L have been available for experimental use since 1986 and were successfully tested in volumes of 0.9-1.2 L/ha against spruce budworm (Wiesner and Kettela 1987; Kettela, unpublished data), and hemlock looper (West *et al.* 1989). Super-high potency formulations thus allow application volumes that are

comparable with or below those for chemical insecticides and are expected to become the mainstay of forest protection programs as soon as registrations are completed (Table 1).

With increasing restrictions on chemical insecticides in recent years, the use of Bt was extended to control several other defoliating species (Table 4). A recent outbreak of jack pine budworm, *Choristoneura pinus pinus*, in Ontario and Manitoba resulted in extensive control operations between 1985 and 1987. Because laboratory bioassays indicated a similar level of susceptibility as the spruce budworm (van Frankenhuyzen and Fast 1989), application of undiluted high-potency formulations was recommended and successfully implemented. Formulations with 12.7 BIU/L applied at 20 BIU in 1.6 L/ha generally provided satisfactory foliage protection.

Operational control of the gypsy moth, *Lymantria dispar*, in Ontario started in 1985 and mushroomed to 100,000 ha in 1986. High-potency formulations were diluted and applied at 30 BIU in 5-6 L/ha because a high volume application rate was considered necessary to obtain adequate coverage of foliage throughout the hardwood canopy. However, studies in 1987 and 1988 demonstrated that application of undiluted Bt provided better canopy penetration and equivalent larval mortality at lower spray deposits than application of diluted Bt in higher volumes (van Frankenhuyzen *et al.* 1989). The 1988 program was consequently conducted with undiluted product applied at 30 BIU/ha in 2.4 L and was the most effective gypsy moth control program conducted to date (G. M. Howse, unpublished data).

Undiluted application of high-potency products was also successful in experimental programs against eastern hemlock looper in Newfoundland (West *et al.* 1987, 1989) and operational use increased from 2-6% in 1985-1987 to 34% in 1988. Foliage protection was generally equivalent to that of fenitrothion (Table 6). Many other defoliators are susceptible to Bt including occasional outbreak species such as forest tent caterpillar, *Malacosoma disstria*, eastern tent caterpillar, *M. americanum*, western spruce budworm, *Choristoneura occidentalis*, black headed budworms, *Acleris* sp., and white marked tussock moth, *Orgyia leucostigma*. Field tests are needed to determine the feasibility of using undiluted Bt for control of these species.

Current Constraints and Future Prospects

After almost 30 years of research and development, Bt has become a major tool in forest protection programs. Operational use of high-potency formulations applied undiluted in low volumes has reduced but not eliminated the initial constraints of high treatment costs and inconsistent efficacy. Operational costs are still higher than for chemical insecticides (Table 3), but the main drawback is that efficacy is more dependent on proper timing of application and favorable postspray weather, which results in a narrower window of application and less predictable efficacy. The 1988 experience in New Brunswick is a good example of this: reduction in defoliation was 61% for fenitrothion compared with 24% for Bt (Table 7). Current research to reduce these constraints is focussed on developing higher potency products with greater foliar persistence, and product improvement by selection of more effective strains and by biotechnological manipulation.

Table 6. Comparative efficacy of Bt and fenitrothion for operational control of the hemlock looper in Newfoundland, 1986-1988.

Year	Product	Dosage/ha	Volume (L/ha)	Area (ha)	% area with defoliation			
					nil	light	mod.	severe
1986 ¹	Fenitro ²	2×210 g ai ³	1.5	79,028	84	3	3	10
	Dipel 132	1×30 BIU ⁴	2.4	5,420	76	0	0	24
1987 ⁵	Fenitro	1×210 g ai	1.5	164,362	84	5	3	8
	Dipel 132	1×30 BIU	2.4	4,183	78	6	1	15
1988	Fenitro	2×210 g ai	1.5	45,138	91	6	<1	2
	Dipel 176	1×30 BIU	1.8	6,473	91	7	<1	7
	Futura XLV	1×30 BIU	2.0	3,704	94	3	<1	3
	Dipel 176	2×30 BIU	1.8	1,372	100	-	-	-
	Dipel 176+	1×30 BIU	1.8					
	+ Fenitro	1×210 g ai	1.5	10,410	97	2	<1	<1

¹About 1.5% of area received single application of fenitrothion.²Fenitrothion (Fenitro).³Grams active ingredient (g ai).⁴Billion International Units (BIU).⁵About 9.6% of area received double application of fenitrothion.

Source: H. Crummey, Newfoundland Dept. Forest Resources and Lands.

Table 7. Comparative efficacy of Bt and fenitrothion for operational control of the eastern spruce budworm in New Brunswick, 1988.

Product	Dosage/ha	Volume (L/ha)	Area (ha)	No. Larvae /45-cm tip	% Mortality	% Reduction in defoliation
Fenitro ¹	2×210 g ai ²	1.4	231,000	21.6	72	61
Futura XLV	1×30 BIU ³	2.0	210,500	26.0	50	24

¹Fenitrothion (Fenitro).²Grams active ingredient (g ai).³Billion International Units (BIU).

Source: Carter (1988).

Improved Formulations

The development of formulations with higher potency and greater residual toxicity is considered the key to short-term improvement of Bt efficacy. The benefit of such formulations is several-fold. Variable tree and insect phenology within large spray blocks make proper timing of a single spray application difficult, particularly when using Bt which has no contact toxicity and limited residual toxicity. Reliability of Bt can be increased by adopting a split application scheme, as is used for chemical insecticides, but high treatment costs have until now prohibited this. However, registration of formulations with much higher potencies in the near future is hoped to permit operational use of reduced dosages at 0.5 L/ha (see "Enhanced atomization application"), which could reduce treatment costs to a level where split applications become competitive. Higher product potency is also expected to decrease the dependance of treatment success on favorable weather after application by ensuring rapid ingestion of a lethal dose (van Frankenhuyzen 1990). Another prerequisite to improving reliability of Bt efficacy is better persistence of spray deposits on the foliage. Residual toxicity of present formulations is limited to a few days, particularly under rainy conditions (van Frankenhuyzen and Nystrom 1989). Increased persistence of spray deposits will minimize adverse effects of postspray rain, thereby allowing more latitude in timing of spray application.

Strain Selection and Modification

An alternative approach to reducing cost and increasing effectiveness of Bt is the commercialization of strains with specific toxicity to forest insects. This can be achieved by identifying natural isolates with the desired activity range or by enhancing natural strains using conventional genetic techniques or genetic engineering techniques.

Of the naturally occurring strains, the HD-1 isolate of current formulations is one of literally thousands of possible candidates (Martin and Travers 1989), but efforts to identify more potent strains have yielded little benefit for forest insect control to date (Morris and Moore 1983; Dubois 1985; Dubois *et al.* 1989). However, new strains are continually being isolated (Travers *et al.* 1987) and the search for more effective ones is actively pursued. One example of possible success in forestry is commercialization of the strain A20 (Biodart, Table 1), although superior performance in the field has yet to be demonstrated.

Genetic enhancement of toxicity is an approach that was used by Ecogen to produce Condor (Table 1). Toxin genes of two strains were combined by employing a native conjugal plasmid transfer system to improve toxicity to spruce budworm and gypsy moth. Condor is currently undergoing field tests for registration. Condor is also an example of a new approach for standardization of product potency, based on quantification of toxin protein content. The proliferation

of target-specific products is making the use of international units obsolete and cumbersome. Because activity against cabbage looper is often not meaningful, each product needs to be labelled with different international units for each target pest. Examples are the recent introduction of units for the Colorado potato beetle, *Leptinotarsa decemlineata*, (Zehnder and Gelernter 1989) and Spodoptera for standardization of products developed specifically against these targets.

The great diversity of natural strains, each with a different activity spectrum, offers opportunities for the application of genetic engineering techniques. Because genes that code for crystal proteins are located on plasmids, they are highly amenable to modification by using recombinant DNA techniques. Modifications include increased toxin yield, altered range of insecticidal activity, or enhanced environmental persistence (Luthy 1986; Wilcox *et al.* 1986; Whiteley and Schnepf 1986). Biotechnological improvement of Bt is enjoying worldwide commercial interest, but most efforts are focussed on the larger agricultural market. In Canada, a research network was established in 1984 with the goal of tailoring an improved Bt product for forest insect control. The network, called Biocide, involves researchers from Forestry Canada, National Research Council, and several universities. Toxin genes from various strains were cloned and sequenced (Hefford *et al.* 1987) and researchers are now elucidating molecular determinants of toxin activity and specificity. Site-directed mutagenesis and chemical modification will then be used to manipulate these determinants to achieve maximum benefit for forestry applications. In addition, a mechanism of sunlight inactivation of the crystal protein has been established (Pozsgay *et al.* 1987) and ways of improving stability in sunlight are being explored. Achievements to date will accelerate the development of novel, target-specific microbial insecticides based on recombinant Bt.

The future of Bt for forest insect control looks bright. Now that operational use is well established, manufacturers are increasingly confident that they face a promising market. Increasing competition (from three main producers in 1985 to seven potential contenders in 1988) is expected to accelerate product improvement and result in further cost reductions in the short term. Genetic engineering offers exciting opportunities in the long term, provided risks associated with the release of genetically-engineered microorganisms (Primentel *et al.* 1989) can be minimized and regulatory requirements can be worked out. After 30 years of research and development, we are just beginning to understand and exploit the real potential of Bt for control of forest insects.

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