Bacillus Thuringiensis: a Logical Weapon for Spruce Budworm Control

W.A. Smirnoff

Laurentian Forest Research Centre,
Canadian Forestry Service,
Department of the Environment,
Sainte-Foy, Quebec G1V 4C7

Abstract

Action of *Bacillus thuringiensis* (B.t.) on spruce budworm, *Choristoneura fumiferana* Clem., was found to be attributable to septicemia with some enterotoxigenesis. Development of a compact economical B.t. formulation, with addition of the enzyme chitinase, increased its efficiency by accelerating larval mortality. Between 1971 and 1975 field tests were conducted in balsam fir stands with different levels of insect density to select the best formulation and methods of application and to identify the major influencing environmental variables. Variability in the results prior to 1975 is attributable to difficulties with calibration of spray systems. With accurate calibration in 1976 and 1977, good results in terms of larval mortality and foliage protection were obtained, thus confirming the value of B.t. for control of spruce budworm.

Résumé

La recherche de base sur le mode d'action de *Bacillus thuringiensis* contre la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* Clem.) révèle que l'action du bacille sur ce lépidoptère est attribuable à une septicémie avec éléments d'entérototoxicose. Le développement d'une formulation compacte et économique du bacille avec addition de l'enzyme chitinase permet d'augmenter l'efficacité de *B. thuringiensis* contre la tordeuse en accélérant la mortalité larvaire. Plusieurs essais en forêt furent réalisés de 1971 à 1975 avec différents modèles d'avions dans de multiples conditions de densité d'insecte, de défoliation et de peuplements de sapins. Ces essais rendirent possible la sélection et l'étude des principaux facteurs de l'environnement influençant l'action du bacille et même la composition des formulations à utiliser. Également, les résultats de ces essais révélèrent que les variations dans les résultats des dispersions de *B. thuringiensis* observées jusqu'en 1975 étaient essentiellement attribuables à une mauvaise calibration des systèmes d'arrosage. Effectivement, les dispersions de 1976 et 1977, réalisées à la suite d'une calibration exacte, donnèrent de bons résultats en terme de mortalité larvaire et de protection du feuillage confirmant ainsi les possibilités d'utilisation opérationnelle de *B. thuringiensis*. Cet article résume les étapes du développement de la recherche ainsi que les résultats des expériences aériennes.

Introduction

The introduction of any innovation usually encounters much resistance. *Bacillus thuringiensis* (B.t.) is no exception. A microbial insecticide proposed for spruce budworm (*Choristoneura fumiferana* Clem.) control, this biological control method is a potential alternative to traditional chemical insecticides¹.

¹ Referring to Fenitrothion, Matacil and Phosphamidon the insecticides which are more commonly used against the spruce budworm in Quebec since banishment of DDT.

Many published articles question the use of B.t. for spruce budworm control. However, since I have been associated with this new form of insect control since the beginning of research on B.t. vs spruce budworm, I now wish to rectify some concepts.

Use of Chemical Insecticides

Foresters are well aware of the spruce budworm's existence and the intensity of its recent infestation (Hardy 1974). They also know that various chemical insecticides are used in enormous quantities in Québec and New Brunswick. However, forest engineers may have limited access to background information on the effects of spraying large quantities of chemical insecticides. Are these spray programs effective? Are the dosages considered effective three or four years ago still adequate today? The answers depend on the user. In Québec, after having spent a considerable amount of money on spraying operations during the past four years, foresters are still questioning the timing of application and consider the possibility of three treatments per year instead of the present two in hope of improving results. But the forest engineer may be even less aware of the potential side effects of chemical insecticides. I am referring here to the intimate interactions within the various ecosystems and also to the creation of resistant insect strains which will require the use of stronger chemical insecticides at higher concentrations, and the potential long and short-term threats these chemicals present to human health (Crocker et al. 1976 and April 1977). It is disturbing to realize that the validity of the methods used for safety tests of chemical insecticides is still questioned. Obviously, the answer to this question will only come through a comprehensive analysis performed by a multi-disciplinary task-force whose work would not be limited to the sampling of birds.

Bacillus Thuringiensis

Biological insecticides have been used for years, particularly the entomopathogenic bacterium B.t. which is produced on a large scale in the United States for the protection of agricultural crops. The commercial preparations of this microbial insecticide, unlike chemical insecticides,

ticides, are absolutely harmless to non-target organisms and man, and are as effective as any chemical insecticides currently used against the spruce budworm. But, because *B.t.* is new, foresters still know little about it. What exactly is this bacterial insecticide? What are its standards of efficiency, its potential, and the possibilities of using it against the spruce budworm? In order to clear up certain misunderstandings regarding its use, I will try to analyze the situation not only as an entomopathologist, but also as a forest engineer.

Mode of Action of the *Bacillus*

The program of research in insect pathology at the Laurentian Forest Research Centre, Department of the Environment, Sainte-Foy, Qué., includes numerous studies on the fundamental and practical aspects of *B.t.* utilization. The object of the fundamental studies is to determine the pathogenic properties of *B.t.* against the spruce budworm. Research has dealt with the mode of action, selection of strains, infection syndrome, influence of environmental factors, and interaction with various natural infections, namely microsporidia. Applied field research is obviously related to the effectiveness of the bacillus in the forest. The main concern is development of an adequate formulation which will meet requirements in terms of international units, volume deposited per unit area, and possibility of being used with modern spraying techniques. All this is closely linked to the reduction of cost in using this bacterium against the spruce budworm.

The first studies demonstrated that when insect mortality is caused by *B.t.*, it is due to a typical septicemia with elements of enterotoxigenesis linked to the possibility of penetration of spores from the gut into the blood (hemolymph) of the larvae (Smirnov 1963).

The action of *B.t.* against the budworm was insufficient because a chitinous layer in the insect intestine makes difficult or impossible the passage of spores into the hemolymph thus considerably reducing the efficiency of *B.t.* on this species. Further work indicated addition of trace amounts of chitinase, an enzyme which hydrolyzes chitin, would facilitate penetration of the *B.t.* spores into the hemolymph, thereby increasing the pathogenicity of the commercial preparations. Laboratory tests had shown that the presence of chitinase in *B.t.* preparations considerably increases its effectiveness on the spruce budworm in populations which do not support a native microsporidia (Smirnov 1971; Smirnov et al. 1973a; Smirnov 1976b). By provoking injuries in gut walls, microsporidian infection facilitates penetration of *B.t.* spores into insect hemolymph. Using these results, for the past seven years, experimental and operational aerial applications of *B.t.* + chitinase have been carried out to evaluate the factors influencing the use of *B.t.* in the field and to determine the possibilities of using this organism for spruce budworm control.

Results of the Aerial Applications

From 1971 to 1977, aerial field tests were conducted with the *B.t.* + chitinase formulation against the spruce budworm. In 1971 using a Stearman aircraft and 1972 using TBM Avengers the same formulation at a rate of 18.81 L/ha was dispersed. (Smirnov et al. 1973b). During the winter of 1972-73 a more compact formulation was developed reducing sprayed volume and the cost of application. The formulation recommended was composed of *B.t.* concentrates, sorbitol (a sugar) as anti-evaporant and Chevron sticker and allowed dispersion of the required amount of bacteria in 4.7 L/ha. It was tested on a small scale in 1973 using a CL-215 aircraft (Smirnov et al. 1974) and over a larger area in 1974 using a DC-6B aircraft (Smirnov et al. 1976). However, during those years larval mortality and foliage protection obtained were unhomogeneous because of improper aircraft calibration. This was particularly evident in 1975 during operations over 92,000 ha of forest. Analysis of the spray deposit showed that only very small amounts of the bacillus were deposited on the ground — seven to nine times less than the sprayed volume. Thus, in 1976 and 1977, particular attention was given to calibration. In 1976 a Sikorsky S-55T helicopter with 4 X 80 μ m Beecomist, and in 1977, a Grumman AgCat with a boom and nozzles spray system were properly calibrated with *B.t.* formulations. This correction produced homogeneous results with a 90% and 77% larval mortality and 72% and 64% foliage protection in 1976 and 1977, respectively. These levels are far above acceptable limits of success for chemical insecticides.

Such results demonstrate the potential of this formulation for the control of spruce budworm as well as the efficiency of the concentrated formulation which has been developed.

Effect of *B.T.* on the Metabolism of Insects

Other valuable and positive information was obtained from the results of *B.t.* treatments. It was shown that *B.t.* has an effect on the physiological condition of the surviving residual population (Smirnov and Valéro 1972, Smirnov 1976a). This residual effect, called remanance, is characterized by strong metabolic disorders which affect the physiological condition and the vitality of insects (Smirnov and Valéro 1972). For example, weight and length of treated larvae and pupae were considerably less than those of untreated larvae and pupae. In pupae, levels of calcium, total proteins and urea nitrogen were also lower. Enzymes of carbohydrate, which are very important during pupal life and adult development, were reduced by 50%, a reflection of the low vitality of residual populations after treatment with *B.t.* This may explain why, in 1973, 1976 and 1977 in the territories treated with *B.t.* in 1972, 1975 and 1976, defoliation was low al-

though in some areas population levels were sufficient to cause severe defoliation. For example in 1972, in the block treated with the *B.t.* + chitinase formulation in 1971, the current year's defoliation was 43%, and foliage potential for 1973, 67%, which clearly indicated that no treatment was required in 1972 to maintain trees in acceptable condition. In nearby untreated areas defoliation was 91% and foliage potential for 1973 only 18%.

Basic Requirements For a Successful Use of *B.T.*

In general it can be concluded that, some 70% foliage protection is ensured when the population of spruce budworm does not exceed 35 larvae per 45 cm branch tip, when foliage potential in the spring is between 80-100%, and when the following procedures are followed.

1. Use the most economical formulation; 50 parts, *B.t.* concentrate; 20 parts, 70% sorbitol solution; 30 parts water; 1/1600, Chevron sticker and 10,000 nephelometric units/ha of chitinase¹, at a rate of 4.7 L/ha.
2. Perform the application at peak third instar larvae, before the buds of *Abies balsamea* are completely open and before the crowns of the conifers are covered by the leaves of deciduous trees.
3. Adequately calibrate the spraying equipment so as to permit a minimum deposit of 30 droplets of formulation per cm² with a droplet spectrum from 100 to 400 μ m, but with a higher proportion of the droplets in the range of 200-300 μ m.

Discussion

The chemical insecticide treatments in 1975 and the *B.t.* treatments were applied with DC-6B aircraft and did not give the expected results. Calibration of the spraying equipment was suspected faulty. The need for special calibration for large aircraft to make *B.t.* application operational over large areas seems obvious but the decision to do this work has not yet been taken. Results of 1976 and 1977 treatments with small aircraft proved beyond any doubt that *B.t.* is highly effective for spruce budworm control and foliage protection provided proper calibration is realized.

The detailed evaluation of the effectiveness of various treatments, based on comparison of results in Québec, has clearly demonstrated that one application of *B.t.* + chitinase can be as effective as a double application of chemical insecticides. Nevertheless, several arguments are advanced against the operational use of *B.t.* One of these is that *B.t.* is rapidly and easily inactivated by solar radiation, which is certainly not

the case. In experiments, which included a detailed analysis of a complex of influencing factors as well as a detailed evaluation of energy transmitted by the UV, IR and visible portion of the solar spectrum, it was shown that *B.t.* is resistant to solar radiation.

It has been stated that commercial *B.t.* products corrode aluminum. My experiments do not confirm this. It is possible that certain lots of *B.t.* may have contained an excess of xylene, which is used as a stabilizer, and that the xylene may have removed some paint from tanks in which *B.t.* formulations are prepared but this bears no relation to the corrosion of metal.

Often arguments against using *B.t.* have been based on its high cost. In Québec in 1977 two applications of chemical insecticide cost about \$4.90/ha, depending on concentration and number of applications, while *B.t.* was \$6.76/ha. This leveling in prices can be attributed to the fact that the cost of chemical insecticides and fuel oil used as a carrier increases considerably each year while *B.t.* and its carrier show only a slight increase or, in some cases, a decrease. Currently there is an alarming increase in the frequency of application of chemical insecticides; up to three applications per year on the same territory. Thus the cost of *B.t.* treatments become equal to or, at most, 1.5 times that of chemical insecticides. Also, cost should decrease once *B.t.* is used in large quantities.

That the final volume sprayed per ha is too large, is another argument often used against *B.t.* This argument, likewise, no longer holds. The concentrated formulation which I have developed allows the desired spray quantity of *B.t.* + chitinase in a final volume of 4.7 L/ha. Chemical insecticides are sprayed at a rate of 150 mL of concentrate in 1.125 L of fuel oil/ha for one application, which in the case of two, three, or four annual applications, would result in a total final volume of 2.24, 3.37 and 4.7 L/ha, respectively.

Therefore, the required final volume of *B.t.* formulation per hectare is no greater than that required for treatment by chemical insecticides. Moreover, fuel oil, which is necessary for the dispersion of chemical insecticides, is not only an energy source in short supply, but also a potentially toxic and polluting substance.

The principal argument in favour of chemical control has always been the presumed, radical effects of chemical insecticides, which supposedly far outweigh their disadvantages as pollutants. However, results of preliminary biochemical analyses revealed that larvae and pupae in plots treated with the chemical insecticide are in better physiological condition than those from untreated plots and in much better physiological condition than the residual populations in *B.t.*-treated plots. Average length and weight, level of calcium, total protein, and urea nitrogen from chemical insecticide-treated plots were higher than those from untreated plots and much higher than those from *B.t.*-treated plots.

¹ Nephelometric units: Index of chitinase activity determined by comparative measurements of turbidity with a standard solution in a nephelometer.

Furthermore, analyses of enzyme activities confirmed that residual populations of spruce budworm from plots treated with chemical insecticides had a metabolic activity superior to that of pupae from plots treated with *B.t.* As a result the use of chemical insecticides ensures a persistency of spruce budworm residual populations with a high index of vitality, and favours the creation of permanent spruce budworm epidemics which have been observed on territories repeatedly treated with chemical insecticides during the past 25 years or more in certain areas of eastern Canada.

B.t., therefore, is superior to chemical insecticides since it is very specific, more effective, and is not, in any way, an environmental pollutant.

Conclusions

Since the results of 1976 and 1977 applications proved *B.t.* + chitinase highly effective for spruce budworm control and foliage protection, no dilemma should exist. Because of their safety, efficiency, and non-polluting characteristics, *B.t.* + chitinase formulations appear to be the logical choice as the first and foremost weapon to be used in combating the spruce budworm.

References

- Aprille, J.R.** 1977. Reye's syndrome: Patient serum alters mitochondrial function and morphology in vitro. *Science* 197: 908-910.
- Crocker, J.F.S., R.L. Ozere, S.H. Safe, S.C. Digout, K.R. Rozee, and O. Hutzinger.** 1976. Lethal interaction of ubiquitous insecticide carriers with virus. *Science* 192: 1351-1353.
- Dorais, L.G. and Y.J. Hardy.** 1976. Méthode d'évaluation de la protection accordée au sapin baumier par les pulvérisations aériennes contre la tordeuse des bourgeons de l'épinette. *Can. J. For. Res.* 6: 86-92.
- Hardy, Y.** 1974. La tordeuse: La lutte qu'on y fait. *For. Con-*
- serv.* 40: 19-22.
- Smirnov, W.A.** 1963. Tests of *Bacillus thuringiensis* var. *thuringiensis* Berliner and *B. cereus* Frankland and Frankland on larvae of *Choristoneura fumiferana* (Clemens). *Can. Ent.* 95: 127-133.
- Smirnov, W.A.** 1971. Effect of chitinase on the action of *Bacillus thuringiensis*. *Can. Ent.* 103: 1829-1831.
- Smirnov, W.A. et J. Valéro.** 1972. Perturbations métaboliques chez *Choristoneura fumiferana* Clemens au cours de l'infection par *Bacillus thuringiensis* seul ou en présence de chitinase. *Can. J. Biol.* 38: 163-169.
- Smirnov, W.A., A.P. Randall, R. Martineau, W. Haliburton and A. Juneau.** 1973a. Field tests of the effectiveness of chitinase additive to *Bacillus thuringiensis* Berliner against *Choristoneura fumiferana* Clem. *Can. J. For. Res.* 3: 228-236.
- Smirnov, W.A., J.J. Fettes and R. Desaulniers.** 1973b. Aerial spraying of a *Bacillus thuringiensis* — chitinase formulation for the control of spruce budworm. *Can. Ent.* 105: 1535-1544.
- Smirnov, W.A., L.V. Larson, A. Juneau and J.R. Valéro.** 1974. Test with a highly concentrated low-volume formulation of *Bacillus thuringiensis* against the spruce budworm. *Bi-Mon. Res. Notes.* 30: 9.
- Smirnov, W.A.** 1976a. Metabolic disturbances in insect during viral and other infections. Proceedings of the first international colloquium on invertebrate pathology. Queen's University at Kingston, Canada.
- Smirnov, W.A.** 1976b. Confirmations expérimentales du potentiel du complexe *Bacillus thuringiensis* et chitinase pour la répression de la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). *Can. Ent.* 109: 351-358.
- Smirnov, W.A., R. Desaulniers, A. Juneau, J. Valéro et M. Pelletier.** 1976. Résultats de la dispersion aérienne d'une formulation concentrée de *Bacillus thuringiensis* avec addition de chitinase contre la tordeuse des bourgeons de l'épinette en 1974. *Ann. Soc. Ent. Québec.* 21: 26-34.

EDITOR'S NOTE:

This paper was written in 1977 and does not include certain recent developments. It deals with a somewhat controversial topic. The author and the editor are fully aware of this and feel that it is important that authors' views be set forth as part of an ongoing discussion.