# *Bacillus thuringiensis* subsp. *kurstaki* Aerial Spray Prescriptions for Balsam Fir Stand Protection Against Spruce Budworm (Lepidoptera: Tortricidae)

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**ABSTRACT** Although commercial formulations of *Bacillus thuringiensis* subsp. *kurstaki* (Btk) are being widely used in forest protection against lepidopteran defoliators, optimal application prescriptions have often yet to be worked out in detail. We conducted field experiments over a 6-yr period (1996–2001) in southwestern Québec to determine application prescriptions for optimal protection of balsam fir, *Abies balsamea* (L.), healthy stands against the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). At moderate larval densities (<30 larvae per 45-cm branch tip), similar foliage protection was achieved with one or two Btk applications of 30 billion international units per hectare (BIU/ha). When larval densities exceeded 30 larvae per branch tip, two successive applications of 30 BIU/ha significantly increased foliage protection. Whether the second application took place 5 or 10 d after the first spray did not affect treatment efficacy. Increasing the application dosage from 30 to 50 BIU/ha did not lead to better foliage protection against high larval densities, but the current standard dosage of 30 BIU/ha saved more foliage than 15 BIU/ha against moderate populations. The recommended dosage of 30 BIU can be applied in lower application volumes (1.5 liters/ha) by using a high-potency product (20 BIU/liter), because we did not observe a reduction in efficacy compared with the application of a lower potency product (12.7 BIU/liter) in 2.37 liters/ha. We also demonstrated that Btk can be applied much earlier in the season without compromising spray efficacy: there was no difference in treatment efficacy of double applications at 30 BIU/ha when the first spray was timed for early third, peak third, or early fourth instars.

**KEY WORDS** *Choristoneura fumiferana*, *Bacillus thuringiensis*, *Abies balsamea*, forest protection, aerial spraying prescriptions

SPRUCE BUDWORM, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), is the most prominent insect defoliator of coniferous forests in eastern North America (Blais 1983, Sanders 1991). Outbreaks of this native insect occur periodically and can last several decades, covering extensive areas; in 1975, ≈55 million ha was infested in Canada alone (Blais 1985, Kettela 1995). Epidemics lead to high coniferous tree volume growth loss and mortality, especially of balsam fir, *Abies balsamea* (L.), and white spruce, *Picea glauca* (Moench) Miller (Blais 1985, MacLean 1985). Boulet (2001) reported tree mortality related losses of 180 million $m^3$ of timber during the last spruce budworm outbreak (1967–1992) in the province of Québec.

During the last spruce budworm epidemic, large-scale aerial spraying programs were conducted in Canada and the northeastern United States to protect forest resources (Dorais et al. 1995, Kettela 1995). However, extensive use of chemical pesticides in forests was so severely questioned by the public that alternative control options were needed (Carrow 1983, Cunningham 1985). During the 1970s, *Bacillus thuringiensis* subsp. *kurstaki* (Btk, isolate HD-1), with its high specificity toward larval Lepidoptera, was tested against the spruce budworm (Carrow 1983, Dorais et al. 1995). Laboratory research and field trials were undertaken in Canada and the United States to improve *Btk* formulations and its application (van Frankenhuyzen 1993, 1995). Higher potency formulations containing 12.7 billion international units/liter (BIU/liter) became available in the early 1980s (Morris 1984). Those formulations could be applied undiluted in low volumes (2.4 liters/ha), which increased efficacy and reduced treatment costs (van Frankenhuyzen 1995). In 1985, the provincial governments of Québec and Ontario approved Btk formulations for operational use in forestry and in 1987 the Québec Ministry of Energy and Resources decided to entirely replace chemical insecticides with this biological insecticide (SOPFIM and SNC-Lavallin 1992, Dorais et al. 1995). Despite operational use for almost a decade

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after that, several issues regarding optimal prescriptions for the application of Btk in forest protection have persisted, as summarized below.

**Dosage Application Rate.** Various treatment prescriptions were tested during the 1980s in response to continued improvements of formulations (Carrow 1983, van Frankenhuyzen 1995, Dorais et al. 1995). In the early 1980s, the recommended dosage rate of 20 BIU/ha in 4.7 liters/ha (Morris 1980) was changed to 30 BIU/ha in 2.4 liters/ha after higher potency products became available and field trials showed increased efficacy at budworm population densities >30 larvae per branch (45-cm tip) (Morris 1984, Morris et al. 1984). However, that application rate was still a compromise between product potency available, the minimum emitted volume considered necessary to obtain adequate spray coverage, and the need to minimize application costs (van Frankenhuyzen 1995). Empirical data on the effectiveness of various application rates above and below the recommended dosage rate are lacking, particularly in relation to budworm larval density.

**High Potency.** Highly concentrated Btk formulations containing 20 BIU/liter became available in the mid-1990s. This permitted application of the recommended dosage rate (30 BIU/ha) in 1.5 liters/ha to further increase spray plane productivity. The use of such low spray volumes requires maximum atomization of the formulation to generate enough droplets for adequate coverage of the target foliage (van Frankenhuyzen et al. 2000a). Foliar deposits and efficacy resulting from such low application volumes need to be validated against the "standard" application rate of 30 BIU in 2.4 liters/ha.

**Single versus Double Applications.** Single applications of 30 BIU in 2.4 liters/ha did not achieve efficient foliage protection when larval densities exceeded 20 larvae per branch in operational sprays in 1987 and 1988 (Carter 1988, Auger and Therrien 1993a). Two sequential applications of 30 BIU/ha improved foliage protection (Auger and Therrien 1993b), but treatment efficacy remained inconsistent (Auger and Therrien 1993c, van Frankenhuyzen 1995). Double applications were spaced 3 d apart, and we postulated that the interval was too short to allow for maximum dose acquisition after the second spray. It is well known that ingestion of a sublethal Btk dose results in temporary cessation of larval feeding, which may last hours to days, depending on the dose ingested (Fast and Régnière 1984, Bauce et al. 2002, Moreau and Bauce 2003) and ambient temperature (van Frankenhuyzen and Nystrom 1987). A second application following too quickly on the first one will not increase treatment efficacy if most larvae that survived the first application are still feeding inhibited. Larvae that recover from sublethal dose ingestion can reinfect themselves, but this is a function of Btk persistence on the foliage (Fast and Dimond 1984). Thus, the efficacy of the second application is likely to depend on the interval between the two sprays. The optimum interval needs to be determined.

**Timing of the First Application.** Spray application for foliage protection is typically timed according to bud development of the host tree. The standard protocol is to apply the first Btk spray when buds are starting to flare to optimize spray droplet deposition and subsequent ingestion (Carrow 1983, Dorais et al. 1995). This usually coincides with the peak fourth instar in larval development. Waiting this late in larval development limits the window for spray application and increases the risk of substantial feeding damage to the foliage before spray application (Blais 1979). Laboratory experiments (Massé et al. 2000) suggested that the first spray can be applied much earlier in larval development (peak third instar) without compromising treatment efficacy, despite that the larvae are feeding mostly inside the swollen buds. This required testing under field conditions.

Between 1996 and 2001, a series of field experiments was conducted in Québec to address these issues with the goal of defining optimal application prescriptions to protect balsam fir healthy stands against spruce budworm feeding damage. The influence of dosage application rate (15, 30, and 50 BIU in 2.37 liters/ha), product concentration (12.7 and 20 BIU/liter, both applied at 30 BIU/ha), and number of applications (single and double, using 15, 30, or 50 BIU/ha) were studied at various initial larval densities (moderate and high). We also compared the efficacy of double applications (30 BIU/ha) spaced 5 or 10 d apart as operationally attractive intervals and investigated differences in efficacy resulting from timing the first spray application on three different stages in larval development (third, mid-third, and fourth instar).

## Materials and Methods

**Objectives.** The objectives varied from year to year, as summarized in Table 1. Efficacy of one versus two applications of 30 BIU/ha was tested at high larval densities (>30 larvae per branch) in 1996 and 1999 and at moderate larval densities (<30 larvae per branch) in 1997 and 1998. The impact of the interval between two successive applications was studied in 1996. Efficacies of high dosage (50 BIU/ha) and low dosage (15 BIU/ha) were compared with that of the current standard dosage (30 BIU/ha) in 1996 and 1997, respectively. Commercial formulations with moderate (Foray 48B) and high potency (Foray 76B) were compared in 1998. From 1996 to 1999, all first applications were conducted when most balsam fir buds were flaring and shoot elongation was initiated (stage 4; see below). The impact of timing on treatment efficacy was examined in 2001, by using three application timings based on insect development: when most spruce budworm larvae had reached third, mid-third, or fourth stadium.

**Site Description.** Experimental sites were located in the valley of the Ottawa River, Québec, Canada (45° 38′–46° 01′ N; 75° 33′–76° 33′ W; 165 m above sea level). Forests in that region are mixed wood stands, where spruce budworm populations have been at epidemic levels since 1992. Experimental plots averaged

**Table 1. Summary of the field trials in the Outaouais region from 1996 to 2001**

| Year | Objectives | Initial larval density[a] | Treatment[b] | No. plots/ treatment | Product (potency) | Volume/ application (liters/ha) | Aircraft type (atomizer spinning speed in rpm) |
|---|---|---|---|---|---|---|---|
| 1996 | | High | Control | 5 | — | — | — |
| | - One vs. two applications | High | 1 × 50 | 5 | Foray 76B | 2.37 | Pawnee PA-25 (6800) |
| | - High dosage | High | 2 × 50 (5 days) | 4 | (20 BIU/l) | | |
| | - Interval between two | High | 2 × 30 (5 days) | 5 | Foray 48B | 2.37 | |
| | applications | High | 2 × 30 (10 days) | 5 | (12.7 BIU/l) | | |
| 1997 | | Moderate | Control | 6 | — | — | — |
| | - One vs. two applications | Moderate | 1 × 15 | 6 | Foray 48B[c] | 2.37 | Dromader M-18 (6500) |
| | - Low dosage | Moderate | 2 × 15 (5 days) | 6 | (6.35 BIU/l) | | |
| | | Moderate | 1 × 30 | 6 | Foray 48B | 2.37 | |
| | | Moderate | 2 × 30 (5 days) | 6 | (12.7 BIU/l) | | |
| 1998 | - One vs. two applications | Moderate | Control | 4 | — | — | — |
| | | Moderate | 1 × 30 | 4 | Foray 48B | 2.37 | Pawnee PA-25 or |
| | | Moderate | 2 × 30 (5 days) | 4 | (12.7 BIU/l) | | Dromader M-18 (7000) |
| | - Intermediate vs. high | Moderate | 1 × 30 | 4 | Foray 76B | 1.5 | |
| | potency | Moderate | 2 × 30 (5 days) | 4 | (20 BIU/L) | | |
| 1999 | | High | Control | 4 | — | — | — |
| | - One vs. two applications | High | 1 × 30 | 4 | Foray 48B | 2.37 | Pawnee PA-25 |
| | | High | 2 × 30 (9 days) | 4 | (12.7 BIU/l) | | Dromader M-18 (6500) |
| 2001 | | High | Control (IDI, 3) | 4 | — | — | — |
| | | High | Control (IDI, 3.5) | 4 | — | — | — |
| | | High | Control (IDI, 4) | 4 | — | — | — |
| 2001 | - Timing of first spray | High | 2 × 30 (7 days) (IDI, 3) | 4 | | | |
| | application based on | High | 2 × 30 (7 days) (IDI, 3.5) | 4 | Foray 76B | 1.5 | EurocopterAs350D |
| | IDI[d] | High | 2 × 30 (7 days) (IDI, 4) | 4 | (20 BIU/l) | | (7500) |

[a] High initial larval density corresponded to ≥30 spruce budworm larvae per 45-cm branch tip and moderate initial larval density corresponded to ≤30 spruce budworm larvae per 45-cm branch tip.
[b] Application rate in billion international units per hectare; interval between first and second application in parentheses
[c] Foray 48B was diluted with water (1:1) to halve its potency.
[d] IDI, insect development index for spruce budworm larvae; peak third instar, 3; mid-third stadium, 3.5; and fourth instar, 4.

25 ha in area and were selected according to the following criteria: high second instar spruce budworm populations in the previous fall, >30% of the basal area in balsam fir, 30- to 50-yr-old fir stands, moderate previous defoliation, and easy access. A different number of plots was selected each year, depending on the annual objectives, as summarized in Table 1. Each treatment, including an untreated control, was replicated four to six times, depending on the year. In each plot, 16 codominant balsam fir trees were randomly selected along lines running perpendicular to the anticipated aircraft flight direction for sampling of spray deposits and assessment of efficacy (see below).

**Formulations and Spray Application.** Two commercial formulations of Btk strain HD-1, Foray 48B, and Foray 76B (produced by Abbott Laboratories, Chicago, IL, on behalf of Valent Bio-Sciences Corporation, Libertyville, IL), with respective nominal potencies of 12.7 and 20.0 BIU/liter, were used during the 5 yr (Table 1). The products were applied undiluted (neat), except in the 1997 tests involving 15 BIU/ha, when a two-fold dilution (with water) of Foray 48B was used to maintain the same application volume as in the 30 BIU/ha treatment (Table 1). Three types of aircraft were used. The Piper-Pawnee PA-25, a Dromader M-18, a Eurocopter As-350 D were flown at 161, 193, and 145 km/h with a 30-, 50-, and 30-m lane separation, respectively. Four (Eurocopter), six (Pawnee), or eight (Dromedar) Micronair AU-5000 rotary atomizers were located on a round boom within 75% of the total wingspan. The flow rates through the atomizer nozzles were calibrated to deliver 1.5 (Foray 76B) or 2.37 liters/ha (Foray 48B). Spray application occurred early in the morning or at dusk under calm and dry weather conditions (wind speed <6 km/h and no rain). Spray applications commenced in mid-May in 1998 and mid- to late May in all other years and were always completed before the mid-June. All missions were carried out under the supervision of an observer flying above the spraying aircraft. Aircraft were equipped with differential global positioning system navigation systems. Key meteorological parameters were monitored on the ground using sensors fitted on a 10-m tower and were transmitted by radio to the guiding aircraft. Before loading the aircraft, food-grade dye (FD & C Blue No. 1, Sensient Food Colors, Kingston, Ontario, Canada) was added to the formulations at 0.3% concentration to aid visual detection of droplets on foliage.

**Evaluation of Treatment Efficacy and Spray Deposition.** In each year, a 45-cm branch tip was collected from the mid-crown of each sample tree (16 trees per plot) 24 h before the first application (day 0). The phenological stage of the buds was described according to the scheme of Auger (cited in Dorais and Kettela 1982, Juneau 1989): stage 1, no apparent bud development (bud in winter condition); stage 2, buds swollen, with 10–35% of the needles visible; stage 3, bud break (all needles visible, but not flaring); stage 4, needles flushed and flaring, and shoot elongation

initiated; and stage 5, shoots are supple and undergoing elongation. On each branch, the number of buds in each stage was recorded and a bud development index (BDI) was calculated as BDI = {Σ (no. buds × class index)}/total number of buds. Branches collected on day 0 also were used for estimating initial larval densities (total number of larvae per 45-cm branch tip) and an insect development index (IDI). Larvae were removed from the bud and quantified by instar (2, instar 2; 3, instar 3; 4, instar 4; 5, instar 5; 6, instar 6; 7, pupa; and 8, adult) to calculate an IDI according to Dorais and Kettela (1982) as IDI = {Σ (no. larvae × class index)}/total number of larvae.

To document mortality associated with one or two successive Btk applications, a 45-cm branch tip was collected from the mid-crown of each sample tree (16 trees per plot) at various intervals after the first application: 5, 10, and 20 d in 1996, 5 and 15 d in 1997, 10 d in 1998, 9 d in 1999, and 8 and 25 d in 2001. In each year, the last postspray sample was taken when 85% of the larvae had reached the pupal stage (pupal stage day). The numbers of living spruce budworm larvae were recorded per branch to estimate larval densities (total number of larvae per 45-cm branch tip). Rates of mortality due to the Btk application corresponded to reductions in larval population densities between day 0,and each of the other collection dates and were calculated as follows: mortality (%) on day X = (day 0 larval density − day × larval density) × 100/day 0 larval density.

Defoliation of current-year growth was estimated by the method of Fettes (1950) (cited in Dorais and Hardy 1976, Sanders 1980) on day 0 (except in 1996 and 1997 when we had to use day 5 samples) and pupal stage day. Defoliation also was assessed on day 10 and 20 in 1996, on day 15 in 1997, on day 10 in 1998, on day 9 in 1999, and on days 8 and 25 in 2001.

To evaluate the quality of spray deposited in a given plot, one mid-crown branch was collected from each sample tree (16 trees per plot) ≈1 h after the first application in most treatments. Two clusters of three buds were removed at random from each 45-cm tip. Two buds from each cluster were used for droplet counting, whereas the third bud was weighed. Droplets were counted under a Wild-Leitz microscope under 12× magnification (van Frankenhuyzen et al. 2000a). Mean numbers of droplets per gram of fresh foliage were calculated per tree and then averaged among trees per plot. A digital length measuring unit (Wild, Heerbrug, Switzerland) was used to measure droplet diameter for a subsample of at least 150 droplets per treatment.

The relationship between spray deposit and larval mortality was examined in foliar bioassays during the 1997 trials that compared efficacy of Foray 48B when applied undiluted (30 BIU/ha) and diluted (15 BIU/ha). Foliage was collected in sprayed plots ($n = 2$ plots for 15 BIU/ha and $n = 4$ plots for 30 BIU/ha) by removing 10 additional bud clusters from each branch ($n = 16$ trees) that was used for assessing spray deposition, as described above. The foliage was transported in plastic bags to the field laboratory where all resident budworm larvae were removed. A total of 20 buds from each branch were placed in a clear plastic cup (Styroware D8) with a damp piece of filter paper to maintain high humidity. Each cup received twenty 1- or-2-d-old fifth instars from the rearing colony maintained at the Great Lakes Forestry Centre (Sault Ste. Marie, Ontario, Canada). The cups were held at 25°C for 5 d. Treatment-induced mortality per cup was then assessed by visually examining dead larvae for signs of Btk infection (cadavers black and soft). Mortality in parallel assays with unsprayed foliage was <1% ($n = 155$).

**Correction of Initial Larval Density Estimates.** Larval densities on any given tree can be lower on day 0 than 5 to 10 d later. This discrepancy can occur because spruce budworm larvae tend to migrate toward the branch tips until they reach the fifth larval stage (Régnière et al. 1989). We have observed that current-year defoliation shows a better correlation with larval densities on day 5 or 10 than on day 0 (unpublished data). Initial larval density and treatment-induced mortality might be underestimated if larval migration is not taken into account. In the control plots, this was done by simply exchanging day 0 densities with the density observed on day 5, if the former was lower. In the Btk-treated plots, larval densities on day 5 are usually lower than on day 0 as a result of Btk-induced mortality. Day 0 densities were corrected by using the mean increase in larval density between day 0 and day 5 as observed in the control plots, which was calculated as follows: mean factor = (mean larval density on day 5 in plot 1)/(mean larval density on day 0 in plot 1) + (mean larval density on day 5 in plot 2)/(mean larval density on day 0 in plot 2) + etc.)/(number of control plots). Day 0 densities in the Btk-treated plots were multiplied by this factor. Over the 5 yr of trials, the correction ranged from 1.1 to 2.7.

**Statistical Analyses.** Data on treatment efficacy were analyzed on a year-by-year basis because numbers of treatments and plots per treatment varied from year to year (Table 1). Plots were the replicates in the experiment, whereas trees within plots (16 trees per plot) were treated as subsamples. In the field trials conducted in 1996, 1997, 1998, and 1999, initial larval density (day 0) data were subjected to an analysis of variance (ANOVA) in a completely randomized design with plots as replicates (PROC GLM, SAS Institute 1988). Defoliation data were submitted to an analysis of covariance (ANCOVA) with initial larval density (day 0) used as covariate in a completely randomized design when the covariate showed a statistically significant effect. Otherwise, data were submitted to the previously described ANOVA model. Initial larval density and defoliation data were normally distributed, with homogeneous variances. The LSMEANS statement (SAS Institute 1988) computed least-squares means and multiple comparisons (least significant difference [LSD]) and in some cases, contrast tests were performed on data. Larval mortality observed on day 5, day 10, etc., were analyzed using a generalized linear model for rate data, termed a Poisson regression-type followed by two-by-two con-

**Table 2. Summaries of the statistical analyses on data observed during the field trials of 1996, 1997, 1998 and 1999 df, *F* values, and *P* values**

| Year | Parameter/sampling day | df | *F* value | *P* value |
|---|---|---|---|---|
| 1996 | Mortality (%)[a] | | | |
| | Day 5 | 4,19 | 24.24 | 0.0001 |
| | Day 10 | 4,19 | 32.61 | 0.0001 |
| | Day 20 | 4,19 | 35.73 | 0.0001 |
| | Pupal stage day | 4,19 | 20.22 | 0.0001 |
| | Defoliation (%)[b] | | | |
| | Day 5 | 4,18 | 1.61 | 0.22 |
| | Day 10 | 4,18 | 5.03 | 0.0067 |
| | Day 20 | 4,18 | 17.8 | 0.0001 |
| | Pupal stage day | 4,18 | 10.45 | 0.0001 |
| 1997 | Mortality (%)[a] | | | |
| | Day 5 | 4,24 | 23.52 | 0.0001 |
| | Day 15 | 4,24 | 4.24 | 0.0098 |
| | Pupal stage day | 4,24 | 12.45 | 0.0001 |
| | Defoliation (%)[b] | | | |
| | Day 5 | 4,23 | 4.57 | 0.0073 |
| | Day 15 | 4,23 | 7.39 | 0.0006 |
| | Pupal stage day | 4,23 | 3.81 | 0.049 |
| 1998 | Mortality (%)[a] | | | |
| | Day 10 | 4,14 | 7.67 | 0.0017 |
| | Pupal stage day | 4,14 | 5.63 | 0.0065 |
| | Defoliation (%)[b,c] | | | |
| | Day 0 | 4,15 | 0.5 | 0.74 |
| | Day 10 | 4,14 | 8.23 | 0.0029 |
| | Pupal stage day | 4,15 | 3.43 | 0.035 |
| 1999 | Mortality (%)[a] | | | |
| | Day 9 | 2,9 | 17.72 | 0.0008 |
| | Pupal stage day | 2,9 | 2.91 | 0.11 |
| | Defoliation (%)[b,c] | | | |
| | Day 0 | 2,9 | 0.37 | 0.7 |
| | Day 9 | 2,9 | 3.85 | 0.062 |
| | Pupal stage day | 2,8 | 21.84 | 0.0006 |

[a] Mortality (%) data were analyzed in a generalized linear model for rate data (Poisson regression-type).

[b] Defoliation (%) data were analyzed using a covariance analysis with initial larval density as covariate in a completely randomized design.

[c] Defoliation (%) data were analyzed using an analysis of variance in a completely randomized design (day 0 and pupal stage day in 1998; day 0 and day 9 in 1999).

trasts to compare differences between treatments (Poisson log-linear model) (Agresti 1996; PROC GENMOD, SAS Institute 1997). In 2001, initial larval density data were subjected to ANOVA in a 2 by 2 factorial design (Btk treatment and timing) with plots as replicates; mortality and defoliation data were submitted to ANCOVA in the same design with initial larval density used as the covariate. Larval density, mortality, and defoliation data had normal distributions and homogeneous variances. The LSMEANS statement (SAS Institute 1988), performed for each effect and interaction, computed least-squares means and multiple comparisons (LSD), with Bonferroni adjustment for the *P* value and confidence limits for the differences of least squares-means (Bonferroni method of pairwise comparisons where the adjusted *P* value corresponds to the traditional *P* value of 0.05 divided by the number of pairwise comparisons; SAS Institute 1988). Mortality data from the foliage bioassays were subjected to probit analysis (LeOra software 1987), by using droplet density as dose.

## Results

In years of high-density populations (1996, 1999), initial larval densities averaged 35 and 63 larvae per 45-cm branch tip, respectively, whereas moderate larval densities in 1997 and 1998 averaged 21 and 28 larvae per branch, respectively. Treatment means and differences between treatments in larval mortality and defoliation are shown in Figs. 1–4. Summaries of the statistical analyses of data observed from 1996 to 1999 are presented in Table 2.

Increasing the dosage application rate at high larval densities from 30 to 50 BIU/ha (by using a higher potency product while maintaining the same application volume) did not significantly improve treatment efficacy (Fig. 1). There were no significant differences in larval mortality or defoliation between double applications (5 d apart) of either dosage rate (Fig. 1). However, reducing the dosage from 30 to 15 BIU/ha in a single application against moderate larval densities resulted in reduced mortality and increased defoliation (Fig. 2). Two applications at the lower dosage rate produced similar mortality and defoliation as a single or double application at 30 BIU/ha (Fig. 2). Overall, the lower dosage rate presented lower efficacy than the current dosage (Posteriori contrasts between 15 and 30 BIU/ha: $F_{1,24} = 4.32$; $P = 0.0485$ for mortality on day 15; $F_{1,23} = 4.31$; $P = 0.049$ for defoliation on

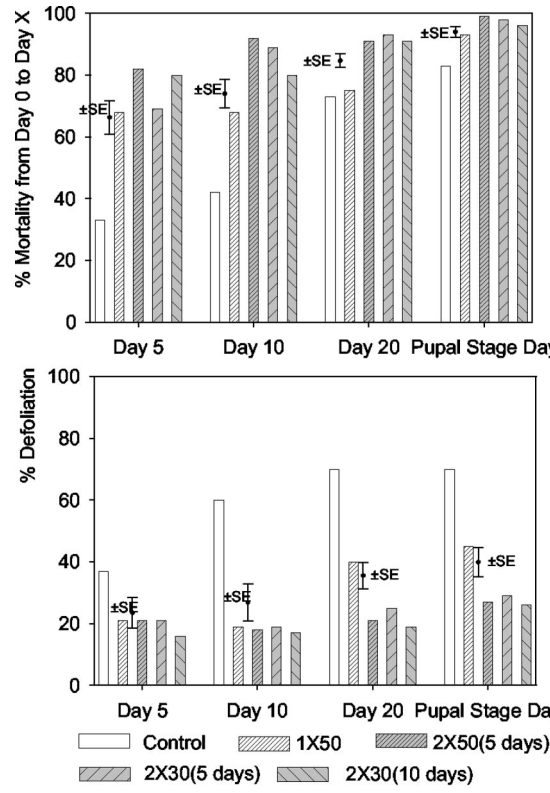


**Fig. 1.** Mortality and defoliation at different sampling dates in 1996 according to different Btk treatments. Initial larval densities were high.

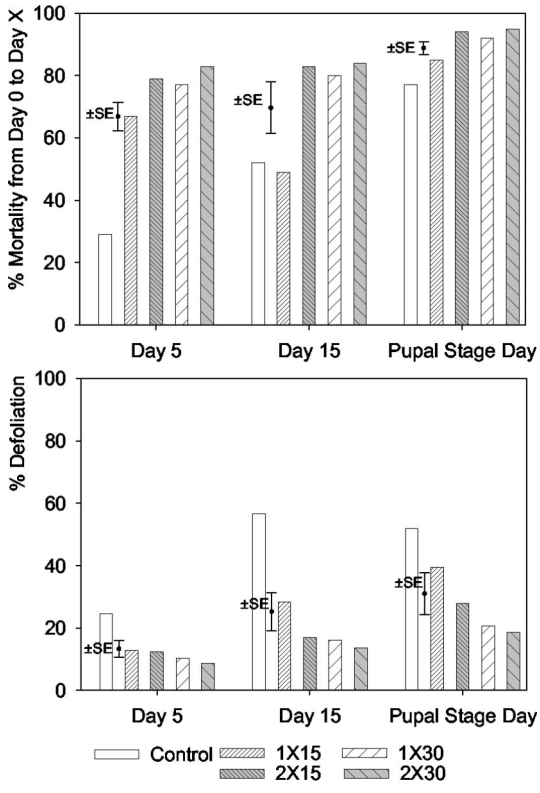


**Fig. 2.** Mortality and defoliation at different sampling dates in 1997 according to different Btk treatments. Initial larval densities were moderate.

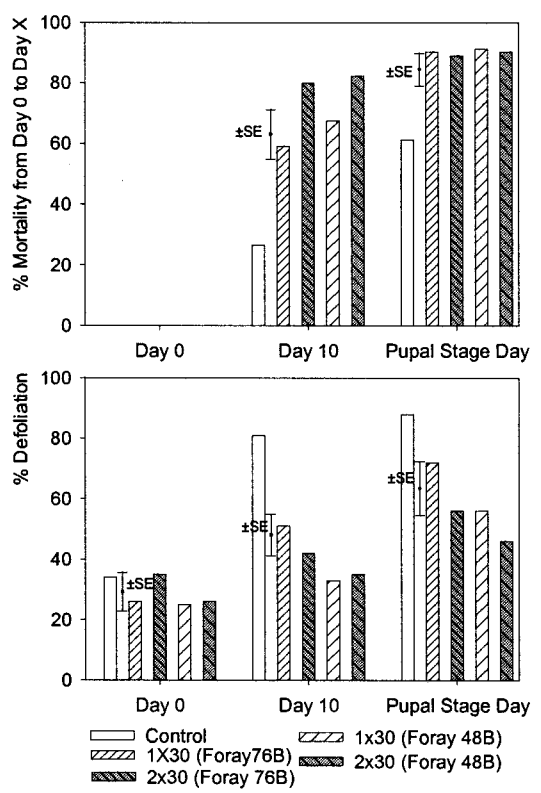


**Fig. 3.** Mortality and defoliation at different sampling dates in 1998 according to different Btk treatments. Initial larval densities were moderate.

pupal stage day). Dilution of Foray 48B from 12.7 BIU/liter (30 BIU/ha) to 6.3 BIU/liter (15 BIU/ha) altered neither the density nor the size spectrum of droplets on the foliage when applied at the same volume rate as the neat product (2.37 liter/ha) (Table 3). Data from the foliar bioassays, however, indicate that dilution reduced the effectiveness of the spray deposits (Fig. 5). On foliage from plots treated with diluted product, 30 (95% CL, 17–87) droplets per bud was required to produce 50% larval mortality in the bioassay compared with 12 (95% CL, 6–24) droplets per bud in plots sprayed with undiluted product.

The high-potency Foray 76B formulation containing 20 BIU/liter applied in 1.5 liter/ha was as efficacious in terms of larval mortality and foliage protection as Foray 48B (12.7 BIU/liter) sprayed at 2.37 liter/ha, at least against moderate larval densities (Fig. 3; posteriori contrasts between Foray 48B and Foray 76B: $F_{1,14} = 0.45$, $P = 0.52$ for mortality on day 10; $F_{1,15} = 2.26$, $P = 0.15$ for defoliation on pupal stage day). The two formulations produced similar and narrow deposited droplet size spectra on foliage (Table 3, 1998).

Similar efficacy was achieved with one or two Btk applications of 30 BIU/ha at moderate budworm larval densities (<30 larvae per branch) (Figs. 2 and 3), whereas treatment efficacy was better achieved with two successive applications of 30 BIU/ha than one spray at high larval densities (>30 larvae per branch)

**Table 3. Droplet densities (mean ± SEM) and droplet diameters observed by microscopic examination of buds collected from the sprayed plots 1 h after spray application (first application only)**

| Year | Product | Treatment | No. Droplets/g foliage (no. of plots) | Median droplet diameter ($\mu$m) |
|---|---|---|---|---|
| 1997 | Foray 48B | 15 BIU/ha | 48.4 ± 7.5 (3) | 51 |
| | Foray 48B | 30 BIU/ha | 44.3 ± 5.2 (6) | 43 |
| 1998 | Foray 48B | 30 BIU/ha | 42.5 ± 8.1 (4) | 54 |
| | Foray 76B | 30 BIU/ha | 22.8 ± 9.1 (4) | 48 |
| 1999 | Foray 48B | 30 BIU/ha | 39.1 ± 6.5 (4) | 45 |
| 2001 | Foray 76B | 30 BIU/ha (IDI, 3.0) | 35.0 ± 9.9 (3) | — |
| | Foray 76B | 30 BIU/ha (IDI, 3.5) | 34.0 ± 9.9 (3) | — |
| | Foray 76B | 30 BIU/ha (IDI, 4.0) | 29.0 ± 9.9 (3) | — |

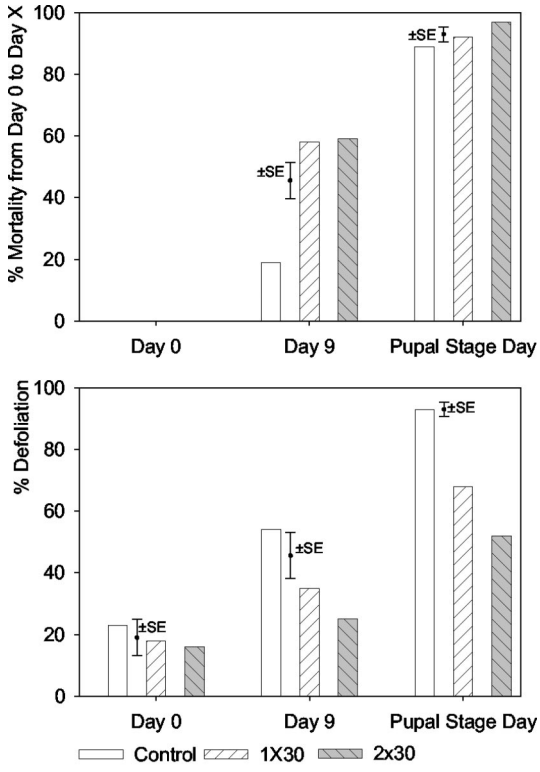


**Fig. 4.** Mortality and defoliation at different sampling dates in 1999 according to different Btk treatments. Initial larval densities were high. Notice that sampling on day 9 was done before the second application.

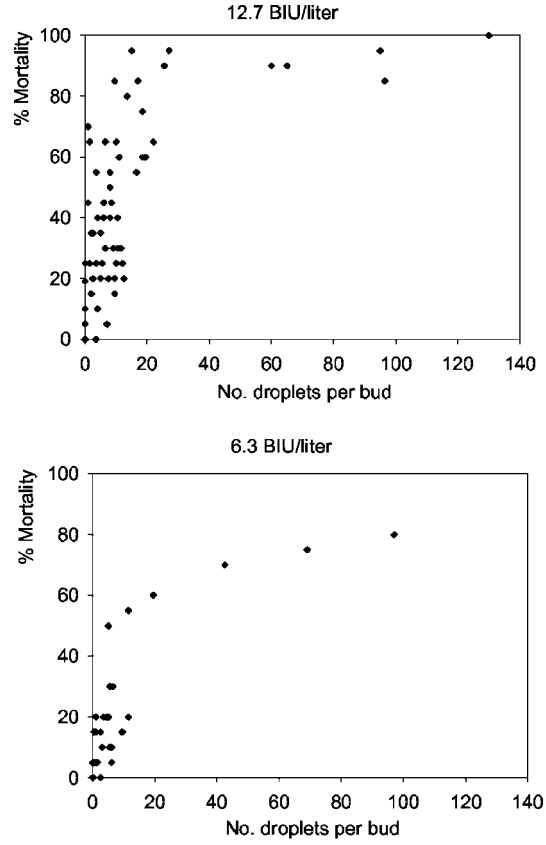


**Fig. 5.** Relationship between spray deposit (number of droplets per bud, 0.25-g average weight) and fifth instar mortality observed in a 5-d bioassay of foliage collected 1 h after application of undiluted (12.7 BIU/liter) and diluted (6.3 BIU/liter) Foray 48B in 2.37 liters/ha.

(Figs. 1 and 4). This is consistent with the results of previous field experiments conducted by Auger and Therrien (1993b): two applications of 30 BIU/ha provided better foliage protection at densities of 35 larvae per branch. At moderate larval densities there were no significant differences in defoliation at the end of the season (pupal stage day) between plots treated with one or two applications of 30 BIU/ha (Figs. 2 and 3). The effect of the second application on larval mortality was variable. At moderate population densities, larval mortality observed 10 or 15 d after the first spray application tended to be higher in the plots that had received two applications (Figs. 2 and 3), but observed differences were not statistically significant. At high larval densities, larval mortality increased significantly after the second application (Fig. 1).

Increasing the interval between two successive applications from 5 to 10 d did not increase treatment efficacy (Fig. 1). There were no significant differences in either larval mortality or defoliation between plots that were sprayed 5 d apart and plots sprayed 10 d apart. Both treatment regimes increased efficacy relative to a single application, at least at high larval densities (see previous section). Even though delaying the second spray to 10 d after the first did not enhance efficacy, it is equally important from an operational perspective that the delay did not compromise spray efficacy.

Timing the first of the two applications for early third instars (actual IDI, 3.0), peak third instar, (actual IDI, 3.4), or early fourth instars (actual IDI, 4.1) did not significantly affect final defoliation or larval mortality (Table 4). On average, treatment with Btk increased mortality to 93% among the three timing treatments from 79% in the corresponding controls, and reduced defoliation from 82% in the controls to 38%. Because initial larval density (day 0) was two times higher in the IDI = 4 treatment than in the two earlier treatments, larval density was used as a covariate in subsequent analyses (Table 4; Fig. 6). Timing of the first spray did not significantly affect larval mortality on day 8 and pupal stage day but influenced larval mortality on day 25. Prespray defoliation was lowest in the earliest treatment, and defoliation was still much lower 8 d after application, but the difference between treatments had disappeared by the day 25 after the first application (Tables 4 and 5).

## Discussion

Five years of experimental spray applications allowed us to more clearly define Btk application pre-

**Table 4. Summaries of the analyses of variance and covariance in a 2 by 2 factorial design (Btk treatment and timing) on data observed during the field trials of 2001 (df, *P* values for *F* tests and the mean square error used for the F-tests [in italics])**

| Sampling day | Larval density | Mortality (%) | | | | Defoliation (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 8 | Day 25 | Pupal stage day | Day 0 | Day 8 | Day 25 | Pupal stage day |
| Sources of variation | | | | | | | | | |
| Larval density on day 0 (covariate) | 1 (0)[a] | — | <0.0001 | <0.0001 | <0.0001 | <0.0099 | 0.018 | 0.0051 | <0.0008 |
| Timing | 2 | 0.032 | 0.89 | 0.0418 | 0.19 | 0.0023 | 0.037 | 0.08 | 0.36 |
| Btk treatment | 1 | 0.15 | 0.0001 | 0.0001 | 0.0004 | 0.10 | 0.043 | 0.0001 | 0.0001 |
| Timing × Btk treatment | 2 | 0.041 | 0.09 | 0.055 | 0.57 | 0.66 | 0.63 | 0.48 | 0.20 |
| Mean square error | 16 (17)[a] | 591 | 93 | 90 | 50 | 43 | 208 | 204 | 210 |

Numbers in bold indicate statistically significant effects at $P < 0.05$. When interactions are significant, the corresponding simple effects are not in bold type.

[a] Degrees of freedom of sources of variation of univariate variance analyses are in parentheses.

scriptions that are likely to 1) improve treatment efficacy for protection of spruce budworm-infested balsam fir stands, or 2) improve the logistics and reduce the cost of large-scale spray programs by using two successive applications.

In the early 1980s, operational trials in Canada and the United States established that application of 30 BIU/ha was more effective than the usual application rate of 20 BIU/ha, especially at high larval population density (Carrow 1983, Morris 1984, van Frankenhuyzen 1995). Although commercial formulations have improved since then, our results suggest that the standard application rate of 30 BIU/ha is still the most effective. We did not obtain better efficacy by increasing the rate to 50 BIU/ha against high larval densities, whereas at moderate densities two applications of 15 BIU/ha were required to achieve the same level of foliage protection as one application of 30 BIU. Our results also confirm earlier operational experience (Auger and Therrien 1993b) that two successive applications are more efficacious than a single application under high population pressure (>30 larvae per branch). Moreover, our data suggest that the window for intervention could be enlarged up to 10 d between applications without loosing efficacy, which is very important for logistical organization of spraying operations.

The development in the late 1980s of higher potency products for undiluted use was a key factor in facilitating large-scale use of Btk in forestry (van Frankenhuyzen 1995). Our results indicate that high-potency formulations such as Foray 76B should allow a further reduction of application costs without loss of efficacy by permitting the use of more economical spray volumes. We did not observe a reduction in treatment efficacy when 30 BIU was applied in 1.5 rather than 2.37 liters/ha.

Optimization of Btk spray application involves a trade-off between an optimal number of droplets on the target foliage (exposure) and the dose in those droplets (droplet size and potency). For example, in the trials with Foray 76B we observed equivalent efficacy as in the Foray 48B treatments but at only half the density of similar-sized droplets. Higher potency of the product reduced the droplet density required. A similar effect of product potency can be seen in the results of the foliar bioassay: dilution of the product resulted in the need for higher spray coverage to maintain a given level of larval mortality (Fig. 5). Further optimization studies with high-potency products are needed to determine the best trade-off between application volume, droplet size, and product potency.

Another important finding of our study is that spray application can begin well before balsam fir buds are flaring. Conventional wisdom has it that bud flare is essential for spray droplet impaction and subsequent dose transfer. Bud flare usually coincides with the fourth instar (IDI, 4.0–4.5) in larval development. Although we know that earlier instars are susceptible

**Table 5. Mortality and defoliation (mean ± SEM) at different sampling dates in 2001 according to Btk treatments timed on three IDI**

| Parameters | Sampling day | Timing | | | Btk treatment | |
|---|---|---|---|---|---|---|
| | | IDI, 3 | IDI, 3.5 | IDI, 4 | Control | 2 × 30 |
| Mortality (%) | Day 8 | 40 ± 4a | 40 ± 4a | 42 ± 4a | 21 ± 6b | 60 ± 6a |
| | Day 25 | 59 ± 4a | 66 ± 4a | 74 ± 4a | 54 ± 3b | 79 ± 3a |
| | Pupal stage day | 86 ± 3a | 91 ± 3a | 83 ± 3a | 79 ± 2b | 93 ± 2a |
| Defoliation (%) | Day 0 | 7 ± 3b | 18 ± 3a | 23 ± 3a | 19 ± 4a | 13 ± 4a |
| | Day 8 | 17 ± 6b | 37 ± 6a | 31 ± 6ab | 35 ± 4a | 21 ± 5b |
| | Day 25 | 43 ± 6a | 59 ± 6a | 59 ± 6a | 76 ± 5a | 31 ± 5b |
| | Pupal stage day | 57 ± 6a | 67 ± 5a | 57 ± 5a | 82 ± 5a | 38 ± 5b |

Means within a line followed by the same letter were not significantly different; multiple comparison test of least significant difference, $P < 0.05$. Means are indicated only when the factor effect was significant

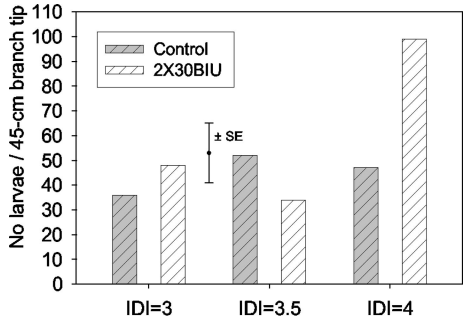


**Fig. 6.** Prespray larval density (No larvae per 45-cm branch tip) in control and plots treated with two applications of 30 BIU/ha (8 d apart) at three IDI.

(Massé et al. 2000), their habit to feed inside the swelling but closed buds makes direct exposure to spray deposits before bud break unlikely. However, larval mortality due to the first Btk spray applied to closed buds was as high as that observed on more fully opened buds, suggesting that third instars are able to acquire an efficacious dose. Two successive applications of 30 BIU were equally efficacious whether the first application coincided with early third instar, peak third instar, or early fourth instar. Our replicated field trial confirms earlier results from laboratory studies under carefully controlled conditions (Massé et al. 2000) and from a previously reported preliminary and nonreplicated field trial (Kettela and Steel 1990). Our data clearly refute the notion that bud flare is essential for spray impaction: we did not observe a difference in spray deposits between flared buds (BDI, 4.5) in the fourth instar treatment and swelling buds (BDI, 2.1) in the early third instar treatment. Elucidating the exact mechanism of dose transfer from external surfaces to larvae feeding inside the buds will require detailed studies of early instar feeding behavior. We suspect that third instars spend a considerable amount of time outside the buds, because one or more buds are often spun together into one feeding shelter. Spray droplet impaction on silk webbing and dose transfer through grooming activities, as observed by Nigam (1987), is also a possible mechanism.

The expected benefit of increasing foliage protection by earlier timing of spray application did not materialize. Despite significantly lower defoliation prespray and before the second spray, defoliation in the third instar treatment plots increased more than in plots treated at the two later timings. Because larval mortality was similar among treatments, we attribute this phenomenon to greater foliage consumption resulting from better recovery in the early treatment. Bauce et al. (2002) showed that fourth instar spruce budworm larvae, which survived Btk ingestion, consumed amounts of food and achieved pupal masses comparable with those of control larvae. When treated at the beginning of the sixth instar, larvae did not recover from Btk injury; they ingested lower amounts of food and presented lower pupal masses than control larvae (Bauce et al. 2002). Sixth instars account for 87% of the food consumed during the spruce budworm larval phase (Miller 1977), and thus most of the insect damage on foliage was incurred during this stadium. In the present field experiment, sixth instars of spruce budworm larvae that survived Btk treatments applied at earlier larval stages probably caused greater foliage damage than larvae treated later in their development, because of their total Btk recovery and their compensatory feeding behavior. Because early treated larvae recovered more fully from Btk ingestion than late-treated larvae and tended to consume much more foliage (Bauce et al. 2002), further studies should be conducted to compare the efficacy of single Btk applications timed for peak third instars and peak fourth to fifth instars in relation to initial larval density.

The projected goal of balsam fir foliage protection varies from 50% in Québec (Auger and Therrien 1993c) to 60% in New Brunswick (Carter 1989, Kettela and Steel 1990), but defoliation must remain <20% to avoid growth losses (MacLean 1985). In the present context of wood resource depletion, a new spruce budworm epidemic could have so huge economical impact that forest managers could decide to have a more intensive foliage protection and these results constitute useful guidelines for improvements in forest protection measures with Btk.

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