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Carry over effects of the entomopathogen *Bacillus thuringiensis* ssp. Kurstaki on *Choristoneura fumiferana* (Lepidoptera: Tortricidae) progeny under various stressful environmental conditions

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Abstract

1 In the present study, we documented the lethal and sublethal effects of the entomopathogen *Btk* on spruce budworm and its progeny under various environmental conditions. We hypothesized that aerial spray of *Bacillus thuringiensis* ssp. *kurstaki* (*Btk*) could affect the biological performance of the surviving spruce budworm (*Choristoneura fumiferana* Clem.) populations and their progenies and that *Btk* sublethal effects could be widened by other types of stress (i.e. temperature conditions and changes in food suitability from year to year).

2 The results from a 3-year field experiment indicated that *Btk* treatments decreased the fitness of the surviving larvae whatever the prevailing temperature and nutritional conditions.

3 The detrimental *Btk* effects on the parental generation carried over to the offspring. The percent of egg hatch and first-instar survival were negatively affected by *Btk* whatever other stress spruce budworm parents underwent.

4 The present study also highlighted the fact that the effects of temperature and nutritional stress suffered by the parents could carry over to the next generation. Balsam fir flowering, which provided larvae with pollen rich in nitrogen, favoured both the parental generation and the fitness of their offspring. Spruce budworm mothers allocated to their progenies large amounts of energy reserves (triglycerides and glycogen) that greatly enhanced the survival of the early stages.

5 Egg hatch and the survival of first-instar larval progeny were drastically affected when their parents had reduced larval growth as a result of exposure to cool temperatures that had desynchronized insect and bud phenology.

6 Budworm mothers submitted to negative impacts of previous defoliation allocated low amounts of energy reserves to their progeny. This lack of energy associated with unfavourable temperature conditions (i.e. high temperatures in late summer and in early fall and an extended cool period in spring) drastically reduced survival of diapausing second-instar larvae.

7 These results highlight the importance of considering the various sources of stress when attempting to evaluate the impact of a control agent on an insect pest population and its progenies.

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Introduction

The spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae) is the most destructive insect defoliator of mixed boreal forest stands in eastern North America (Blais, 1983; Sanders, 1991). The insect feeds primarily on the three spruce tree species [*Picea glauca* (Moench) Voss, *P. mariana* (Mill.) and *P. rubens* Sarg], and balsam fir, *Abies balsamea* (L.) Mill, which is the most vulnerable host species to *C. fumiferana* (MacLean, 1980). Spruce budworm adult moths emerge in July and lay egg masses, each containing approximately 50 eggs, on host tree needles. The first-instar larva builds a hibernaculum in bark cracks or in old staminate flower scars, moults to second-instar and overwinters until early spring. Because first-instar and diapausing second-instar larvae do not feed, their survival is strongly dependent upon the amount of energy reserves provided by their parents (Carisey & Bauce, 2002). Second-instar larvae emerge from diapause 2–3 weeks prior to vegetative budbreak and mine old foliage. At budbreak, larvae feed on current-year foliage and undergo four additional larval stadia before turning into pupae in early July. Balsam fir trees die from budworm defoliation after 3–4 years of severe defoliations (>65%), whereas white spruce and red spruce trees die after 4–5 years of severe defoliation. Black spruce appears to be more resistant to budworm defoliation than the other host tree species although budworm-related mortality and growth reduction have been reported in certain regions on this tree species (Hix *et al*., 1987).

From the mid-1980s omwards, protection of coniferous stands against spruce budworm has relied on aerial sprays of commercial formulations of *Bacillus thuringiensis* ssp. *kurstaki* (*Btk*) (van Frankenhuyzen, 1995). This gram-positive soil bacterium produces a proteinaceous crystal that is specifically toxic against Lepidoptera (Beegle & Yamamoto, 1992; Dent, 1993). The larva that ingests *Btk* droplets stops feeding and then dies within a few hours or days, or recovers and resumes feeding, depending on the *Btk* dose ingested and its vulnerability (Retnakaran *et al*., 1983; Fast & Régnière, 1984; van Frankenhuyzen & Nystrom, 1987). The most frequently reported *Btk* sublethal effect is an increase in development time of surviving larvae both under laboratory (Fast & Régnière, 1984; Ramachandran *et al*., 1993; Erb *et al*., 2001) and field conditions (Morris, 1976; Dubois *et al*., 1988; Régnière & Cooke, 1998). The results reported in the literature are contradictory regarding *Btk* sublethal effects on pupal weight and fecundity. Some studies mention no impact of sublethal exposure to *Btk* for *C. fumiferana* (Fast & Régnière, 1984; Pedersen *et al*., 1997) and *Lymantria dispar* (Lepidoptera: Lymantriidae) (Dubois *et al*., 1988), whereas others report negative impacts for several insect species such as spruce budworm (Smirnoff, 1974; Morris, 1976; Smirnoff, 1983; van Frankenhuyzen & Nystrom, 1987; Pedersen *et al*., 1997), *Agrostis ypsilon* Hufnagel (Lepidoptera: Noctuidae) (Salama & Sharaby, 1988) and *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) (Dulmage & Martinez, 1973). Bauce *et al*. (2002) showed that *Btk* ingestion reduced pupal weight when spruce budworm larvae were contaminated during their late larval development (sixth instar), but not when they ingested a *Btk* sublethal dose earlier in larval development (fourth instar) and had sufficient time to recover. The carry-over effects of *Btk* sublethal exposure of the parental generation to the offspring have only been studied partially. Except for a study by Oatman & Legner (1964) reporting an increase in overwintering mortality of *Spilonota ocellana* Denis & Schiffermüller (Lepidoptera: Tortricidae), most studies have dealt with the effects of parental exposure to *Btk* on egg hatch. Ali & Watson (1982) for *H. virescens* and Dubois *et al*. (1988) for *L. dispar* reported that egg hatch was not negatively affected by *Btk* exposure of the parents. However, Carrière *et al*. (2001) showed that *Perctinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) moths that had eaten *Bt* toxin (Cry1Ac) as larvae had reduced embryo development and egg eclosion. Egg hatch was also negatively affected when *A. ypsilon* larvae were fed on a diet containing *Btk* product (Hafez *et al*., 1993).

According to Benz (1987) and Hare (1992), the vulnerability of insect herbivores to entomopathogens depends on host plant suitability, and many factors were reported to affect the availability and suitability of balsam fir trees for spruce budworm larvae. For example, balsam fir flowering provides larvae with pollen that is rich in nitrogen and amino acids that increase larval growth rate (Carisey & Bauce, 1997a). High larval densities force larvae to feed on 1-year-old foliage of poor nutritional quality when the current-year foliage is depleted, which increases larval development time and decreases pupal weight (Carisey & Bauce, 1997b). Bauce & Hardy (1988) reported that, after a year of severe defoliation, balsam fir trees produce foliage of low nutritional quality, which negatively affects spruce budworm biological performance. Under laboratory conditions, Carisey & Bauce (2002) showed that *C. fumiferana* progeny fitness was influenced by parental nutrition; the parental generation, reared on food depleted in nitrogen, gave rise to progeny with a reduced survival of the early stages (eggs and first instar) compared with well-fed parents. The effect of parental nutrition and other stresses impacting on the parental generation with respect to the fitness of offspring is of major interest in the prediction of spruce budworm population densities, particularly because spruce budworm larvae do not feed from egg hatch in mid-summer to diapause emergence in the next spring. Spruce budworm overwintering survival relies on the nutritional reserves provided by the female parent (Harvey, 1985; Han & Bauce, 2000; Carisey & Bauce, 2002) and

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probably on defensive agents, toxins, hormones and enzymes that all depend on the parental genetic inheritance and the environmental experience of the parents (Mousseau & Dingle, 1991; Rossiter, 1996; Carisey & Bauce, 2002).

Therefore, we hypothesized that ingestion of *Btk* sublethal doses by the parental generation could affect not only the performance of the parents, but also their offspring. Moreover, we hypothesized that these effects could depend on other stresses experienced by the parental generation (temperature conditions and nutritional stress through changes in balsam fir suitability) and by their progeny during their early stages of development (temperatures in late-summer, fall and early spring).

Materials and methods

To test our hypotheses, a 3-year experiment (1997–99) was undertaken in the Ottawa River valley (Quebec) where a spruce budworm outbreak has occurred from 1992 onwards. After *Btk* aerial sprays [Foray 48B: strain HD-1 (Valent Bio-Sciences Corporation, Abbott Laboratories, Abbott Park, Illinois) at nominal potency of 12.7 BIU/L], spruce budworm pupae were collected each year and various biological parameters were measured regarding the parental generation (pupal weight, fecundity, development time) and the offspring [egg hatch, first- and second-instar mortality, energy reserves (glycogen, triglyceride and glucose) and cryoprotectant (glycerol)]; these traits were analysed according to year and *Btk* treatment (0, 1 or 2 *Btk* applications at 30 BIU/ha). Living conditions (spring temperatures, larval density, previous defoliation and balsam fir flowering) of the parental generation and the progeny (temperatures from late-summer to early spring) were reported and compared with the biological performance observed each year. This approach allowed us to document the lethal and sublethal effects of the entomopathogen *Btk* on spruce budworm and its progeny under various levels of stressful environmental conditions.

Site description

The experimental site is located in the Outaouais region, Quebec, Canada (45°38′, 46°01′N; 75°33′, 76°33′W; 165 m asl). In this region, forests comprise mixed-wood stands and spruce budworm populations have been at epidemic levels after 1992. Plots were selected according to the following criteria: high second-instar spruce budworm populations in autumn (>30 larvae per 75-cm long branches), more than 30% of the basal area in balsam fir, 30–50-year-old fir stands, and easy terrestrial access. On average, plots measured 25 ha. In 1998, balsam fir trees presented an abundant production of staminate flowers.

Experimental design

The experiment was replicated over three years (i.e. in 1997, 1998 and 1999). Numbers of *Btk* applications [i.e. 0, 1 or 2 applications of 30 billion International Units per hectare (BIU/ha)] and their sublethal effects on the spruce budworm population were systematically studied for each of the three years. In 1997 and 1999, four plots per treatment were selected whereas, in 1998, three plots per treatment were used. Different plots were used each year. The first *Btk* applications were carried out when most balsam fir buds had reached the fourth phenological stage (needles flaring and start of shoot elongation) [Auger's classification in Dorais & Kettela (1982) and Juneau (1989)]. The first applications were carried out by SOPFIM (Société de Protection des Forêts contre les Insectes et Maladies) between 29 May and 2 June 1997, between 13 and 15 May 1998 and between 20 and 21 May 1999. The second applications were sprayed 5 days later in 1997 and 1998, and 9 days later in 1999. The second *Btk* treatments, sprayed 5 days after the first ones were reported to be as efficient as those sprayed 10 days later (Bauce *et al*., 2004).

One day before the first *Btk* spray and when 85% of the larvae had reached the pupal stage, a mid-crown 45-cm branch tip was collected on 12 balsam fir trees per plot to evaluate: (i) spruce budworm initial larval density and defoliation of the previous year and (ii) current-year defoliation at the end of the spruce budworm feeding. Previous and current-year defoliations were estimated by the Fettes method (Fettes, 1950; Dorais & Hardy, 1976; Sanders, 1980).

Temperature conditions

Each year, from 1 April to 30 June, daily minimum and maximum air temperatures were recorded with an automatic weather station installed according to the World Meteorological Organization standards for agro-meteorological observations in forested areas (Turner & Lawson, 1978) in La Pêche, Québec, Canada (45°37N, 76°01′W; 206 m asl), the nearest meteorological station to the experimental plots.

Each year, from 1 August to 30 April, daily minimum and maximum air temperatures were recorded at the Québec airport nearby the outdoor insectarium where insects overwintered (46°47N, 71°18′W).

Btk formulation applications

Foray 48B, a *Btk* strain HD-1 commercial formulation (Valent Bio-Sciences Corporation, Abbott Laboratories) at nominal potency of 12.7 BIU/L was used during each of the 3 years of field experiments. The aircraft used, a Dromader M-18, was equipped with eight atomizers (Micronair AU-5000 Micronair, NJ). Micronair atomizers were located on a round boom within 75% of the total wingspan. The Dromader was flown at 193 km/h with a 50 m lane separation. The flow rates through the nozzles were calibrated to deliver 2.37 L/ha. As documented by Bauce *et al*. (2004) spray deposit (number of droplets/g foliage, median droplet diameter) was similar between years and between treated plots.

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Collection of spruce budworm pupae

When 50% of the spruce budworm population had reached the pupal stage of developement, pupae were collected from balsam fir trees, located along lines perpendicular to the flight direction of the aircraft. Depending on the *Btk* treatment, approximately 200 or less pupae per plot were harvested between 22 June and 7 July 1997, 30 May and 20 June 1998 and 10–19 June 1999. In the field laboratory, pupae were weighed using a Sartorius LA120S (Sartorius AG, Goettingen, Germany) balance [0.1 mg reproducibility (SD)], placed in a cool box at 10°C and then, sent to the laboratory of forest entomology at Laval University (Québec) to arrive 12 h later.

Parental generation

On arrival at the laboratory, spruce budworm pupae were placed immediately in a rearing room at 20°C, 65% relative humidity (RH) and under an LD 16 : 8 h photoperiod. Adult emergence was monitored every morning; the date of emergence was recorded (Julian day) and moths were sexed. One female and one male were placed in a clear plastic vial (9.5 cm high × 4.5 cm in diameter), covered at the top by a piece of cheesecloth and at the bottom by a plastic cap. Moths could drink from a cotton ball that had been saturated with distilled water placed inside the vial. Newly-emerged female moths were matched with 48- h-old male moths. Vials were maintained at the same previously described rearing conditions. Forty to 80 moth couples were prepared per plot. Percent fertility was calculated as: (number of couples that produced a minimum of one live larva/the total number of couples) × 100.

Offspring

Throughout the oviposition period, the eggs laid by each female were collected every 2 days using a fine brush. Eggs were incubated at 23°C, 65% RH and under an LD 16 : 8 h photoperiod for 1 week and were enclosed in clear plastic boxes (4 × 2.5 × 1.5 cm) whose lids were lined with cheesecloth that could be used by first-instar larvae for building hibernacula. One week after oviposition, larvae hatched, and spent 1 week at 23°C and two weeks at 18°C. This moderate temperature enabled first-instar larvae to moult to the second larval stage and prepare for diapause (Han & Bauce, 2000). To evaluate percent egg hatching and realized fecundity (the number of eggs laid by an individual female), unhatched eggs, live and dead first- and second-instar larvae were counted 4 weeks after oviposition. After the count, unhatched eggs and dead larvae were discarded from the plastic box and the cheesecloth containing live larvae was transferred into a 30-mL clear plastic cup.

Larvae (>50 larvae) from 10 to 40 spruce budworm mothers were randomly chosen per plot to study overwintering survival. The cups, each containing larvae from one mother, were placed in an outdoor insectary near Laval University (46°47N, 71°18W) and exposed to natural temperatures. At the end of April, cups were placed in a growth chamber at 18°C, 65% RH and under an LD 16 : 8 h photoperiod. This temperature allowed living postdiapause second-instar larvae to leave the hibernacula that they had built in the cheesecloth. Mobile second-instar larvae and dead second-instar larvae (i.e. larvae that had not exited their hibernacula after 5 weeks at 18°C) were counted. Some first-instar larvae were found dead in the hibernacula, indicating that these larvae had not succeeded in moulting to the second instar and had probably died when transferred to the outdoor insectary; these larvae were also counted. Using criteria described by Han *et al*. (2000), first- and second-instar larvae were distinguished based on the presence/absence of green faecal pellets and first-instar head capsules in hibernacula, and differences in head capsule morphology and size. Finally, first- and second-instar mortality was recorded from time of hatch to the end of diapause.

Fifty second-instar larvae from each of 10–15 spruce budworm mothers were randomly chosen per plot and frozen to determine their content in energy reserves (glycogen, triglycerides and glucose) and cryoprotectant (glycerol) (50 larvae per sample). Glycogen, glucose, triglycerides and glycerol were quantified according to methods described by Han & Bauce (1993).

Statistical analysis

Data on initial larval density, 1-year-old and current-year defoliations were submitted to an analysis of variance in a 2 × 2 factorial design (*Btk* treatment and year). Plots and the 12 sampled trees per plot were used as replications and subsample units, respectively (PROC GLM; SAS Institute Inc., 1988). Data had normal distributions and homogeneous variances. The LSMEANS statement (SAS Institute Inc., 1988), performed for each effect and interaction, calculated least-squares means and multiple comparisons (least significant difference). Bonferroni adjustment for the *t*-value and confidence limits for the differences of least squares-means were calculated for multiple comparisons (SAS Institute Inc., 1988).

Male and female spruce budworm pupae and offspring of each moth couple were used as subsample units in each plot, each of which was considered as a replicate. Data on pupal weights, realized fecundity, adult emergence (Julian day), energy reserve and cryoprotectant contents were subjected to an analysis of variance in a 2 × 2 factorial design (*Btk* treatment and year) (PROC GLM; SAS Institute Inc., 1988). Data had normal distribution and homogeneous variances. Bonferroni adjustment for the *t*-value and confidence limits for the differences of least squares-means were calculated for multiple comparisons. Percent fertility, percent of egg hatch, and first- and second-instar mortality were analysed in a 2 × 2 factorial design (*Btk* treatment and year) using a generalized linear model for rate data, termed a Poisson regression-type, followed by 2 × 2 contrasts to compare differences between treatments [Poisson log-linear model; Agresti, (1996); PROC GENMOD; SAS Institute Inc. (1997)].

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Mean daily temperatures were calculated. To compare temperatures between years at La Pêche station, mean temperature data per period of 15 days were subjected to an analysis of variance in a completely randomized design (PROC GLM; SAS Institute Inc., 1988) with year as factor. Maximum and mean temperature data per period of 15 days, from the Québec station, were submitted to the same statistical analysis.

Results

Parental generation

Field conditions. Initial spruce budworm larval density in 1997 and 1998 was moderate (23 ± 7 larvae/45-cm branch tip; mean ± 2SE) and lower than that observed in 1999 (49 ± 7 larvae/45-cm branch tip; mean ± 2SE) ($F_{2,24} = 19.7$; $P = 0.0001$). Larval densities were similar between *Btk* treatments ($F_{2,24} = 1.23$; $P = 0.31$), indicating that plots were well-randomized within *Btk* treatments.

Defoliation of the 1-year-old foliage, in plots selected in 1997 and 1998, was 43% on average, and was significantly lower than that measured in 1999 (81%) ($F_{2,24} = 11.55$; $P = 0.0003$). Defoliation of the 1-year old foliage was similar among *Btk* treatments ($F_{2,24} = 2.91$; $P = 0.074$), indicating that plots were well-randomized within *Btk* treatments.

Current-year foliage defoliation, in plots selected in 1998 and 1999, was 70% on average, and was 112% higher than that measured in 1997 (33%) ($F_{2,24} = 17.52$; $P = 0.0001$). Two *Btk* sprays achieved better foliage protection than one *Btk* spray (37 and 56% current-year foliage defoliation, respectively) ($F_{2,24} = 15.84$; $P = 0.0001$). On average, defoliation in control plots (79%) was significantly higher than that in plots treated with one or two *Btk* applications ($F_{2,24} = 15.84$; $P = 0.0001$).

In the Outaouais region, spring temperatures from April to mid-June were similar in 1998 and 1999, except for the last 2 weeks of April when mean temperatures in 1998 were significantly warmer (2.5°C) than those in 1999 (Table 1). Early spring temperatures in 1997 were colder than those in 1998 and 1999 (Table 1). The first 15 days of April in 1997 were 5–6°C colder than those in 1998 and 1999. Temperatures of the second half of April in 1997 were similar as those prevailing in 1999. Mean temperatures in 1997 were 8–10°C, and 3–4°C under those of 1998–99, for the first 15 days and the last 15 days of May, respectively (Table 1). Temperature conditions in June were similar in 1997, 1998 and 1999.

Table 2 summaries all the field conditions prevailing each year during the parental generation development and indicates the levels of temperature and nutritional stress.

Spruce budworm moth emergence. *Btk* applications delayed both male and female development but their impacts depended on the current-year conditions (Table 3). Each year, two applications of 30 BIU/ha significantly delayed insect development from 8 to 14 days compared with the control (Fig. 1). One application of 30 BIU/h a significantly delayed insect development compared with the control in 1997 and 1998, but not 1999. In 1998 and 1999, emergence of adult moth collected in *Btk*-treated plots occurred at the same time in the season (i.e. between 20 and 25 June). Emergence of adult moths collected in control plots in 1998 occurred on 10 June, the earliest date over the 3-year experiment, whereas moths emerged very late in 1997 (i.e. at the beginning of July) (Fig. 1).

Spruce budworm male pupal weight. The effect of *Btk* treatments on pupal weight depended on the experimental year (Table 3). Male pupal weights in control plots in 1999 were significantly lower than those recorded in 1997, whereas spruce budworm pupae collected in 1998 had similar weights to those collected in 1997 and 1999 (Fig. 2). In 1997 and 1998, male pupae from plots treated with one or two *Btk* sprays had 29% lower weights on average than those collected in control plots. In 1999, one *Btk* spray did not negatively affect male pupal weight, but two *Btk* sprays led to a 25% decrease in male pupal weight.

Spruce budworm female pupal weight. In 1997, spruce budworm female pupae were significantly 16% heavier than those collected in 1999 (Table 4). Female pupae collected in 1998 had similar weights to those collected in 1997 and 1999. One or two *Btk* applications led to a 20% decrease in female pupal fresh weight compared with the control (Table 4).

Fecundity. In 1997 and 1998, spruce budworm female moths laid 24% more eggs than those in 1999 (Table 4). Females collected in plots treated with one or two *Btk* applications had a 28% lower fecundity than those from control plots.

Table 1 Mean temperatures (°C)[1] in 1997, 1998 and 1999 from 1 April to 15 June (per period of 15 days) registered at the meteorological station of La Pêche (Québec, Canada), nearby the field experiment

	Mean temeperature (°C)[2]			
Period	1997	1998	1999	
April – first 15 days	−1.19 ± 1.94[b]	5.78 ± 1.38[a]	4.34 ± 1.48[a]	$F_{2,30} = 17.47$; $P = 0.0001$
April – last 15 days	6.65 ± 1.54[b]	9.47 ± 1.54[a]	6.93 ± 1.50[b]	$F_{2,43} = 4.05$; $P = 0.0244$
May – first 15 days	6.35 ± 1.88[b]	16.12 ± 1.88[a]	14.03 ± 1.82[a]	$F_{2,37} = 30.04$; $P = 0.0001$
May – last 15 days	11.30 ± 1.88[b]	14.52 ± 1.62[a]	15.56 ± 1.76[a]	$F_{2,49} = 5.94$; $P = 0.0049$
June – first 15 days	18.20 ± 1.82	18.56 ± 1.96	19.74 ± 1.82	$F_{2,37} = 0.78$; $P = 0.46$

[1]Data were analysed using an univariate analysis of variance in a completely randomized design.
[2]Means ± 2SE followed by the same superscript letter do not differ significantly ($P < 0.05$, LSMEANS comparison test).

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Table 2 Summary of the field conditions, temperature and nutritional stress prevailing in the Outaouais region during the parental generation development from 1997 to 1999. Summary of the temperature conditions in the outdoor insectary (Quebec city) during the early larval development of the offspring

	Parental generation							Offspring		
Year	Larval density	One-year-old defoliation	Current-year defoliation	Spring temperature[b]	Temperature stress	Balsam fir suitability and availability	Nutritional stress	Late summer and autumn temperatures[f]	Winter and early spring temperatures[g]	Temperature stress
1997	Moderate (22 larvae/45-cm branch tip)	Moderate (43%)	Low (33%)	Cold	Negative	Asynchrony between bud and insect phenology[c]	Negative	Normal	Normal	No
1998	Moderate (24 larvae/45-cm branch tip)	Moderate (43%)	High (70%)	Normal	No	Balsam fir flowering[d] (pollen available)	Positive	Warm	Normal	No
1999	High (49 larvae/45-cm branch tip)[a]	High (81%)	High (70%)	Normal	No	Potential lower current-year foliage quality and depletion in current-year foliage[e]	Negative	Warm	Cold	Negative

[a] Risk of intraspecific competition.
[b] See Table 1.
[c] Lawrence *et al.* (1997).
[d] Bauce & Carisey (1996). Carisey & Bauce (1997a).
[e] Bauce & Hardy (1988). Carisey & Bauce (1997b).
[f] See Tables 5 and 6.
[g] See Table 7.

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Table 3 Variance analyses of biological performance parameters of the parental generation of *Choristoneura fumiferana*: d.f., *P*-values for *F*-tests and the mean square error used for the *F*-tests (*in italic*)

	Male		Moth emergence Male	
Sources of variation	d.f.	Pupal weight	Male	Female
Year	2	0.98	0.0001	0.0001
Btk treatment	2	0.0001	0.0001	0.0001
Year × *Btk* treatment	4	0.04	0.0079	0.0115
Mean square error	24	21.6	5.06	3.83

Percent fertility. In 1998, 23% more couples were fertile than in 1997 and 1999 (Table 4). Two *Btk* applications negatively affected fertility, whereas one *Btk* application had no impact. (Table 4).

Offspring

Temperature conditions at the outside insectary. From 1 August to 31 December, mean and maximum daily temperatures were higher in 1998 and 1999 than in 1997 except during the first 2 weeks of August and October (Table 5). Thus, during late summer and autumn, the offspring of 1998 and 1999 were submitted for a longer period to high temperatures than those of 1997; second-instar larvae in 1998 and 1999 spent on average 23 and 21 days at temperatures above 23°C and between 0 and 3°C, respectively, whereas larvae in 1997 only spent 13 and 11 days, respectively (Table 6). During winter and early spring, the offspring of 1999 spent 39% of the time before emergence from diapause under temperatures between 0 and 6°C, whereas the offspring of 1997 and 1998 spent 23 and 27% of this time, respectively, under these temperatures (Table 7). This extended cool period in 1999, with temperatures that correspond to thresholds for postdiapause spruce budworm development, has been suggested to induce overwintering mortality, especially when larvae had been exposed to high temperatures before winter (Han & Bauce, 1998).

Temperature conditions and stress prevailing during the early larval development of the offspring are summarized in Table 2.

Egg hatch. The percentage of eggs hatching in 1998 was significantly 11 and 30% higher than that in 1999 and 1997, respectively (Table 4). The percentage of eggs hatching in 1997 was significantly 15% lower than that in 1999. One or two *Btk* sprays negatively affected egg hatching compared with the control (Table 4).

First-instar mortality. Although most of the newly emerged larvae died before they reached the second instar in 1997, first-instar mortality was extremely low in 1998 and 1999 (Table 4). When the spruce budworm parental generations were submitted to one or two *Btk* applications, their progeny had lower survival during their first instars compared with those whose parents had not ingested *Btk* (Table 4).

Second-instar mortality. In 1999, nearly three quarters of the progenies died between the second-instar moult and the end of diapause, which was significantly (170%) higher than that observed in 1998. Second-instar mortality recorded in 1997 was negligible compared with that recorded in either 1998 or 1999 (Table 4). *Btk* treatment had no significant impact on second-instar mortality (Table 4).

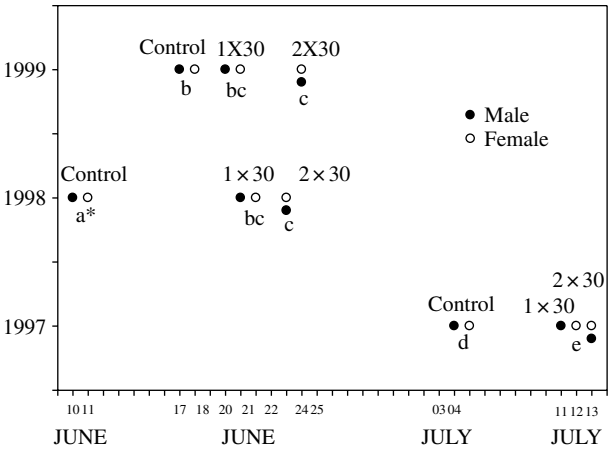

Figure 1 Moth emergence dates of male (•, 2SE = 2.43) and female (○, 2SE = 2.12) spruce budworm according to years and *Btk* treatments (Control, 1 × 30, and 2 × 30, respectively, 0, 1 and 2 applications of 30 BIU/ha). *Moth emergence dates followed by the same letter do not differ significantly according to the Bonferroni pairwise comparison method.

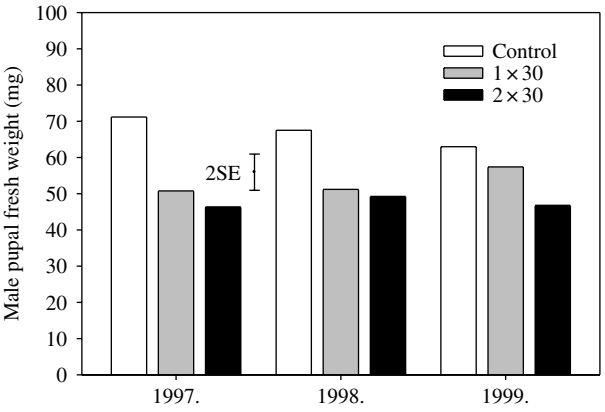

Figure 2 Fresh weight (mg) of male spruce budworm pupae according to years and *Btk* treatments (Control, 1 × 30, and 2 × 30, respectively, 0, 1 and 2 *Btk* aerial applications of 30 BIU/ha).

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Table 4 Statistical significant effects (at $P < 0.05$) of *Btk* treatment (control, one (1 × 30) and two (2 × 30) *Btk* applications of 30 billion International Units/(ha) or year on biological performance parameters of the spruce budworm parental generation and its offspring

	Year				*Btk* treatment			
	1997	1998	1999		Control	1 × 30	2 × 30	
Parental generation								
Female pupal fresh weight (mg)[1]	83.4 ± 5.6[a]	78.2 ± 6.5[ab]	71.9 ± 5.6[b]	$F_{2,24} = 4.14$; $P = 0.029$	90.0 ± 5.9[a]	72.4 ± 5.9[b]	71.1 ± 5.9[b]	$F_{2,24} = 12.72$; $P = 0.0002$
Fecundity[1]	156 ± 16[a]	163 ± 18[a]	121 ± 16[b]	$F_{2,24} = 7.45$; $P = 0.003$	181 ± 16[a]	136 ± 16[b]	123 ± 16[b]	$F_{2,24} = 13.74$; $P = 0.0001$
Fertility (%)[2]	57 ± 8[b]	75 ± 5[a]	65 ± 7[b]	$F_{2,23} = 7.95$; $P = 0.002$	70 ± 6[a]	70 ± 7[a]	57 ± 8[b]	$F_{2,23} = 3.7$; $P = 0.041$
Offspring								
Egg hatch (%)[2]	63 ± 4[c]	82 ± 2[a]	74 ± 3[b]	$F_{2,24} = 36.78$; $P = 0.0001$	76 ± 2[a]	70 ± 3[b]	71 ± 4[b]	$F_{2,24} = 5.53$; $P = 0.011$
First-instar mortality (%)[2]	87 ± 11[a]	6 ± 1[b]	8 ± 3[b]	$F_{2,24} = 246.97$; $P = 0.0001$	12 ± 3[b]	18 ± 4[a]	20 ± 4[a]	$F_{2,24} = 5.31$; $P = 0.005$
Second-instar mortality (%)[2]	0.5 ± 1.2[c]	27 ± 3[b]	73 ± 8[a]	$F_{2,24} = 88.48$; $P = 0.0001$	13 ± 5	7 ± 14	10 ± 11	$F_{2,24} = 0.05$; $P = 0.95$

[1]Data were analysed using a univariate analysis in a 2 × 2 factorial design (*Btk* treatment and year). Interaction between *Btk* treatment and year was not significant for these variables. Mean ± 2SE followed by the same superscript letter do not differ significantly according to the Bonferroni pairwise comparison method.
[2]Data were analysed in a generalized linear model for rate data (Poisson regression type). Interaction between *Btk* treatment and year was not significant for these variables. Means (%) ± 2SE followed by the same superscript letter do not differ significantly (LSMEANS comparison test, $P < 0.05$).

Energy reserves and cryoprotectants. Three-week-old offspring in 1998 contained 67 and 152% more triglycerides than those in 1997 and 1999, respectively (Table 8 and Fig. 3A), whereas 1997 progeny had significantly 51% more triglycerides than in 1999. The amount of triglyceride per mg (larval dry weight) was not significantly affected by *Btk* treatment.

In 1998, spruce budworm progeny had higher glycogen reserves (Table 8 and Fig. 3B) than those in 1997 and 1999. In 1997, it appeared that progeny whose parents were treated with one or two *Btk* applications contained 35% less glycogen than progeny from non-*Btk*-treated parents. However, *Btk* treatments did not affect glycogen content of 3-week-old progeny in 1998 and 1999. Offspring of control parents in 1997 had similar amounts of glycogen compared with offspring in 1999 (Fig. 3B).

Three-week-old offspring in 1997 contained significantly 43% more glycerol than those in 1998 and 1999 (Table 8 and Fig. 3C). Glycerol content was not influenced by *Btk* treatment (Table 8) indicating no impact of *Btk* on spruce budworm cryoprotectant levels.

Glucose content of 3-week-old progeny was not affected by *Btk* treatment and did not change between the 3 years (Table 8 and Fig. 3D).

Discussion

For the first time, we show that *Btk* sublethal effects on spruce budworm parental generations could be carried over to the next generation. Furthermore, the results from the present 3-year field experiment demonstrat that *Btk* sublethal effects on spruce budworm fitness are unrelated to other stress suffered by the parental generation, such as changes in balsam fir suitability, in terms of food quality for the insect, and temperature conditions. Moreover, our results confirm those reported by Carisey & Bauce (2002), indicating that nutritional stress suffered by the parental generation could also carry over to the next generation.

The present study clearly demonstrated that spruce budworm populations surviving *Btk* aerial applications developed more slowly than untreated budworm populations. A few field studies have also reported similar *Btk* effects for the gypsy moth (Weseloh & Andreadis, 1982; Dubois *et al.*, 1988) and spruce budworm (Morris, 1976; Régnière & Cooke, 1998). This increase in larval development could be the result of a combination of three phenomena. First, when larvae ingested *Btk* sublethal droplets, feeding could be inhibited, the duration of which depends on temperature (van Frankenhuyzen & Nystrom, 1987), exposure duration (Fast & Régnière, 1984), dose ingested (Fast & Régnière, 1984; Bauce *et al.*, 2002) and food quality (Bauce *et al.*, 2002). This period would enable the larva to degrade the toxin and repair the midgut epithelium cells (Retnakaran *et al.*, 1983; Spies & Spence, 1985). Second, larvae that resumed feeding tended to extend their development time to compensate for the energy reserves lost during the feeding inhibition period (Bauce *et al.*, 2002). Third, in the field, the period of anorexia (feeding inhibition) tended to

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Table 5 Mean and maximum temperatures (°C)[1] in 1997, 1998 and 1999 from 1 August to 31 December (per period of 15 days) registered at the meteorogical station of the Québec airport (Québec, Canada), nearby the outdoor insectary

		Year			
	Temperature (°C)[2]	1997	1998	1999	
August – first 15 days	Mean	18.0 ± 1.4^{ab}	19.6 ± 1.5^{a}	16.9 ± 1.4^{b}	$F_{2,41} = 3.55$; $P = 0.038$
	Max	24.1 ± 1.6^{ab}	25.9 ± 1.6^{a}	21.8 ± 1.6^{b}	$F_{2,42} = 6.18$; $P = 0.0044$
August – last 15 days	Mean	16.7 ± 1.1^{b}	17.0 ± 1.1^{b}	18.9 ± 1.1^{a}	$F_{2,45} = 4.97$; $P = 0.011$
	Max	24.1 ± 1.3^{b}	22.0 ± 1.3^{b}	24.8 ± 1.3^{a}	$F_{2,45} = 7.82$; $P = 0.0012$
September – first 15 days	Mean	15.6 ± 1.1^{b}	15.0 ± 1.1^{b}	18.9 ± 1.1^{a}	$F_{2,42} = 14.02$; $P = 0.0001$
	Max	19.7 ± 1.8^{b}	19.8 ± 1.8^{b}	24.5 ± 1.8^{a}	$F_{2,42} = 10.29$; $P = 0.0002$
September – last 15 days	Mean	10.2 ± 1.6^{b}	12.4 ± 1.6^{ab}	13.0 ± 1.6^{a}	$F_{2,41} = 3.58$; $P = 0.037$
	Max	15.4 ± 1.8^{a}	17.5 ± 1.8^{a}	17.9 ± 1.8^{a}	$F_{2,41} = 2.4$; $P = 0.1$
October – first 15 days	Mean	10.1 ± 1.4^{a}	7.9 ± 1.4^{b}	6.2 ± 1.4^{b}	$F_{2,41} = 8.12$; $P = 0.0011$
	Max	15.0 ± 1.8^{a}	11.9 ± 1.8^{b}	11.2 ± 1.8^{b}	$F_{2,41} = 5.79$; $P = 0.0061$
October – last 15 days	Mean	3.3 ± 1.4^{b}	6.7 ± 1.4^{a}	4.5 ± 1.4^{b}	$F_{2,44} = 5.75$; $P = 0.006$
	Max	7.4 ± 1.8^{b}	11.2 ± 1.8^{a}	8.7 ± 1.8^{ab}	$F_{2,44} = 4.62$; $P = 0.015$
November – first 15 days	Mean	2.9 ± 2.4^{a}	1.8 ± 2.4^{a}	2.2 ± 2.4^{a}	$F_{2,40} = 0.24$; $P = 0.8$
	Max	6.3 ± 2.8^{a}	4.8 ± 2.8^{a}	6.3 ± 2.8^{a}	$F_{2,40} = 0.39$; $P = 0.7$
November – last 15 days	Mean	-5.0 ± 2.0^{b}	-0.8 ± 2.0^{ab}	1.8 ± 2.0^{a}	$F_{2,41} = 12.5$; $P = 0.0001$
	Max	-0.7 ± 2.0^{b}	2.0 ± 2.0^{b}	5.2 ± 2.0^{a}	$F_{2,41} = 8.53$; $P = 0.0008$
December – first 15 days	Mean	-7.1 ± 2.0^{b}	-1.8 ± 2.0^{a}	0.1 ± 2.0^{a}	$F_{2,42} = 13.3$; $P = 0.0001$
	Max	-2.8 ± 2.0^{b}	1.6 ± 2.0^{a}	2.2 ± 2.0^{a}	$F_{2,42} = 8.37$; $P = 0.0009$
December – last 15 days	Mean	-9.4 ± 2.7^{a}	-9.3 ± 2.7^{a}	-11.1 ± 2.7^{a}	$F_{2,45} = 0.56$; $P = 0.6$
	Max	-4.8 ± 2.7^{a}	-4.7 ± 2.7^{a}	6.7 ± 2.7^{a}	$F_{2,52} = 0.73$; $P = 0.5$

[1]Data were analysed using a univariate variance analysis in a completely randomized design.
[2]Means ± 2SE followed by the same superscript letter do not differ significantly ($P < 0.05$, LSMEANS comparison test)

Table 6 Pre-winter temperature exposure of *Choristoneura fumiferana* progeny (from 1 August to 31 December) in the outdoor insectary nearby the Québec Airport according to year

	Duration of exposure to maximum daily temperatures (days and percentage of time)						
	≥23 °C	23–18 °C	18–13 °C	13–8 °C	8–3 °C	3–0 °C	<0 °C
1997	13 (9%)	36 (24%)	18 (12%)	19 (13%)	15 (10%)	11 (7%)	37 (25%)
1998	22 (15%)	26 (17%)	20 (13%)	26 (17%)	15 (10%)	18 (12%)	24 (16%)
1999	24 (16%)	27 (17%)	21 (14%)	21 (14%)	19 (12%)	24 (16%)	17 (11%)

desynchronize insect and bud phenology and might have led the larvae to feed on unsuitable foliage. Lawrence *et al.* (1997) showed that a delay in synchrony of spruce budworm larvae and white spruce tree phenology induced higher larval mortality, longer larval development time and lower pupal dry weight.

The present study, which gave consistent results over 3 years, tended to confirm the findings of Morris (1976) and Smirnoff (1974, 1983), by showing that one or two *Btk* applications reduced male and female spruce budworm pupal weights and fecundity under field conditions. Fast & Régnière (1984) showed that larvae repeatedly recovering from sublethal *Btk* dose ingestion and feeding inhibition periods tended to have lower pupal weights under laboratory conditions. These authors concluded that improvements in *Btk* formulation for better persistence could increase *Btk* lethal and sublethal effects. During the last 15 years, formulations of *Btk* products have been improved

Table 7 Winter and spring temperature exposure of *Choristoneura fumiferana* progeny (from 1 January to 30 April) in the outdoor insectary nearby the Québec Airport according to year

	Duration of exposure to mean daily temperature (days and percentage of time)			
	≥6 °C	6–3 °C	3–0 °C	0 °C
1998	16 (14%)	12 (10%)	16 (13%)	76 (63%)
1999	8 (7%)	14 (12%)	18 (15%)	78 (66%)
2000	5 (4%)	24 (20%)	23 (19%)	69 (57%)

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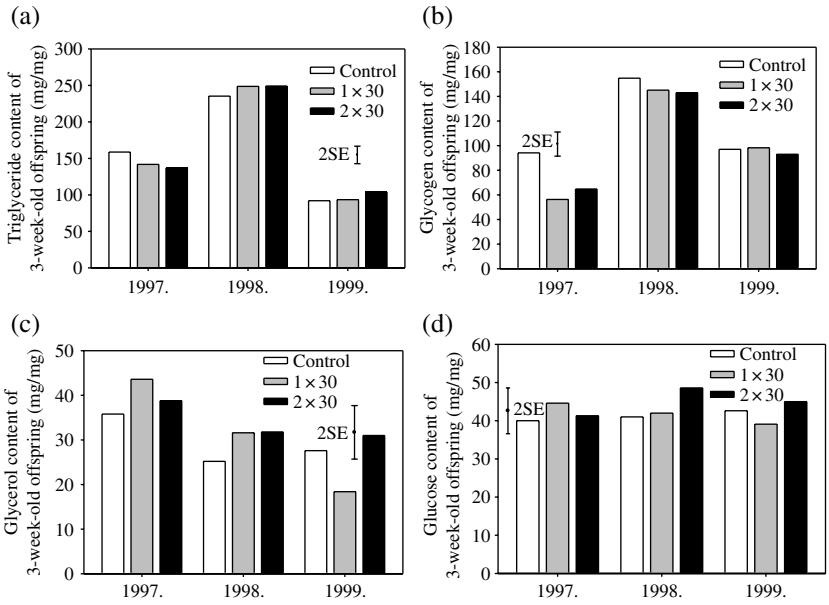

Figure 3 (a) Triglyceride, (b) glycogen, (c) glycerol and (d) glucose content of 3-week-old spruce budworm offspring (μg/mg larval dry weight) whose parents were collected in 1997, 1998 and 1999 and submitted to three different *Btk* aerial spray treatments (Control, 1 × 30 and 2 × 30, respectively, 0, 1 and 2 *Btk* application of 30 BIU/ha).

(van Frankenhuyzen, 1995) and van Frankenhuyzen *et al.* (1998) demonstrated that approximately 30–35% of the toxin, resulting from an application of Foray 48B at 30 BIU/ha, persisted on foliage after 4 days, and still had insecticide proprieties (van Frankenhuyzen *et al.*, 2000). Thus, in the field, spruce budworm larvae might have been reinfected and recovered from several feeding inhibition periods, which could have had detrimental effects on larval growth and pupal weight. Moreover, as larvae grew up and neared the sixth instar, the opportunity to gain normal larval biomass tended to decrease after an ingestion of *Btk* sublethal dose. Under laboratory conditions, several studies have demonstrated that spruce budworm larvae, exposed as fourth instars, had normal female pupal weights, whereas those exposed as sixth instars had reduced pupal weights (van Frankenhuyzen & Nystrom, 1987; Pedersen *et al.*, 1997; Bauce *et al.*, 2002) These larvae had probably less time to recover and presumably had to allocate a large part of the assimilated nutrients to epithelial cell repairs instead of weight gain (Bauce *et al.*, 2002). As previously mentioned, feeding on unsuitable foliage arising from a delay between insect and bud phenology might have also affected larval growth (Lawrence *et al.*, 1997). The greater negative *Btk* impact on male than on female pupal weights observed in the present study has also been reported for spruce budworm (Pedersen *et al.*, 1997) and gypsy moth (Moldenke *et al.*, 1994; Erb *et al.*, 2001).

A reduction in fecundity after sublethal exposure to *Bacillus thuringiensis (Bt)* has been reported previously for several species: *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) (Dulmage & Martinez, 1973; Gould & Anderson, 1991), *Agrotis ypsilon* Hufnagel (Lepidoptera: Noctuidae) (Salama & Sharaby, 1988), *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (Costa *et al.*, 2000) and *C. fumiferana* (Pedersen *et al.*, 1997). This effect might be the consequence of the observed effect on female pupal weight because realized fecundity was found to be proportionally related to female pupal

Table 8 d.f., *P*-values for *F*-tests and the mean square error used for the *F*-tests (*in italic*) from the variance analyses of energy reserves and cryoprotectant in *Choristoneura fumiferana* offspring whose parents were collected in 1997, 1998 or 1999 and submitted to three different *Btk* treatments

		Content of 3-week-old offspring (μg/mg)			
Sources of variation	d.f.	Triglycerides	Glycogen	Glycerol	Glucose
Year	2	0.0001*	0.0001	0.0001*	0.74
Btk treatment	2	0.93	0.0006	0.3	0.33
Year × *Btk* treatment	4	0.06	0.0045*	0.051	0.42
Mean square error	24	166.89	82.69	40.86	35.6

*Statistically significant effects at $P < 0.05$. When interactions are significant, the corresponding simple effects are not in bold type.

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weight for *L. decemlineata* (Costa *et al*., 2000) and *C. fumiferana* (Bauce & Carisey, 1996; Pedersen *et al*., 1997). However, some studies have reported no relationship between spruce budworm female pupal weight and realized fecundity (Delisle & Hardy, 1997; Carisey & Bauce, 2002). Some other factors might be linked to the decrease in realized fecundity. Costa *et al*. (2000) mentioned that reduced longevity of *L. decemlineata* adults after exposure to *Bt. tenebrionis* may have negatively affected oviposition rate. Moreover, the detrimental *Btk* impact on spruce budworm male pupal weight might have lowered male reproductive potential (Savalli & Fox, 1998; Erb *et al*., 2001) and, thus, reduced realized fecundity (Carisey & Bauce, 2002; Delisle & Hardy, 1997). Smirnoff & Valéro (1972) and Smirnoff (1983) reported that spruce budworm pupae sampled from *Btk*-treated areas had lower potential activity (i.e reduced content in enzymes, calcium, proteins and total lipids). Such changes in insect physiology could influence sperm production, nutrient contribution in the spermatophore and mating behaviour. These types of factors might have also contributed to reduce fertility among budworm population surviving to two *Btk* applications.

In the present study, the consistent results obtained in each of the three successive years clearly demonstrated that spruce budworm parental effects resulting from *Btk* ingestion tended to slightly decrease egg hatching (7% reduction) and increase first-instar mortality up to 58%. The negative effects of *Btk* infection of the parents on their progeny probably depended on changes in the physiology of the parental generation. Except for a 35% *Btk* treatment-related reduction in glycogen in 1997, energy reserves in spruce budworm 3-week-old offspring (triglycerides, glycogen, glucose) were not influenced by the *Btk* exposure experienced by their parents. Consequently, we can hypothesize that, in 1998 and 1999, the normal production and transmission of parental gene products to offspring (enzyme, proteins, resistance factors, self-made defensive compounds, parental mRNA) (Mousseau & Dingle, 1991; Rossiter, 1996) might have been jeopardized by *Btk* sublethal dose ingestion. When spruce budworm larvae succeeded in moulting to the second instar and entering diapause, *Btk* infection of the parents did not influence overwintering survival. Parental effects on first-instar mortality probably selected for individuals that were similarly provided with energy reserves and physiological active compounds (Carisey & Bauce, 2002).

In the present 3-year experiment, the best performance for both the parental generations and their progeny was observed in 1998, the year of balsam fir flowering. Fertility rate in 1998 was the highest over the 3 years. In 1998, fecundity and male and female pupal weights were similar to those obtained in 1997, and spruce budworm larvae fed on balsam fir flowering trees in control plots reached the pupal stage faster (i.e. at the beginning of June). Carisey & Bauce (1997a) previously demonstrated that spruce budworm larvae fed pollen and then current-year foliage from balsam fir flowering trees developed 9 days faster than those fed on current-year foliage from nonflowering trees whereas larvae from both types of trees had similar pupal weights and fecundities. The high larval performance was associated with the high content of nitrogen in pollen and the low content of total monoterpenes in current-year foliage of a balsam fir flowering tree (Carisey & Bauce, 1997a). Larvae from the *Btk*-treated plots in 1998 reached the pupal stage at the same period (between 20 and 25 June) as those observed in 1999 (Fig. 1). Nitrogen content in current-year foliage from balsam fir flowering trees was reported to be lower than that from nonflowering trees and tended to decrease rapidly throughout the season (Carisey & Bauce, 1997a). Thus, larvae that recovered from *Btk* ingestion and feeding inhibition in 1998 might have encountered current-year foliage of low nutritional quality and, thus, increased their larval development time to compensate for nitrogen depletion (Carisey & Bauce, 1997a).

Furthermore, it is apparent that the positive effects of pollen ingestion by spruce budworm parents could carry over to the next generation and improve progeny fitness. Feeding on pollen rich in nitrogen favoured egg hatch. In addition, females that fed on balsam fir flowering trees produced offspring with very high amounts of triglycerides and glycogen, which clearly improved second-instar survival during winter by 170% compared with progeny whose parents were fed on nonflowering trees. Triglycerides are used for maintenance, especially in fall and spring (Danks, 1978; Han & Bauce, 2000). Glycogen is the supplier for glycerol (cryoprotectant) production during winter and the success of spruce budworm postdiapause development in spring was associated with the amount of glycogen available in spring (Han & Bauce, 2000).

Spring temperatures in 1997 were lower than those of 1998 and 1999. The 1997 temperature conditions delayed the emergence of postdiapausing second-instar larvae and slowed down larval development. In control plots, spruce budworm larvae did not reach the adult stage before early July 1997 whereas moths from control plots emerged on 10 and 17 June in 1998 and 1999, respectively. Pupal weight, fecundity and fertility were not negatively affected, but male and female physiology might have been disturbed by these temperature conditions because progeny fitness was severely affected (reduced percent of egg hatch and first-instar survival). Cold temperatures might have desynchronized larval and bud phenology, so that the nutritional needs of spruce budworm larvae may not have been satisfied. Nitrogen, phosphorous and potassium contents in coniferous current-year foliage tend to decrease rapidly after budbreak (Carisey & Bauce, 1997b; Lawrence *et al*., 1997) and progeny fitness (egg development and first-instar survival) was shown to be negatively affected by food poor in nitrogen on which the parental generation was reared during the sixth instar (Carisey & Bauce, 2002). Thus, depletion in nitrogen might have affected progeny fitness in 1997. Moreover, reserves in glycogen were lower in progeny from *Btk*-treated plots than from control plots, which could have been related to the delay in larval development and the unavailability of suitable foliage in early July 1997. Most of the progeny died as first instars, and larvae that succeeded in moulting to the second instar and entering diapause survived during winter and were not

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influenced by stress suffered by the parental generation (Carisey & Bauce, 2002).

In 1999, larval density was nearly twice that recorded in 1997 and 1998 and, at the end of the season, current-year foliage defoliation was very high (i.e. 95% in control plots and 71% in plots treated with one *Btk* application) (Bauce *et al*., 2004). In 1999, spruce budworm competition for suitable foliage might have occurred and forced larvae to feed on 1-year-foliage. That type of feeding behaviour has been shown to have detrimental effects on spruce budworm performance (Bauce & Carisey, 1996; Carisey & Bauce, 1997b). Moreover, previous severe defoliation in 1998 might have affected the nutritional quality of the current-year foliage and led to low pupal weight and fecundity (Bauce & Hardy, 1988). This potential nutritional depletion did not affect moth fertility, egg hatch and first-instar survival, but the conjunction of parental nutritional effects and temperature conditions might have contributed to very high second-instar larval mortality (73%) during winter. First, females in 1999 provided their offspring with the lowest amounts of triglycerides over the 3 years. Second, temperatures in late summer and autumn were higher than those observed in 1997 and, third, their progeny encountered extended cool weather conditions in spring 2000. Extended periods of high temperatures during late summer and autumn are known to be detrimental to spruce budworm larvae, causing depletion of reserves used during diapause (Han & Bauce, 2000). Moreover, the negative impacts of such environmental conditions are particularly detrimental to postdiapausing larvae when they are combined with cool temperatures (approximately 0–6°C) during the subsequent early spring (Han & Bauce, 2000).

The present study highlights the fact that prediction of larval density in spring, based on the evaluation of overwintering second-instar larval populations in the autumn, would not be influenced by previous *Btk* treatments. The main effect of *Btk* aerial sprays conducted in spring is demonstrated by a significant reduction in the second-instar larval population in the subsequent autumn. This phenomenon results from both direct effects of *Btk* (larval mortality) and indirect effects (decrease in fecundity, reduced egg hatch and increase in first-instar progeny mortality). Moreover, Weseloh *et al*. (1983) reported an increase in larval parasitism due to the longer larval development time of the surviving population. The results of the present study also indicate that nutrition and temperature stress encountered by the spruce budworm parental generation could affect the early larval development of the progeny.

In future studies, and to improve models for predicting the population dynamics of *C. fumiferana*, knowledge of the parental effects on progeny performance related to nutrition and temperature conditions should be taken into account, as well as the overwintering conditions of the offspring.

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