# EFFICACY AND FIELD PERSISTENCE OF *BACILLUS THURINGIENSIS* AFTER GROUND APPLICATION TO BALSAM FIR AND WHITE SPRUCE IN WISCONSIN

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**Abstract**

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*Bacillus thuringiensis* was applied at three dosages (1.0 BIU/tree, 0.1 BIU/tree, and .01 BIU/tree) to balsam fir, *Abies balsamea* (L.) Mill., and white spruce, *Picea glauca* (Moench) Voss, with mist blowers. Crystalline proteins were detected on balsam fir foliage for a maximum of 16 days (d) after *B. thuringiensis* was applied at 1.0 BIU/tree. Higher levels of crystalline proteins were detected on white spruce foliage treated with Thuricide 16B than on that treated with Dipel 4L. On balsam fir, the situation was the opposite. Mist-blower-treated foliage collected for up to 16 d posttreatment caused mortality of spruce budworm, *Choristoneura fumiferana* (Clemens), larvae. Viable endospores of *B. thuringiensis* were recovered on white spruce foliage collected 1 year after treatment.

**Résumé**

*Bacillus thuringiensis* a été appliqué à 3 doses (1.0 BIU/arbre; 0.1 BIU/arbre et .01 BIU/arbre) sur le sapin, *Abies balsamea* (L.) Mill., et l'épinette blanche, *Picea glauca* (Moench) Voss, à l'aide de brumisateurs. Les cristaux protéiques ont été détectés sur le feuillage pendant 16 jours (d) au maximum après l'application de 1.0 BIU/arbre. Des niveaux plus élevés de cristaux protéiques ont été détectés sur le feuillage de l'épinette blanche traité au Thuricide 16B que sur celui traité au Dipel 4L. Sur le sapin baumier, la situation inverse s'est présentée. Du feuillage traité au brumisateur et prélevé jusqu'à 16 jours après le traitement a causé de la mortalité chez des larves de tordeuse, *Choristoneura fumiferana* (Clemens). Des endospores viables de *B. thuringiensis* ont été trouvées sur du feuillage d'épinette blanche prélevé 1 an après le traitement.

## Introduction

The entomopathogenic bacterium *Bacillus thuringiensis* Berliner has been used to suppress populations of spruce budworm, *Choristoneura fumiferana* (Clemens), although efficacy has been inconsistent and results often inconclusive (Harper 1974; Morris 1980, 1981). The Canada/United States Spruce Budworms Program (CANUSA) sponsored a field test in 1980 to compare the efficacy of two *B. thuringiensis* var. *kurstaki* formulations, Thuricide 16B and Dipel 4L, for spruce budworm in northern Wisconsin. Small fixed-wing aircraft equipped with 8004 Flat Fan Tee-jet nozzles were used to deliver a dosage of 20 billion international units (BIU)/ha at a rate of 9.35 L/ha in a single application (Reardon *et al.* 1982). In 1981, densities of spruce budworm larvae, levels of defoliation, and total field persistence of *B. thuringiensis* were monitored in 12 of the 15 plots established in 1980 (Reardon and Haissig 1983). Also, in 1981, three dosages of *B. thuringiensis* were applied by ground application to obtain baseline data on the field persistence of *B. thuringiensis* in northern Wisconsin. This paper reports a study of the field persistence of *B. thuringiensis* after application of Dipel 4L and Thuricide 16B to balsam fir and white spruce with mist blowers.

## Materials and Methods

**Population levels**. Three dosages (1.0 BIU/tree, 0.1 BIU/tree, and 0.01 BIU/tree, the equivalent per tree at 20 BIU/ha) of Dipel 4L and of Thuricide 16B were applied to 3–5 m tall balsam fir, *Abies balsamea* (L.) Mill., and white spruce, *Picea glauca* (Moench) Voss,

with mist blowers (KWH-model 75). Both untreated and treated (Dipel blank for Dipel 4L, and water for Thuricide 16B) check trees were included for each formulation and tree species. All sprays were applied the morning of 31 May 1981. There were six trees of each species per treatment for a total of 120 trees. Approximately 480 mL of tank mix was applied per tree for the 1.0 BIU and 0.1 BIU dosages and 48 mL for the equivalent-per-tree at 20 BIU/ha dosage. No sticker was added. Spruce budworm larvae and foliage were collected at 24–48 h pretreatment and at 6 h, 2, 4, 8, 16, and 24 d posttreatment. Living and dead/moribund larvae, and new vegetative shoots were counted on two 38-cm branch samples removed from the midcrown of each tree per sample time. The aggregate population estimate for the two branches was expressed as numbers of living and dead/moribund larvae/100 shoots/tree.

**Persistence of *B. thuringiensis*.** Three techniques were used to determine the field presence of *B. thuringiensis*: (1) an enzyme-linked immunosorbent assay (ELISA) technique to detect and quantitate parasporal crystalline proteins (Hammock 1980; Voller *et al.* 1980; Wie *et al.* 1982), (2) a culture technique to detect viable endospores (Pinnock *et al.* 1971), and (3) an insect bioassay technique to determine total field persistence, i.e., duration of pathogenicity in the field (Leong *et al.* 1980).

All three techniques used spruce budworm larvae or foliage, or both, collected at 24–48 h pretreatment and at 6 h, 2, 4, 8, 16 and 24 d posttreatment from a subsample of the sample trees.

*Elisa technique*: A maximum of 10 larvae were collected from each of three randomly selected trees per species per mist blower treatment. Larvae were separated by tree, sorted into living or dead/moribund categories, weighed, placed in vials, and frozen. A 10-g subsample of new foliage was removed from the larval branch samples, placed in plastic bags, and frozen. Also, a sample of each *B. thuringiensis* lot and mist blower tank mix was placed in a plastic container and frozen. Larvae, foliage, and lot and tank mix samples were shipped frozen to the University of California, Davis. A 2-g subsample of foliage was taken from each 10-g sample, washed in 10 mL of 0.2% $Na_2CO_3$ (w/v) with 0.02% sodium azide (pH 12), diluted with PBS-TWEEN (pH 7.4) 1:10 (200 ng foliage/mL), and 1:100, and the extracts were analyzed. Larvae were processed in a similar manner using 0.5 g of larvae per sample. The cross-reactivity of various purified toxins from different *B. thuringiensis* serotypes was also examined with *kurstaki* toxin as the coating antigen to determine if ELISA was specific to the *kurstaki* K-1 crystalline proteins.

*Culture technique*: A random subsample of new foliage collected from the same branch samples used for the ELISA was used in the culture technique: needle impression on Tryptic soy agar (GIBCO, Madison, Wisconsin). Four shoots collected from each branch were impressed on each agar plate, the plates stored at room temperature for 48 h, *Bacillus*-like colonies identified, and spore stains (Benz and Borusiewicz 1963) made of a random subsample of these colonies.

*Insect bioassay technique*: For each sample time, five additional shoots of new foliage were removed from the same two branch samples for the ELISA and culture techniques. The base of each shoot was embedded in agar in a glass tube, and the open end of the tube was covered with a metal cap. There were five tubes (1 shoot/tube) per treatment per time and three larvae were collected from untreated trees and placed in each tube. After 10 d, numbers of living and dead/moribund larvae were recorded. Dead/moribund larvae were examined for vegative rods, endospores, and crystals.

## Results and Discussion

**Population levels.** Pretreatment densities of living spruce budworm larvae per 100 shoots for trees treated with mist blowers were lower for balsam fir ($\bar{X} = 1.4 \pm 0.7$) than for white spruce ($\bar{X} = 12.6 \pm 1.0$). Populations of living larvae on balsam fir were too low

(<3 larvae/100 shoots); population reduction results are not presented. These low populations of larvae are often associated with balsam fir regeneration in northern Wisconsin. For white spruce, the uncorrected population reduction was 81% for the 1.0 BIU/tree dosage of Thuricide 16B and 64% for the same dosage of Dipel 4L (Table I). The 0.1 BIU/tree dosage reduced larval population as effectively as the 1.0 BIU/tree dosage for Thuricide 16B; although, for Dipel 4L the 0.1 BIU/tree dosage was ineffective when compared with the 1.0 BIU/tree dosage. The 0.1 BIU dosage of Dipel 4L did not reduce the larval population. The posttreatment (8-day) means of dead/moribund larvae per 100 shoots were higher than the pretreatment means for the 1.0 BIU/tree dosage of Dipel and the 1.0, 0.1, and 20 BIU/ha equivalent dosages of Thuricide.

**Persistence of *B. thuringiensis*.** *Elisa technique*: Preliminary laboratory studies demonstrated that ELISA sensitivity to *B. thuringiensis* was about 3 μg/g of needle tissue, because interfering substances in the needle tissue caused variable (5–30%) background inhibition. These inhibition levels did not consistently correlate with tree species, age of foliage, or optical density of the extract. *B. thuringiensis* crystalline proteins (antigen) were detected on balsam fir and white spruce foliage treated with 0.1 and 1.0 BIU/tree a maximum of 16 d posttreatment (Table II). On white spruce foliage, the ELISA detected much higher levels of antigen from the Thuricide 16B formulation than from the Dipel 4L formulation. The antigen levels for Dipel 4L sprayed at 1.0 BIU/tree were low and at 0.1 BIU/tree were not above background. The antigen levels in the Thuricide 16B formulation were high for both dosages.

Antigen levels decreased with time. On white spruce foliage, Dipel was not detected after 4 d; whereas, Thuricide was detected 8 d after spraying. For balsam fir, the situation

Table I. Population densities (larvae/100 shoots) and percent population reduction of spruce budworm larvae on white spruce before and 8 days after application of *Bacillus thuringiensis* with mist blowers, Wisconsin 1981

| Treatment (BIU/tree) | | White spruce | | | | |
|---|---|---|---|---|---|---|
| | | Pretreatment | | Posttreatment | | Reduction (%) |
| | | L[1] | D | L | D | |
| **Dipel 4L** | | | | | | |
| 1.0 | $\bar{X}$ | 13.9 | 1.5 | 5.0 | 7.7 | 64 |
| | $S\bar{x}$ | 3.9 | 0.6 | 2.1 | 4.4 | |
| 0.1 | $\bar{X}$ | 10.2 | 1.8 | 10.2 | 0.7 | 0 |
| | $S\bar{x}$ | 1.1 | 0.3 | 1.9 | 0.5 | |
| 20 BIU/ha equiv. | $\bar{X}$ | 19.3 | 2.7 | 17.4 | 1.0 | 10 |
| | $S\bar{x}$ | 6.5 | 1.2 | 3.7 | 0.6 | |
| Check | $\bar{X}$ | 11.5 | 1.0 | 14.5 | 0.6 | 0 |
| | $S\bar{x}$ | 3.8 | 0.2 | 2.7 | 0.4 | |
| Blank | $\bar{X}$ | 12.8 | 1.7 | 12.9 | 0.6 | 0 |
| | $S\bar{x}$ | 1.6 | 0.9 | 3.3 | 0.6 | |
| **Thuricide 16B** | | | | | | |
| 1.0 | $\bar{X}$ | 10.1 | 1.0 | 2.0 | 8.0 | 81 |
| | $S\bar{x}$ | 1.2 | 0.3 | 1.0 | 1.1 | |
| 0.1 | $\bar{X}$ | 15.7 | 1.5 | 3.8 | 4.4 | 76 |
| | $S\bar{x}$ | 3.3 | 0.6 | 1.6 | 1.6 | |
| 20 BIU/ha equiv. | $\bar{X}$ | 9.1 | 0.3 | 11.8 | 2.1 | 0 |
| | $S\bar{x}$ | 1.4 | 0.3 | 3.2 | 0.7 | |
| Check | $\bar{X}$ | 14.0 | 2.4 | 18.5 | 0.8 | 0 |
| | $S\bar{x}$ | 3.7 | 1.2 | 3.3 | 0.5 | |
| Water | $\bar{X}$ | 9.4 | 1.0 | 12.6 | 1.3 | 0 |
| | $S\bar{x}$ | 1.7 | 0.4 | 2.4 | 0.7 | |

[1]L, living; D, dead/moribund.

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Table II. Enzyme-Linked Immunosorbent Assay (ELISA) to detect *Bacillus thuringiensis kurstaki* parasporal crystalline proteins on balsam fir and white spruce foliage, Wisconsin, 1981

| Treatment (BIU/tree) | Intensity of reaction[1] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | White spruce | | | | | | Balsam fir | | | | | |
| | 6 h | 2 d | 4 d | 8 d | 16 d | 24 d | 6 h | 2 d | 4 d | 8 d | 16 d | 24 d |
| **Dipel 4L** | | | | | | | | | | | | |
| 1.0 | + | + | + | – | – | – | + + + | + + + | + + | + + | + | – |
| 0.1 | – | – | – | – | – | – | + + | + + | + | – | – | – |
| 20 BIU/ha equiv. | – | – | – | – | – | – | – | – | – | – | – | – |
| Blank | – | – | – | – | – | – | – | – | – | – | – | – |
| **Thuricide 16B** | | | | | | | | | | | | |
| 1.0 | + + + | + + + | + + | + + | – | – | + + | + + | + + | + | – | – |
| 0.1 | + + | + + | + + | + | – | – | + | + | + | – | – | – |
| 20 BIU/ha equiv. | – | – | – | – | – | – | – | – | – | – | – | – |

[1] +, positive lowest intensity reaction; + + +, highest intensity reaction; –, no reaction.

was reversed; antigen levels on trees sprayed with Dipel were higher and remained at detectable levels longer than on those sprayed with Thuricide. In this case, though, antigen levels in both formulations were fairly high at both the 0.1 and 1.0 BIU/tree dosages. Antigen from Dipel was detected for a maximum of 16 d, but antigen from Thuricide could not be detected after 8 d. Negative readings for the foliage collected from trees that received 20 BIU/ha equivalent per tree indicate the need to improve assay sensitivity.

Interfering substances were present in extracts of spruce budworm larvae. Only the larvae collected from white spruce were analyzed because less than 0.5 g of larvae were available for collection from balsam fir for each sample time. *B. thuringiensis* crystalline proteins were detected 6 h and 2 d posttreatment in larvae treated with Thuricide at 1.0 BIU/tree but not for those treated with Dipel at the same dosage.

The amount of *kurstaki* toxin in tank mixes prepared from Dipel lot no. 14-706CF and Thuricide lot no. 2WO162 were proportional to the amounts the formulations added to the samples. For example, for the Dipel tank mixes, toxin levels were 33 μg/mL at 1 BIU/tree, 2.2 μg/mL at 0.1 BIU/tree, and were not detectable at the equivalent per tree for 20 BIU/ha (Wie *et al.* in press).

The cross-reactivity of various purified toxins from different *B. thuringiensis* serotypes was highest (high inhibition reflects high concentration of toxin) for *kurstaki* crystal type K-1 and next to highest for crystal type K-73. When the toxicities of *B. thuringiensis* varieties were placed in order of pathogenicity for spruce budworm larvae (Yamvrias and Angus 1969), highly to moderately pathogenic varieties (*alesti, entomocidus,* and *galleriae*) and moderately pathogenic varieties (*kenyae* and *dendrolimus*) were comparable in inhibition; all were significantly greater in inhibition than the slightly pathogenic variety (*finitimus*). The only exception was *sotto*: it was identified as highly to moderately pathogenic but produces a very small crystal and was comparable to *finitimus* in inhibition. The intensity of reaction reflects the concentration of crystalline proteins although the ELISA appears to detect crystalline proteins common to several *B. thuringiensis* serotypes. This cross-reactivity would not be a problem in an area sprayed with *B. thuringiensis* as one could assume the reactivity was due to the *B. thuringiensis* that was sprayed.

*Culture technique*: Several *Bacillus*-like colonies were cultured from the needle impressions. There appeared to be two species of *Bacillus* based on morphological characteristics of endospores and presence and location of crystals. The developing colonies identified as *B. thuringiensis* by spore stain were verified as *B. thuringiensis* serotype 3a, 3b-*kurstaki* by Donaldson (SEA, Brownsville), and as a strain of *B. thuringiensis* in which occasional cells fail to produce a parasporal body (Thomas, Univ. of California, Berkeley). Viable *B. thuringiensis* endospores were recovered from all branch samples receiving

Table III. Results of bioassay using foliage collected from trees treated with *B. thuringiensis* (*B.t.*), Wisconsin 1981

| Treatment (BIU/tree) | Incidence (%) and fate of spruce budworm larvae | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Dead/moribund | | | | Dead/moribund | | |
| | | *B.t.* | Parasites | Other | | *B.t.* | Parasites | Other |
| | Living | White spruce | | | Living | Balsam fir | | |
| **Thuricide 16B** | | | | | | | | |
| 1.0 | 7 | 48 | 2 | 43 | 35 | 39 | 3 | 23 |
| 0.1 | 33 | 31 | 5 | 31 | 15 | 37 | 7 | 40 |
| 20 BIU/ha equiv. | 67 | 19 | 4 | 11 | 72 | 9 | 14 | 5 |
| Water | 80 | 0 | 16 | 4 | 74 | 0 | 15 | 12 |
| Check | 79 | 0 | 13 | 8 | 76 | 0 | 12 | 12 |
| **Dipel 4L** | | | | | | | | |
| 1.0 | 51 | 25 | 6 | 18 | 0 | 68 | 2 | 30 |
| 0.1 | 71 | 4 | 14 | 11 | 19 | 43 | 6 | 32 |
| 20 BIU/ha equiv. | 80 | 2 | 11 | 7 | 46 | 43 | 7 | 5 |
| Blank | 81 | 0 | 4 | 15 | 73 | 0 | 9 | 18 |
| Check | 84 | 0 | 14 | 2 | 77 | 0 | 7 | 16 |

Thuricide or Dipel treatments. On 26 June 1982, approximately 1-year posttreatment, branch samples were collected from the same white spruce and needle impressions made on Tryptic soy agar. Viable *B. thuringiensis* endospores were recovered from white spruce that received either Dipel or Thuricide treatment in 1981.

*Insect bioassay technique*: The foliage remained in excellent condition as evidenced by larval feeding, and mortality in checks was minimal. Fates of spruce budworm larvae collected from balsam fir and white spruce treated with mist blowers were determined (Table III). The incidences of dead/moribund larvae attributable to *B. thuringiensis* were higher on balsam fir than on white spruce. For white spruce, the 1.0 BIU/tree dosage of Thuricide had the highest incidence of *B. thuringiensis*; and comparable incidences for the 0.1 BIU/tree and 20 BIU/ha equivalent dosages of Thuricide and 1.0 BIU/tree dosage of Dipel. For balsam fir, the 1.0 BIU/tree dosage of Dipel 4L had the highest incidence of *B. thuringiensis*; incidences were similar for the 0.1 BIU/tree and 20 BIU/ha equivalent dosages of Dipel and 1.0 BIU/tree and 0.1 BIU/tree for Thuricide. Spruce budworm larvae that fed on white spruce and balsam fir foliage collected pretreatment did not show any evidence of *B. thuringiensis* mortality whereas larvae from all posttreatment sample times up to a maximum of 16 d showed evidence of *B. thuringiensis* mortality.

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