SUSCEPTIBILITY AND VULNERABILITY OF THIRD-INSTAR LARVAE OF THE SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE) TO *BACILLUS THURINGIENSIS* SUBSP. *KURSTAKI*

AURÉLIE MASSÉ

École nationale des ingénieurs des travaux de l'horticulture et du paysage,
49045 Angers Cédex 01, France

and KEES VAN FRANKENHUYZEN[1] and JOHN DEDES

Great Lakes Forestry Centre, Canadian Forest Service, Natural Resources Canada,
P.O. Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

Abstract

The Canadian Entomologist **132**: 573 – 580 (2000)

A droplet-imbibing assay was used to assess the susceptibility of third-instar larvae of the spruce budworm, *Choristoneura fumiferana* Clemens, to Foray 48B, a commercial formulation of *Bacillus thuringiensis* subsp. *kurstaki* containing 12.7 billion international units (IU) per litre. We observed an LD_{50} of 1.17 IU/larva for third instars, as compared with 3.96 IU/larva for fifth instars. Comparison with previously published data on susceptibility of later instars revealed that third instars were two- to three-fold more susceptible to Foray 48B than fourth and fifth instars and about eightfold more susceptible than sixth instars. Vulnerability of third instars to simulated aerial spray deposits was investigated by using potted balsam firs, *Abies balsamea* L. Potted trees were infested in the greenhouse when the buds were starting to swell, using a density of about one newly emerged second-instar larva per bud. When 90% of the larvae had reached the third instar and 52% of the buds were breaking (4 d after infesting), infested twigs were harvested and sprayed with undiluted Foray 48B in a spray chamber. Spray droplets on the buds measured between 25 and 125 µm in diameter, with 80% having a diameter of 80 µm or less. A density of 4.2 ± 1.0 droplets per bud resulted in spruce budworm mortality of 83.4 ± 4.0% and a corresponding reduction in larval density of 86.5 ± 3.9% (means ± SD, $n = 6$) after 5 d at 25°C. Results of the spray chamber test suggest that third-instar spruce budworms were able to acquire a lethal dose, despite their concealed feeding habits.

Massé A, van Frankenhuyzen K, Dedes J. 2000. Sensibilité et vulnérabilité des larves de troisième stade de la Tordeuse des bourgeons de l'épinette (Lepidoptera : Tortricidae) à *Bacillus thurengiensis* subsp. *kurstaki*. *The Canadian Entomologist* **132** : 573–580.

Résumé

Une expérience d'absorption de gouttelettes a été utilisée pour évaluer la susceptibilité des larves de 3e stade de la Tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* Clemens), au mélange Foray 48B, une préparation commerciale de *Bacillus thuringiensnsis* subsp. *kurstaki* contenant 12,7 milliards d'unités internationales (IU)/litres. Nous avons observé un LD_{50} de 1,17 IU/larve dans le cas des larves de 3e stade, comparativement à 3,96 IU/larve dans le cas des larves de 5e stade. La comparaison avec des données publiées antérieurement sur la sensibilité des stades avancés a révélé que les larves de 3e stade sont de deux à trois fois plus sensibles à la préparation Foray 48B que les larves de 4e ou 5e stades et environ huit fois plus sensibles que les larves de 6e stade. La vulnérabilité des larves de 3e stade à des simulacres de résidus d'arrosage aérien a été examinée sur des sapins baumiers *Abies balsamea* L. en pot. Les arbres en pot ont été infestés dans la serre au moment où les bourgeons se sont mis à gonfler, à raison d'environ une larve de 2e stade

[1] Author to whom all correspondence should be addressed (E-mail: kvanfran@nrcan.gc.ca).

fraîchement émergée par bourgeon. Lorsque 90 % des larves ont atteint le 3e stade et que 52 % des bourgeons se sont ouverts (4 jours après le début de l'infection), les rameaux infectés ont été recueillis et arrosés de Foray 48B non dilué dans une enceinte de vaporisation. Les gouttelettes du produit sur les bourgeons mesuraient de 25 à 125 µm et 80 % mesuraient moins de 80 µm de diamètre. Une densité de 4,2 ± 1,0 gouttelettes par bourgeon a entraîné une mortalité de 83,4 ± 4,0 % des tordeuses, ce qui correspond à une réduction de la densité des larves de 86,5 ± 3,9 % (moyenne ± écart type, $n = 6$) après 5 jours à 25°C. Les résultats du test dans l'enceinte de vaporisation indiquent que les larves de tordeuse de 3e stade sont en mesure d'absorber des doses létales, en dépit du fait qu'il ne soit pas possible d'observer directement leur alimentation.

[Traduit par la Rédaction]

Introduction

Efficacy of spray products containing *Bacillus thuringiensis* Berliner subsp. *kurstaki* (Bacillaceae) against spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), larvae can now be simulated with a process-oriented model (Cooke and Régnière 1996, 1999; Régnière and Cooke 1998). The model simulates development, feeding, and fate of individual larvae in the presence of *B. thuringiensis* spray deposits, which are defined in terms of droplet size spectrum and number of droplets per unit foliage. The probability of a larva ingesting *B. thuringiensis* is determined by the density of spray droplets on new-growth needles and an individual's feeding rate, whereas the probability of death is determined from instar-specific dose–response functions.

Field validation of the model in 1996 led to the observation that additional information was needed with respect to susceptibility and exposure (vulnerability) of early-instar larvae (Régnière and Cooke 1998). The model requires LD_{50} and LD_{95} estimates for fourth, fifth, and sixth instars as inputs. Because dose–response functions were not available for earlier instars, they were assumed to be equally susceptible as fourth instars. Because earlier studies revealed that susceptibility to *B. thuringiensis* decreased with larval stage between instars 4 and 6 (van Frankenhuyzen *et al.* 1997), we postulated that third instars are more susceptible than later instars.

In addition to innate susceptibility to the pathogen, larval vulnerability needs to be taken into consideration. Because early instars often mine in old needles or swelling buds (Atwood 1944), they are assumed to be less likely to encounter spray deposits. In fact, operational spray application of *B. thuringiensis* is timed expressly to avoid early instars and targeted at peak fourth instar when larvae are more open feeders (Carter 1991). Having more concealed feeding habits does not necessarily mean that larvae are not likely to encounter and ingest *B. thuringiensis* spray droplets. Exact mechanisms of dose transfer from spray droplets to feeding budworm larvae are incompletely understood and may involve various nonfeeding behaviours (Nigam 1987). We postulated that third-instar larvae are vulnerable to *B. thuringiensis* spray deposits despite their concealed feeding habits.

In this paper we quantified susceptibility of third-instar larvae to a commonly used forestry formulation of *B. thuringiensis* (Foray 48B) in a discrete-dose ingestion (droplet-imbibing) bioassay, and investigated their exposure and vulnerability to simulated aerial spray deposits on potted balsam fir, *Abies balsamea* L. (Pinaceae).

Methods

Susceptibility. Susceptibility of third-instar larvae from the colony maintained at the Great Lakes Forestry Centre (Grisdale 1970) was determined by enticing individual

larvae to imbibe a droplet of Foray 48B (Novo Nordisk, Copenhagen Denmark; lot BBN 6296 F850), a commercial formulation containing 12.7 billion international units (IU) per litre. The imbibing assay and associated procedures were described in detail by van Frankenhuyzen *et al.* (1997). In brief, Foray 48B was diluted in buffer (phosphate-buffed saline, pH 8.0) to obtain six threefold dilutions. Droplets of each dilution were applied to the bottom of 250-µL microcentrifuge tubes, using a Tracor Atlas Microjector syringe drive interfaced with a digital Microdoser. One-day-old third-instar larvae (mean wet weight of 0.57 mg) readily imbibed 0.0625-µL droplets of the Foray–buffer suspension, which were the smallest droplets that could be generated with the microdoser. The dilution series started at a nominal dose (calculated from the labelled potency) of 3.13 IU per 0.0625-µL droplet for third instars or 14.5 IU per 0.250-µL droplet for fifth instars. Fifth-instar larvae were included to relate observed third-instar response to dose–response functions of later instars to the same product which were determined previously (van Frankenhuyzen *et al.* 1997). We used fifth instars rather than fourth or sixth instars because the former are used in our routine product testing bioassays, which provide a solid baseline for assessing normal variation in larval susceptibility. Each tube received one droplet and one larva within 24 h of moulting into the appropriate instar. For each dilution we used between 70 and 90 third or fifth instars from the same rearing batch. Larvae that had completely imbibed the droplet within 2 h were transferred to artificial diet (Grisdale 1970) and held at 25°C, 60% RH, and a 16L:8D photoperiod. Mortality on the diet was recorded after 5 d. Mortality of control larvae, which were reared in the same manner after imbibing a droplet of dilution buffer, was 1.1% for third instars ($n = 90$) and 1.4% for fifth instars ($n = 72$). Mortality data were subjected to the POLO-PC probit analysis program (Russell *et al.* 1977; LeOra Software 1987). Significant differences between lethal dose values were determined from the non-overlap of the 95% confidence limits. Comparison of the regression lines between instars were made with likelihood-ratio tests of parallelism (common slopes) and equality (common slopes and intercepts) at the $\alpha = 0.05$ level (LeOra Software 1987).

Vulnerability. Vulnerability of third instars to *B. thuringiensis* spray deposits that are representative for an aerial application was assessed by treating infested twigs from potted balsam fir trees in a spray chamber. Fifty 3- to 5-year-old trees were infested in the greenhouse when the buds were swelling (stage 2, see later in this section). Second instars that had just emerged (<2 d) from their hibernaculae were transferred individually from rearing cups onto the swollen buds with a soft brush at a rate of approximately one larva per bud. The larvae were allowed to establish feeding sites and develop under ambient greenhouse conditions for 4 d. On the fifth day, when most of the larvae had reached the third stadium, all buds were harvested by removing branch sections of about 5 cm in length with one or more bud clusters (hereafter referred to as twigs). The twigs were randomly distributed over eight cafeteria trays (60 per tray). Two trays of twigs were left unsprayed and used as controls. The remaining six trays were sprayed in a De Vries track sprayer with undiluted Foray 48B, containing 0.2% Erio-Acid Red XB400 dye (AS Patterson, Willowdale, Ontario) as a tracer. The sprayer was equipped with a Mini Ulva spinning disk (Micron Corporation, Houston, Texas) set to produce droplets predominantly in the <100-µm size range. Each tray was sprayed individually with three passes at a formulation flow rate of 0.75 mL/min to obtain a total of six replicated treatments. The sprays were applied under ambient conditions (about 20°C, 50–60% RH), using a droplet falling distance of about 1 m.

A subsample of 12 twigs (20% of total) was randomly removed from each tray immediately after spraying to obtain estimates of larval density and instar distribution at the time of application and to assess resulting spray deposits. For each twig we

recorded the number of buds and the number of larvae in each instar. Spray droplets on each bud and associated budworm webbing were counted under 10-fold magnification. Droplet diameters were measured for a subsample of 150 droplets, using a Digital Length Measuring Unit (Wild, Heerbrugg, Switzerland) at 31-fold magnification. Droplets were grouped in 10 μm diameter classes to calculate the number and volume median diameters of the deposited droplet size spectrum.

The remaining twigs were divided over plastic rearing cups (Styroware D8), with tight-fitting lids and a piece of damp filter paper to maintain high humidity, using three or four twigs per cup for 14–16 cups per replicate. Variation in bud development within each cup was minimized by first grouping the sprayed twigs from each tray into three broad bud-development categories (*i.e.*, majority of buds swollen, at early bud break, or at full bud break) so that each cup received twigs with similar bud phenology. Bud development in each cup was then quantified by using a refinement of Auger's scale (Dorais and Kettela 1982) to distinguish the following developmental stages: 1, bud resinous, tightly enclosed in membrane; 2, buds swollen, membrane thinning; 3, early bud break, membrane separating and exposing new needles; 4, full bud break, membrane present only as scales around base and as cap on tight bud. An average bud development index (BDI) was calculated for each cup as follows:

$$\text{BDI} = \frac{\sum(\text{no. buds} \times \text{class index})}{\text{total no. of buds}} \quad [1]$$

All cups were kept at 25°C, 60% RH, and a 16L:8D photoperiod for 5 d. Under those conditions, foliar quality was retained during the entire incubation period. On the sixth day, twigs were examined and buds were dissected to count the number of live and dead larvae. To obtain an estimate of natural larval mortality during the 5-d incubation period, twigs from the two unsprayed control trays were randomly divided over three replicates of 12 cups and were held under the same conditions as those for the treated twigs.

Results and Discussion

Susceptibility. Third-instar spruce budworm larvae were more susceptible to Foray 48B than fifth instars in a discrete-dose ingestion bioassay, as was evident from the significantly lower LD_{50} estimate (Table 1). The hypothesis of the two instars having equal dose–response regressions was rejected ($\chi^2_2 = 65.4$, $P < 0.001$). Third-instar larvae were 3.4-fold more susceptible than fifth instars at the 50% response level. The difference in susceptibility was consistent across dose levels because the slopes of the dose–response regressions were not significantly different (hypothesis of parallelism accepted, $\chi^2_2 = 1.7$, $P = 0.183$).

We previously investigated differences in susceptibility to Foray 48B among fourth, fifth, and sixth instars from the same spruce budworm colony. In those tests, fifth instars had an intermediate susceptibility of 2.2 (1.4–3.6) (95% CL) IU/larva, compared with 1.8 (1.4–2.4) IU/larva for fourth instars and 5.1 (4.1–6.3) IU/larva for sixth instars (see Table 3 in van Frankenhuyzen *et al.* 1997). The third-instar estimates obtained in this study can be related to those estimates by taking into account that the larvae used in the earlier tests were slightly more susceptible, as was evident from the 1.8-fold lower fifth-instar LD_{50} (2.2 IU/larva versus 3.9 IU/larva in this study). Such variation in LD_{50} over time is to be expected because "responses of a group of insects tested at any one time will never be exactly the same as responses of another group tested at either the same time or at a different time, regardless of the extent to which bioassay techniques are standardized" (Finney 1964, pp. 91–92; see also Robertson *et al.* 1995).

TABLE 1. **Susceptibility of third- and fifth-instar *Choristoneura fumiferana* larvae to Foray 48B in a droplet-imbibing assay.**

Instar	*n*	Slope ± SE	LD_{50} (95% CL)	LD_{95} (95% CL)
3	466	2.2±0.36	1.17(0.67–1.93)	6.39(3.61–25.03)
5	500	2.8±0.29	3.96(3.37–4.66)	15.00(12.12–20.07)

NOTE: LD_{50} and LD_{95} expressed as IU/larva, based on labelled product potency of 12.7 billion IU/L.

The less than twofold difference is well within the limits of normal variation in *B. thuringiensis* susceptibility between rearing batches of our spruce budworm colony (K van Frankenhuyzen, unpublished data) and between field populations (van Frankenhuyzen *et al.* 1995). Because the difference in susceptibility between instars was consistent among rearing batches (van Frankenhuyzen *et al.* 1997), we used the ratio of the fifth-instar LD_{50} (2.2/3.9 = 0.56) to correct for variation in susceptibility between the two tests, yielding a corrected LD_{50} of 1.17 × 0.56 = 0.66 IU/larva for third instars for comparison with the previously published lethal dose estimates. Thus, third instars were about two- to three-fold more susceptible than fourth and fifth instars and about eightfold more susceptible than sixth instars. The efficacy model has been modified to take this into account (Régnière and Cooke 1999), and improvements to the model's outputs are being tested.

Vulnerability. Although more susceptible than later instars, third-instar larvae might not be exposed to *B. thuringiensis* spray deposits because of their concealed feeding habits. Vulnerability of third instars was examined in a spray chamber test using twigs from potted balsam fir trees at a phenological stage that typically coincides with the presence of early-instar budworm larvae under field conditions. Buds on the twigs used for the bioassay were mostly in stages 2 (swollen, 31%) and 3 (early bud break, 52%), with smaller proportions in stages 1 (not yet swelling, 6%) and 4 (full bud break, 12%) (n = 1317 buds, six replicates combined) at the time of spray application. The average bud development index varied between replicates from 2.4 to 2.9 (Table 2). About 90% of the larvae on the subsample of twigs dissected after spraying were third instars, whereas 10% were still second instars (n = 153 larvae, six replicates combined) (Table 2). All larvae had well-established feeding sites at the time of spray application; most of the third instars were found inside feeding shelters spun between adjacent stage 3 and stage 4 buds, whereas most of the second instars were mining inside stage 2 buds. Twigs were handled carefully during and after application to avoid larvae evacuating their feeding sites.

Droplets on the buds were mostly hemispherical and measured between 25 and 125 µm in diameter, with 50% of the droplets being below 60 µm and 80% below 80 µm. The deposited droplet size spectrum was characterized by a number median diameter of about 60 µm and a volume median diameter of about 100 µm. This corresponds closely to droplet size spectra on foliage typically observed after aerial forestry applications (*e.g.*, Régnière and Cooke 1998; van Frankenhuyzen *et al.* 2000). Ninety percent of the droplets (n = 1380) were found on buds, the majority (75%) on stage 3 buds, with smaller proportions on stages 2 (15%) and 4 (10%) buds. Ten percent of the droplets were found on webbing associated with budworm feeding sites. The mean number of droplets per bud ranged from 2.9 to 5.8, with an overall mean (± SD) of 4.25 ± 1.0 droplets per bud (Table 2).

Larval mortality resulting from the Foray 48B treatment was estimated from the ratio of dead and live larvae found on the foliage 5 d after spraying and averaged 83.4 ±

https://doi.org/10.4039/Ent132573-5 Published online by Cambridge University Press

TABLE 2. Density and mortality of third-instar *Choristoneura fumiferana* after 5 d of feeding on balsam fir shoots treated with Foray 48B in a spray chamber.

Replicate	Bud development index (BDI)	Third instars (%)	No. of drops/bud	Mortality (%)*	$n^{\dagger}$	No. of survivors/bud	Population reduction (%)‡
			Sprayed				
1	2.7	92.3	4.2	81.8	149	0.12	83.3
2	2.9	88.9	5.8	88.8	134	0.07	90.2
3	2.8	89.2	4.5	79.4	170	0.14	80.5
4	2.7	100.0	4.5	87.8	123	0.07	90.3
5	2.5	97.7	2.9	84.3	89	0.08	88.8
6	2.4	79.2	3.3	79.7	123	0.10	86.1
Mean	2.7	90.6	4.2	83.4		0.1	86.5
SD	0.18	6.3	1	4		0.03	3.9
			Unsprayed				
1	2.8	—	—	1.4	137	0.7	—
2	2.6	—	—	3.1	97	0.65	—
3	2.9	—	—	3.4	118	0.72	—
Mean	2.8	—	—	2.6		0.72	—
SD	0.14			1		0.07	

* Calculated as [(no. of larvae found dead on foliage)/n] × 100.

† Total number of larvae retrieved from foliage (dead + alive).

‡ Calculated as [(no. of survivors per bud in control – no. of survivors per bud in treated)/(no. of survivors per bud in control)] × 100.

4.0% (SD) (Table 2). The dead larvae displayed typical symptoms of *B. thuringiensis* infection. This direct mortality estimate was corroborated by a mean (± SD) population reduction estimate of 86.5 ± 3.9%, as calculated from the difference in the day-5 density of surviving larvae between unsprayed (0.72 ± 0.07) and sprayed twigs (0.10 ± 0.03, $n = 6$) (Table 2). Correction for natural mortality was not necessary because larval density on the unsprayed twigs did not change appreciably during the 5-d interval between prespray and postspray counts (from 0.74 to 0.72 larvae per bud). Low natural mortality was also evident from the low proportion of dead larvae on unsprayed foliage (2.6%, Table 2).

The spray chamber test results indicate that third-instar spruce budworm larvae are vulnerable to *B. thuringiensis* spray deposits, and that their concealed feeding habits do not necessarily preclude acquisition of a lethal dose. We feel that experimental procedures giving rise to disturbance feeding (Retnakaran 1983) or other behaviours that might have artificially increased exposure to spray droplets did not make a major contribution to dose acquisition. We did not find any abandoned feeding shelters or wandering larvae on the twigs that were examined immediately after spray application, indicating that handling of the twigs did not cause undue disturbance. This raises the question of how dose transfer took place. Transfer of droplets from webbing onto feeding surfaces through larval movement and preening is one possible mechanism (Nigam 1987), but probably did not make a large contribution to mortality in this study because only 10% of the droplets were found on budworm silk. Because 85% of the remaining droplets were found on stages 3 and 4 buds, we suspect that most of the observed mortality was attributable to third instars acquiring a lethal dose by feeding on contaminated surfaces associated with their feeding shelters. More detailed observations on

feeding behaviour of early budworm instars are needed to elucidate the exact mechanisms of dose transfer.

The spray chamber test was conducted as an intermediate step between the discrete-dose ingestion assay and the real budworm world. Results of the imbibing assay show that third-instar budworms are highly susceptible to *B. thuringiensis*, and the foliage assay demonstrates that they do encounter and ingest spray droplets. The high level of larval mortality observed in the spray chamber test may not be attainable under field conditions, considering that the test was conducted under conditions that were optimum for feeding (constant temperature of 25°C) and dose acquisition (no weathering of spray deposits). Far from being a perfect simulation of field conditions, the spray chamber data do corroborate results of earlier field tests that the window for aerial *B. thuringiensis* sprays for spruce budworm control can be expanded by earlier timing of application (Kettela and Steel 1990). Currently, spray application is usually timed for peak-fourth or early-fifth instar abundance, when larvae are most likely to be directly exposed to spray deposits. This poses a severe constraint on the level of foliage protection that can be obtained, since feeding damage done by early instars before bud flare can lead to extensive damage (Blais 1979). The results of our study underline the need for further field tests to quantify spray deposition on buds in early developmental stages and resulting larval mortality after aerial application of *B. thuringiensis* targeted against third instars.

Acknowledgments

We gratefully acknowledge financial assistance from Forest Protection Limited (Fredericton, New Brunswick) and Société de protection des fôrets contre les insectes et maladies (Québec) through the Spray Efficacy Research Group.

References

Atwood CE. 1944. The feeding habits of young spruce budworm larvae. *The Canadian Entomologist* **76**: 64–6

Blais JR. 1979. Rate of defoliation of balsam fir in relation to spruce budworm attack and timing of spray application. *Canadian Journal of Forest Research* **9**: 354–61

Carter NE. 1991. Efficacy of *Bacillus thuringiensis* in New Brunswick, 1988–1990. pp. 113–6 *in* Preprints of the 72nd Annual Meeting, Woodlands Section, Canadian Pulp and Paper Association, Montréal, Quebec

Cooke BJ, Régnière J. 1996. An object-oriented, process-based stochastic simulation model of *Bacillus thuringiensis* efficacy against spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *International Journal of Pest Management* **42**: 291–306

—— 1999. Cooke's efficacy model: user's guide to the decision-support tool for control of spruce budworm populations with *Bacillus thuringiensis*. *Natural Resources Canada Laurentian Forestry Centre Information Report* **LAU-X-124**

Dorais L, Kettela EG.1982. A review of entomological survey and assessment techniques used in regional spruce budworm surveys and in the assessment of operational spray programs. Eastern Spruce Budworm Council, Quebec Department of Energy and Natural Resources, Québec

Finney DJ. 1964. *Statistical method in biological assay.* London: Griffin

Grisdale DG. 1970. An improved method for rearing large numbers of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *The Canadian Entomologist* **102**: 1111–7

Kettela EG, Steel V. 1990. An account of spray trials conducted to evaluate the efficacy of Bt against high spruce budworm populations. *Canadian Forest Service Spray Efficacy Research Group Report* **1990/01**

LeOra Software. 1987. *POLO-PC: a user's guide to probit or logit analysis*. Berkeley: LeOra Software

Nigam PC. 1987. Dose transfer and spruce budworm behaviour during operational application of fenitrothion. pp. 281–4 *in* GW Green (Ed.), Proceedings of Symposium on the Aerial Application of Pesticides in Forestry. *National Research Council Canada* **AFA-TN-18**

Régnière J, Cooke BJ. 1998. Validation of a process oriented model of *Bacillus thuringiensis* variety *kurstaki* efficacy against spruce budworm (Lepidoptera: Tortricidae). *Environmental Entomology* **27**: 801–11

Retnakaran A. 1983. Spectrophotometric determination of larval ingestion rates in the spruce budworm (Lepidoptera: Tortricidae). *The Canadian Entomologist* **115**: 31–40

Robertson JL, Preisler HK, Ng SS, Hickle LA, Gelernter WD. 1995. Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. *Journal of Economic Entomology* **88**: 1–10

Russell RM, Robertson JL, Savin SE. 1977. POLO: a new computer program for probit analysis. *Bulletin of the Entomological Society of America* **23**: 209–13

van Frankenhuyzen K, Nystrom C, Tabashnik BE. 1995. Variation in tolerance to *Bacillus thuringiensis* among and within populations of the spruce budworm (Lepidoptera: Tortricidae) in Ontario. *Journal of Economic Entomology* **88**: 97–195

van Frankenhuyzen K, Gringorten L, Dedes J, Gauthier D. 1997. Susceptibility of different instars of the spruce budworm (Lepidoptera: Tortricidae) to *Bacillus thuringiensis* var. *kurstaki* estimated with a droplet-feeding method. *Journal of Economic Entomology* **90**: 560–5

van Frankenhuyzen K, Nystrom C, Dedes J, Seligny V. 2000. Mortality, feeding inhibition, and recovery of spruce budworm (Lepidoptera: Tortricidae) larvae following aerial application of a high-potency formulation of *Bacillus thuringiensis* subsp. *kurstaki*. *The Canadian Entomologist* **132**: 505–18

(Date received: 15 February 2000; date accepted: 29 August 2000)