Monitoring Nontarget Lepidoptera on *Ribes cereum* to Investigate Side Effects of an Operational Application of *Bacillus thuringiensis* subsp. *kurstaki*

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ABSTRACT The abundance of nontarget Lepidoptera on the shrub *Ribes cereum* Douglas was monitored from 1997 to 2000 in an Interior Douglas-fir forest in British Columbia to assess potential side effects of an operational program to control the western spruce budworm, *Choristoneura occidentalis* Freeman. The treatment was a single application of *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) (Foray 48B) at the rate of 30 BIU/ha in 2.4 liter/ha. The guild of leaf-feeding Lepidoptera on *R. cereum* was dominated by *Gelechia ribesella* Chambers, and *Euhyponomeutoides gracilariella* Busck, which made up 24 and 62% of the guild, respectively. The remaining 14% of the guild comprised numerous sparsely distributed species (at least 45 different species based on morphotypes). Total larval abundance was significantly lower on plants that were sprayed with *Btk* than on plants that were covered to exclude *Btk*. Covering the plants was a novel approach that enabled us to replicate the treatment within a single spray area. *G. ribesella* and *E. gracilariella* were significantly reduced by *Btk*, but a modest reduction of 'sparsely distributed species' was not significant. Both *G. ribesella* and *E. gracilariella* appeared to make a full recovery within 2 yr of the *Btk* spray, but as a group the abundance of the sparsely distributed species was lowest in the year 2000 in both the *Btk* sprayed area and an untreated comparison area. This suggests a general decline independent of the treatment. Microscopic examination of cadavers of the two major nontarget species showed the presence of *Btk* in some of the larvae reared from the treated plot, but *Btk* was absent in larvae from the reference plot.

KEY WORDS *Bacillus thuringiensis* subsp. *kurstaki*, nontarget Lepidoptera, Douglas-fir, western spruce budworm, *Choristoneura occidentalis*

THE WESTERN SPRUCE budworm, *Choristoneura occidentalis* Freeman, is possibly the most destructive defoliator of Douglas-fir trees, *Pseudotsuga menziesii* (Mirbel) Franco, in western North America (Markin 1982). Young Douglas-fir trees in the understory are frequently killed by western spruce budworm defoliation resulting in loss of regeneration in multi-aged stands. Mature Douglas-fir trees are rarely killed by western spruce budworm, but the effects of defoliation include radial growth loss and height loss, deformation of stems, reduced cone and seed production, and increased attack by secondary insect pests and decay organisms (Alfaro 1985, Shepherd et al. 1995). In addition, damaged stands are avoided as recreation areas and can be a fire hazard.

For these reasons, forest managers aim to suppress western spruce budworm populations in selected areas. Chemical pesticides have not been used against the western spruce budworm in British Columbia (Shepherd et al. 1995). The control method of choice is the aerial spraying of the microbial insecticide *Bacillus thuringiensis* subsp. *kurstaki (Btk)*, which is specific to Lepidoptera. Although *Btk* is toxic to many lepidopteran species, its use has become popular because it is far more specific than chemical and molt-inhibiting insecticides (Wagner and Miller 1995). Nevertheless, there are risks to nontarget Lepidoptera associated with the large-scale use of *Btk* (Johnson et al. 1995). Concerns include nontarget lepidopteran species that are ecologically important, rare, or endangered (Orton 1987; Miller 1990, 1992). Therefore, studies aimed at determining the success of an insecticide treatment should be accompanied by studies that document the side effects of the pesticide used on nontarget species so that the environmental costs and economic benefits can be evaluated.

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Few studies have assessed the impacts of *Btk* on nontarget Lepidoptera after experimental sprays (Rodenhouse and Holmes 1992, Johnson et al. 1995, Sample et al. 1996, Wagner et al. 1996) and operational spray programs targeting either the western spruce budworm or the gypsy moth, *Lymantria dispar* L. (Miller 1990, 1992; Whaley et al. 1998, Boulton 1999). However, in three different studies, (1) Miller (1990, 1992) reported that *Btk* caused reductions in the density of lepidopteran guilds for up to 1–2 yr; (2) Wagner et al. (1996) showed that *Btk* suppressed *Malacosoma disstria* Hübner (Lasiocampidae) for at least 1 yr, and *Phoberia atomaris* Hübner (Noctuidae) for at least 2 yr (*M. disstria* is generally considered a pest but in this study it was considered a nontarget species because it was not the direct target of the *Btk* application); and (3) using light traps, Sample et al. (1996) found reduced total abundance of macro- and micro-Lepidoptera 1 yr after *Btk* application. These impacts, although apparent only after 1 yr after treatment, should be considered immediate impacts because many of the captured individuals were probably present as larvae when the *Btk* was applied.

In general, spraying *Btk* appears to be hazardous to susceptible nontarget Lepidoptera, which have the alkaline conditions in the gut required for infection. Further data are needed because the immediate impact of *Btk* is influenced by weather conditions during and after application, species composition, terrain, and various spray parameters (Reardon et al. 1994, Cadogan and de Groot 1995, Schauber et al. 1997). Moreover, nontarget Lepidoptera have received little attention in British Columbia until recently (T. S. Boulton, D. A. Rohlfs, and K. L. Halwas, unpublished data), and to date no Canadian studies have been published on nontarget effects. Hence, this study was initiated to gather information about the response of nontarget Lepidoptera to a single, operational application of *Btk* (30 BIU/ha in 2.4 liter/ha [BIU = billion international units]) in 1998 to control the western spruce budworm near Merritt, British Columbia.

Materials and Methods

Study Sites. In November 1996, western spruce budworm egg-mass surveys were conducted in the Kamloops Forest Region in budworm infested *Pseudotsuga menziesii* variety *glauca* (Beissner) Franco (Douglas-fir) stands that had never received *Btk* treatments in the past. The purpose of these surveys was two-fold: to locate stands of Douglas-fir with western spruce budworm infestations severe enough to require treatment with *Btk* and that also had a high density of *Ribes cereum* Douglas shrubs in the understory. *Ribes cereum* was chosen as the study plant because a pilot study in 1995 revealed that this shrub was the only plant species in the area consistently hosting a large number of nontarget Lepidoptera.

Two suitable areas (≈100 ha each) were found and a study plot (≈50 ha) was established in each. The 'treatment plot' received a single *Btk* application of 30 BIU/ha. At present *Btk* is registered for western spruce budworm control at 30 BIU/ha in Canada. The treatment plot was located on a ridge (1,040–1,160 m) 2 km directly north of the city of Merritt (Fig. 1). The other plot did not receive any *Btk* applications before or during this study, and it was used as a 'reference plot,' as opposed to a 'control plot.' We use the term *reference plot* because of the problem of pseudoreplication (see Hurlbert 1984, Heffner et al. 1996). True replication, unfortunately, is frequently not feasible to achieve in field studies. Pseudoreplication was unavoidable in our case because only two areas were found that fit our selection criteria. The reference plot was situated on a different ridge (1,020–1,140 m) ≈8 km northeast of the treatment plot near the southwest end of Nicola Lake (Fig. 1). Both the treatment and reference plots had a relatively high density of *R. cereum*, and they were similar with respect to plant species composition, aspect, and percent canopy cover.

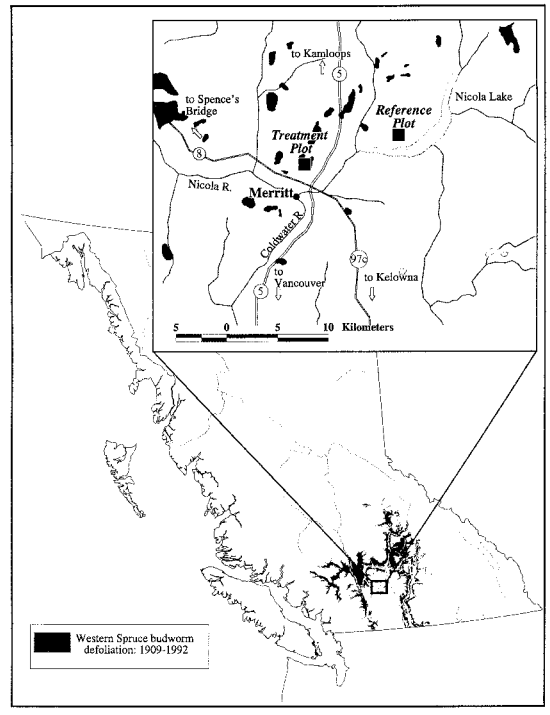

Fig. 1. Location of study plots and distribution of past western spruce budworm defoliation in British Columbia (1909–1992).

***Ribes cereum* Abundance.** The density of *R. cereum* was established by conducting systematic surveys in both the treatment and reference plots and counting each plant. *R. cereum* plants grow in patches of variable sizes, mainly in openings or under the more open-grown trees in the forest. A rough estimate of the total amount of *R. cereum* leaves, available to lepidopteran herbivores as a habitat, was obtained by measuring the

height of each plant, placing each plant into one of five height categories (<0.5 m, 0.5–1.0 m, 1.0–1.5 m, 1.5–2.0 m, 2.0–2.5 m) and estimating the average dry mass of leaves per shrub for each height category. The average dry mass of leaves per *R. cereum* plant was estimated using data from an earlier study (Boulton 1999) in which all leaves were removed, including petioles, from 30 randomly selected *R. cereum* plants in each height category. The removed foliage was air-dried for 2 mo and then oven-dried for 36 h at 80°C, allowed to cool to room temperature, and then weighed immediately.

Sample Design in the Reference Plot. Within the reference plot, 12 contiguous but variable-in-size patches of *R. cereum* were selected, and within each patch 10 plants were chosen for sampling. Each plant was paired with an adjacent plant that was judged to be similar in size and structure. Each pair was randomly assigned to a different position in the sample schedule (one of five sample dates). Then, one plant within each pair was randomly selected for sampling in 1997 and the other plant was sampled in 1998.

Sample Design in the Treatment Plot. Because the reference plot provided pseudoreplicates only, 60 *R. cereum* plants were covered in the treatment plot with a synthetic wrap (Spunguard, Kimberly-Clark, Dallas, TX) over a metal/wooden frame constructed around each plant to provide true controls by excluding *Btk* spray droplets. The Spunguard material was chosen because although it is impermeable to the finest *Btk* spray deposits, it is not airtight and was not expected to significantly alter temperature, humidity and other variables that could affect larvae. The covers were removed ≈1–2 h after the *Btk* application (between 0700 and 0730 hours) by which time all the *Btk* spray droplets had settled in the plot (Boulton 1999).

In the treatment plot, 12 contiguous but variable-in-size patches of *R. cereum* were selected and within each patch 15 similar *R. cereum* plants were chosen for sampling. Each plant was grouped with two adjacent plants that were judged to be similar in size and structure. Each group of three plants was randomly assigned to a different position in the sampling schedule (one of five sample dates), and then each plant within each group of three was randomly assigned to one of three different subplot treatments: 'baseline,' 'sprayed,' and 'covered.' The baseline samples collected in 1997 were used to estimate the natural density of nontarget Lepidoptera in the treatment plot. Sample plants from the sprayed and covered samples were collected in 1998 after the operational application of *Btk*. The 1998 sample dates (13 June, 23 June, 3 July, 13 July, 19 July) were timed to match the phenology of larvae collected in 1997 (17 June, 27 June, 7 July, 17 July, 23 July). The resulting split-plot design had 12 replicates with the whole-plot treatment factor of sample date in a completely randomized block design with spray treatment as the subplot treatment factor.

A total of 2,060 *R. cereum* plants was counted within the treatment plot. Of these, 361 had a height equal to or >1 m, the minimum height specified for the study plants. The foliage removed from *R. cereum* plants in the treatment plot the previous year (1997) comprised an estimated 4.2% of the total amount of *R. cereum* leaves originally present. From the remaining *R. cereum* plants, an estimated 6.1% of the leaves were removed during 1998 and an estimated additional 4.5% was removed during 2000.

A total of 3,252 *R. cereum* plants was counted within the reference plot. Of these, 443 had a size equal to or >1 m. The foliage removed from *R. cereum* plants in the reference plot during 1997 comprised an estimated 2.9% of the total *R. cereum* leaf habitat. Of the remaining *R. cereum* leaf habitat, an estimated 3.4% was removed during 1998 and an estimated additional 3.2% was removed during 2000.

Long-Term Impacts. The experiment was originally designed to sample *R. cereum* in 1997 and 1998 only. In 2000, however, we had the opportunity to sample both plots for a third year to assess the long-term impacts of *Btk*. To do this, additional plants were randomly selected from the remaining (previously unsampled) plants in the same patches that were used in 1997 and 1998. These plants were not covered during the 1998 *Btk* application. The sample dates in 2000 (12 June, 21 June, 3 July, 13 July, 23 July) were timed to match the phenology of larvae collected in the previous 2 yr.

Insect Sampling. All plants in the study were of similar size (1–2 m tall) and general appearance, and each was sampled only once during the 3 yr of the study. Each plant was sampled with equal effort for 25 min (or less for smaller plants), and the same person (T.J.B.) performed all the sampling throughout the study to minimize variation. At each sample date, all study plants were sampled within a 3-d period. Covered plants were sampled within 5 min of their paired, sprayed plant.

Samples were obtained from four regions in the canopy of each sample shrub corresponding to the four cardinal directions. Care was taken to sample similar amounts of foliage from the lower and higher branches of each shrub. The 25-min sampling effort included clipping the branches with pruning shears and striking them briskly against a 1 m long steel pole, directly over a 3 by 2-m white nylon sheet (see Harris et al. 1972). The dislodged caterpillars were counted and collected easily from the sheet. Time constraints prevented us from rearing all but a subsample of specimens. All larvae that were not reared were immediately placed in 70% ethanol and recorded as the number of larvae collected per 25 min sampling effort. The preserved larvae were identified by comparing their size and color pattern to those larvae that were reared to adults. Voucher specimens of unidentified larvae and some adults have been placed in the permanent collection at Pacific Forestry Center, Victoria, British Columbia, Canada.

Statistical Analysis. The percent reduction of nontarget Lepidoptera was estimated by taking the aver-

age difference in larval abundance between *Btk*-sprayed plants and covered plants between 5 and 41 d postspray. This approach may have provided conservative estimates of larval mortality for the following two reasons: (1) some of the larvae collected from the sprayed plants early in the season might have died from *Btk* at a later date if we had left them in situ; and (2) sublethal dosages slow the rate of larval development in some species (Whaley et al. 1998), which would tend to inflate estimates of abundance in the treatment plot toward the end of the season.

Statistical analyses were performed as described below ($\alpha = 0.05$) to determine the immediate impact of *Btk* on the entire guild of leaf-feeding Lepidoptera on *R. cereum.* Analyses were also conducted on the two most abundant Lepidoptera species, *Gelechia ribesella* Chambers (Gelechiidae) and *Euhyponomeutoides gracilariella* Busck (Yponomeutidae) (see Kyrki 1990). All remaining species, subsequently referred to as sparsely distributed species, were pooled and analyzed as a group. The sparsely distributed species were collected too infrequently to allow statistical analyses to be performed at any taxonomic level. We did not attempt to identify each of the sparsely distributed species through rearing because that would have required rearing efforts beyond our means.

Preliminary examination of data confirmed linearity of the relationship between larval abundance and each of the main effects, and favored the use of analysis of variance (ANOVA). Split-plot ANOVA (Statistical Sciences 1995) was used to compare larval abundance on *Btk*-sprayed plants with that on covered plants in 1998. The first postspray sample date was not included in the *E. gracilariella* ANOVA because very few *E. gracilariella* larvae were free-feeding at that time, and the last postspray sample date was not included in the *G. ribesella* ANOVA because most *G. ribesella* larvae had already pupated by that time. All data were square root transformed so that each produced a straight line in a quantile/quantile plot of the residuals (Statistical Sciences 1995).

The problem of pseudoreplication prevented us from using statistical tests to compare both spatial variation between the treatment and the reference plot, and temporal variation between 1997, 1998, and 2000. In addition, plants that were sampled in 2000 were selected after the original randomization. Thus, the 2000 samples are not independent of the earlier samples and they cannot be compared with the earlier samples using statistical inference. Nevertheless, we report the results of the 2000 data, because the 2000 samples were well interspersed in both plots and they provide the best possible estimate of nontarget Lepidoptera populations 2 yr after the *Btk* spray. The data from each year are presented graphically, and the apparent differences between years should be considered indicative rather than conclusive.

***Btk* Treatment.** In June 1998, the British Columbia Ministry of Forests conducted an aerial *Btk* spray operation to control the western spruce budworm. Several areas totaling 1,259 ha in the Kamloops Forest Region were sprayed with *Btk* using 30 flat fan nozzles on a symplex spray system boom that was mounted on an 802 fixed-wing aircraft. The spray system was calibrated on the ground before application to deliver Foray 48B (Abbott, Chicago, IL) at a rate of 30 BIU/ha in 2.4 liter/ha, the registered dose to control western spruce budworm in Canada. Our treatment plot was situated within a 410-ha sprayed area. *Btk* was applied as soon as the Douglas-fir buds flushed completely and western spruce budworm larvae were free-feeding. Spraying is ineffective before bud flush because budworm larvae mine the interior of buds or partly open shoots, where they are protected from spray deposits. Kromekote (Smart Papers, Hamilton, ON) cards were placed on the ground beside each *R. cereum* shrub slated for sampling, as well as inside the covers of covered plants to check for *Btk* spray deposit. Automated weather stations (Campbell Scientific with CR-10 data logger, Edmonton, AB) measured ambient temperature, precipitation, and wind speed in the treated plot.

Mortality of *G. ribesella* and *E. gracilariella*. A small rearing experiment was conducted in 1997 to compare the mortality rates of 65 late-instar *G. ribesella* larvae with 65 early-instar and 65 late instar *E. gracilariella* larvae. The larvae were collected from *R. cereum* by cutting stems 4–6 h after an operational application of *Btk* (30 BIU/ha in 2.4 liter/ha). Each larva was kept on the stem from which it was collected, each stem was individually placed in a glass vial with a water source, and the larvae were reared outdoors in full shade. After 5 d, each dead larva was removed from its container and a section including its mid-gut was smeared on a glass slide. The gut contents were examined with a phase contrast microscope at 1,000× magnification. The presence of *Btk* spores and/or crystals were interpreted as evidence that *Btk* was the cause of death (Misra and Singh 1993).

Results and Discussion

***Btk* Deposit Analyses and Meteorological Conditions.** The *Btk* spray was applied to the treatment plot on 8 June 1998, at 0530–0600 hours under dry and mainly clear conditions. The average wind speed was <1 kph and the average temperature was 7.4°C. The first rain (light drizzle, 3 mm) occurred more than 1 wk after the *Btk* was applied. Heavy rain (i.e., >10 mm/24 h) did not occur during the first 2 wk of the *Btk* treatment. Thus, spray droplets would not have been washed off the study plants. A visual inspection of the Kromekote cards, placed next to each sample plant, confirmed that each uncovered *R. cereum* plant received *Btk* residues, and most droplets were 100–120 μm in size. Kromekote cards placed within the protective enclosures over the control plants showed no spray deposits, confirming

Table 1. Total number of nontarget Lepidoptera collected, per 25-min effort, from treatment (T) and reference (R) plots during each year of the study and from covered (C) and sprayed (S) treatment subplots in 1998 near Merritt, Kamloops Forest Region, BC, Canada

Year	*G. ribesella* (T/R)	*E. gracilariella* (T/R)	Sparsely distributed species (T/R)	*R. cereum* guild (grand total) (T/R)
1997	296/352	1,902/300	251/239	2,449/891
1998	$\frac{C292}{S127}$/338	$\frac{C909}{S696}$/313	$\frac{C177}{S133}$/311	$\frac{C1,378}{S946}$/962
2000	461/678	1,734/623	196/204	2,391/1,505
Total	1,166/1,368	5,241/1,236	757/754	7,164/3,358

Each total is the sum of 60 samples.

that the covers indeed provided an effective barrier to the *Btk* treatment.

Total Abundance Of Nontarget Lepidoptera. A total of 10,522 lepidopteran larvae was collected from *R. cereum* foliage throughout the entire study (Table 1). The guild was numerically dominated by *E. gracilariella* and *G. ribesella*, which made up 62% and 24% of the total collected insects, respectively. The remaining 14% of the guild was composed of sparsely distributed species, at least 45 different species based on morphotypes. Of these, 12 were collected only once, nine twice, five three times, and seven four times. The most abundant of the sparsely distributed species (two of the 45 morphotypes) were reared and the emerged adults were identified as *Filatima* sp. (Gelechiidae) and *Itame bitactata* (Walker) (Geometridae), which made up 3 and 2.5% of the total sample, respectively. The other species could not be identified because financial and time limitations precluded a thorough rearing program of all morphotypes, which is necessary for the identification of most microlepidopterans.

Population Trends and Density of Nontarget Lepidoptera. Within-year population trends for *G. ribesella*, *E. gracilariella*, and the entire caterpillar guild of leaf-feeding Lepidoptera on *R. cereum*, were similar in each plot and in each of the 3 yr of the study (Fig. 2). Guild abundance was highest during early July and began to decline quickly near mid-July (Fig. 2 G and H). The density of *G. ribesella* was greatest during late June and declined throughout July (Fig. 2 A and B). *E. gracilariella* was more abundant in July than in June (Fig. 2 C and D). During the period of 5–41 d after the application of *Btk*, the density of all species in the leaf-feeding guild on sprayed *R. cereum* plants was 31% lower than on paired control plants (Table 1). *G. ribesella* and *E. gracilariella* were reduced by an estimated 60 and 23%, respectively. All of these declines were significant ($\alpha = 0.05$, Table 2). The density of the sparsely distributed species peaked during late June in 1997 and 2000, but not until mid-July in 1998 (Fig. 2 E and F). The peak abundance of caterpillars over the 3 yr is shown in Fig. 3. The sparsely distributed species declined by an estimated 12% (Table 1), but this decline was not statistically significant (Table 2).

In Situ Observations on *G. ribesella* and *E. gracilariella*. The size of *G. ribesella* ranged from 1.5 mm after hatch to 15.1 mm before pupation. The size of *E. gracilariella* ranged from 1.3 to 17.2 mm. Both species are yellow in the early larval stages and green in the late larval stages. All instars of *G. ribesella* are solitary and consume leaf edges. The hatching period of *G. ribesella* lasted ≈2–3 wk, from late May until early June. Thus, *G. ribesella* was almost fully emerged and free-feeding when the *Btk* was applied. Hatching of *E. gracilariella* lasted ≈4–5 wk, from early June to early July. Thus, some *E. gracilariella* individuals were present as early instars when the *Btk* was applied but most individuals emerged 5–15 d later. Observations showed that early instars of *E. gracilariella* mine the mesophyll tissue of a single leaf, usually near the outer branch tips. Mid-instars sometimes tie leaves together and remain in the shelter, feeding on the upper surface of one leaf and on the lower surface of the other. Later instars often aggregate as groups of 5–12 individuals, and construct silk shelters, presumably to deter predators and parasitoids. Feeding damage by later instars consists of large, irregular shaped holes in the center of leaves, usually not extending to the leaf edge. Later instars feed both inside and outside their silk shelters.

Mortality of *E. gracilariella* and *G. ribesella*. Microscopic examination of *E. gracilariella* cadavers in the rearings showed that there were no *Btk* infections in the guts of the early (leaf-mining) instars that were reared in 1997. However, *Btk* infection was found in 15 of 65 (23.1%) of the late instar larvae. *Btk* infection was found in 22 of 65 (33.8%) late instar *G. ribesella* larvae. *Btk* infection was absent in larvae collected before the *Btk* application and it was also absent in larvae collected from the reference plot. To our knowledge this is the first study in which larvae that died in the rearings were microscopically examined for the presence of *Btk* infection.

Immediate Impacts of *Btk* on Nontarget Lepidoptera. Our results from larval rearings and our field observations showed conclusively that *Btk* is toxic to both *G. ribesella* and *E. gracilariella*. Their larval populations were reduced by 60% and 23%, respectively (Table 1). The relatively low reduction (23%) of *E. gracilariella* population levels was expected because most individuals did not emerge and begin feeding until 5–15 d after the *Btk* was applied. Because the half-life of *Btk* is typically ≈3–5 d in the field (Beckwith and Steltzer 1987, Sundaram et al. 1997), most *E. gracilariella* larvae likely received only slight (i.e., sublethal) or no exposure to *Btk* spray residues. In

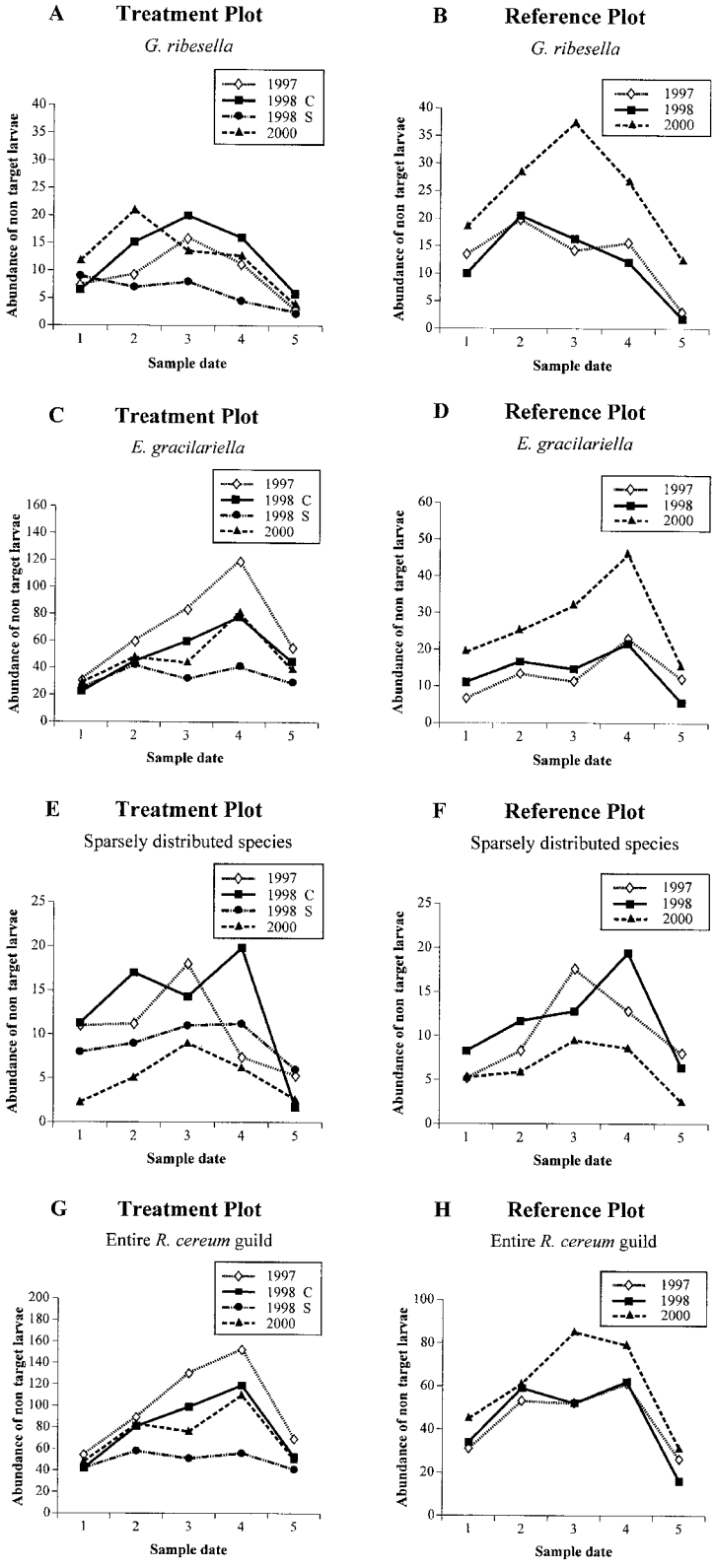

Fig. 2. Number of non–target Lepidoptera larvae collected per 25-min sampling at each sample date in each year of the study (C, covered; S, sprayed). Sample dates were based on insect phenology (see text for details of sample dates).

Table 2. Results of split plot ANOVA: abundance of nontarget Lepidoptera collected from *R. cereum* in the treatment plot in 1998: covered versus sprayed plants, $P = P(F > F_0)$

Lepidoptera group	Source of error	df	Sum of squares	F_0	P
G. ribesella	Whole plot				
	Patch (replicate)	11	63.27		
	Sample date	3	68.38	13.84	0.0000005
	Residuals	33	54.34		
	Subplot				
	Spray (treatment)	1	19.68	12.16	0.0011
	Sample date*spray	3	2.69	0.55	0.6482
	Residuals	44	71.21		
E. gracilariella	Whole plot				
	Patch (replicate)	11	298.05		
	Sample date	3	22.39	0.91	0.4472
	Residuals	33	270.88		
	Subplot				
	Spray (treatment)	1	43.44	8.58	0.0054
	Sample date*spray	3	3.32	0.66	0.5841
	Residuals	44	5.06		
Sparsely distributed species	Whole plot				
	Patch (replicate)	11	10.07		
	Sample date	3	31.42	7.19	0.0008
	Residuals	33	48.04		
	Subplot				
	Spray (treatment)	1	2.81	2.13	0.1514
	Sample date*spray	3	3.09	0.78	0.5114
	Residuals	44	1.32		
Entire *R. cereum* Guild	Whole plot				
	Patch (replicate)	11	164.49		
	Sample date	3	1.90	0.64	0.9647
	Residuals	33	231.38		
	Subplot				
	Spray (treatment)	1	73.24	18.29	0.0001
	Sample date*spray	3	3.83	0.318	0.8121
	Residuals	44			

addition, those individuals that were feeding when the *Btk* was applied were early instars, which feed exclusively on the under surfaces of *R. cereum* leaves where they are unlikely to encounter lethal doses (see Boulton 1999). Other studies on nontarget Lepidoptera (Martinat et al. 1988, Sample et al. 1996, Wagner et al. 1996) have also linked low levels of mortality among microlepidoptera to their tendency to feed in concealed microhabitats. Thus, it appears that the different mortality rates of *G. ribesella* and *E. gracilariella* were the result of differences both in phenology and feeding behavior. The two species may also differ in their physiological susceptibility to *Btk* but we did not conduct laboratory bioassays to test this hypothesis.

The estimated 12% population reduction of the sparsely distributed species group lacks precision because the numbers in the samples were small and highly variable. In addition, some of these species may be generalist herbivores, which may experience different mortality rates on different host plants due to variation in their habitat and diet (Maksymiuk 1970, Smith and Bouse 1981). The spray-droplet collection efficiency of a plant is affected by the size, shape, texture, and orientation of its leaves (Markin 1982, Bora et al. 1994, Falchieri et al. 1995). Consequently, it is ideal when larvae can be collected from more than one host plant species. However, this was not possible in our study because only *Ribes cereum* was consistently a host to a relatively high number of nontarget Lepidoptera of the commonly occurring shrubs in our plots.

The statistically nonsignificant decline observed among the sparsely distributed species may be the result of small and variable sample sizes and should not be interpreted as evidence that most were unaffected by the *Btk* spray. There were probably variable responses to the *Btk* application within this group because toxicity of *Btk* varies greatly among Lepidoptera species, even within genera (van Frankenhuyzen and Fast 1989, Peacock et al. 1993, Ali and Young 1996). The efficacy of *Btk* is also influenced by phenology and many aspects of herbivore behavior (Jepson 1988). Although too few sparsely distributed species were collected to deduce their phenologies, some species, including *Itame bitactata*, appeared to complete their development relatively early in the season, which may reduce their exposure to *Btk*. Several other species, including *Filatima* sp., were present in the feeding stage when *Btk* was applied, and others appeared shortly thereafter. Thus, some of these 'later-feeding' species were likely harmed by the *Btk* spray. In theory, the species most likely to be harmed are those that are both physiologically susceptible to *Btk* and present in

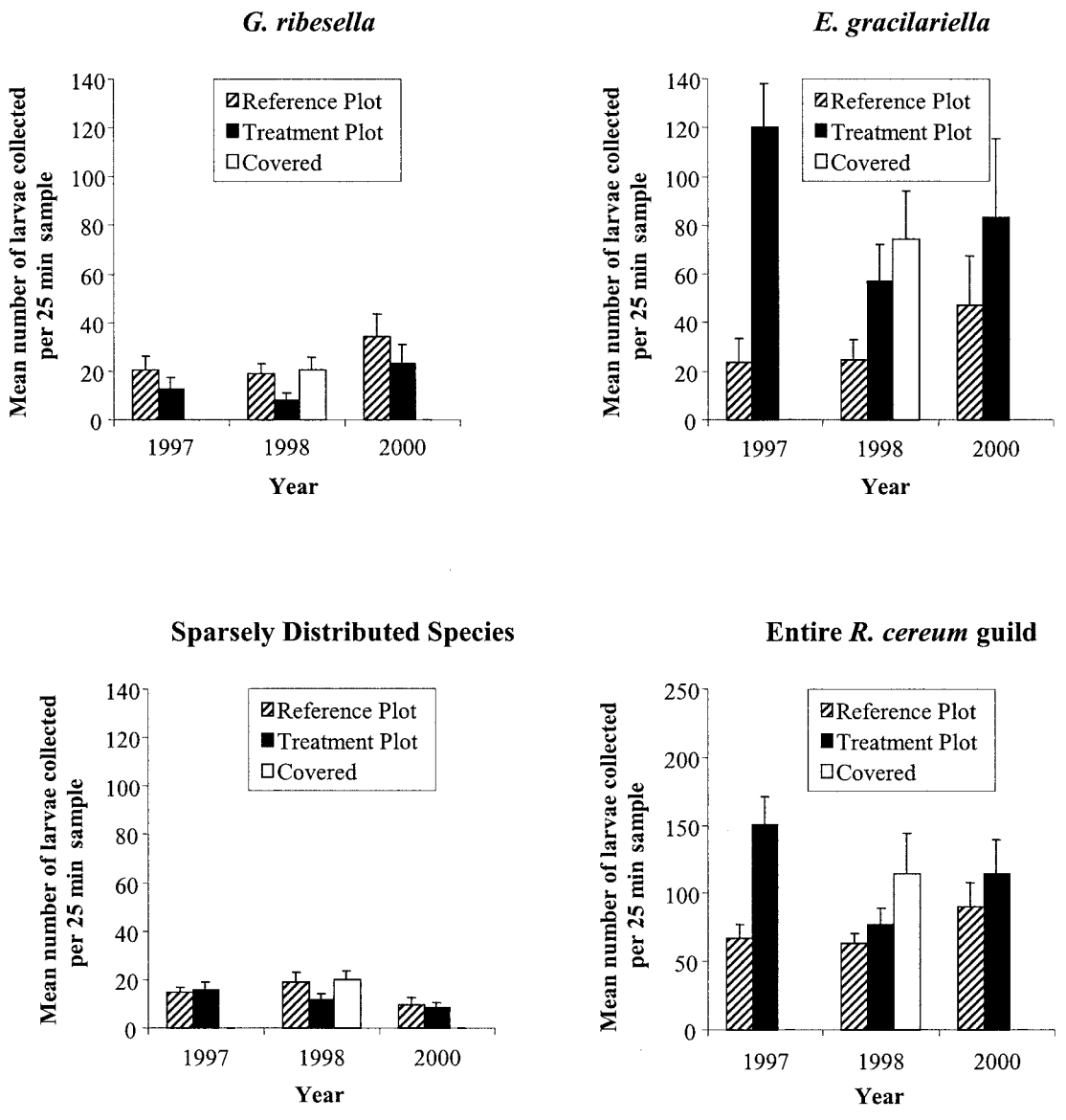

Fig. 3. Mean abundance of nontarget Lepidoptera larvae, collected per 25-min sampling, from *R. cereum* foliage in the treatment plot (both covered and sprayed plants) and the reference plot. Each bar represents the mean, during peak abundance, and the error lines indicate the 95% confidence interval.

the feeding stage when *Btk* is applied. Univoltine species may be at particularly high risk (Miller 1992), especially cohorts that develop with a high degree of synchrony. Multivoltine species, and univoltine species with an extended period of larval emergence, are unlikely to be harmed by *Btk* applications if enough larvae feed outside the time when residues are active (Pascual 1994).

Long-Term Impacts of *Btk* on Nontarget Lepidoptera. The guild-level data collected in 2000 indicates that, as a group, the nontarget Lepidoptera feeding on *R. cereum* had fully recovered to their initial population density in the treatment plot within 2 yr of the *Btk* application. However, analyzing a group of species may conceal important effects on individual species (Aldridge and Carter 1992). Indeed, the apparent recovery at the guild level was the result of the recovery of the two most common species: *G. ribesella* and *E. gracilariella*. Some of the sparsely distributed species may not have recovered to the same degree.

The summer of 1998 was the warmest and one of the driest in Canada on record (Lahn 2000). The average summer (July and August) temperature in Merritt was

3.2°C above normal. Thus, the coincidence of *Btk* application and the drought could have caused some nontarget species to experience particularly severe population reductions. The assessment of *Btk* side effects by Sample et al. (1996) was also confounded by aberrant weather. They believed that hot-dry weather in 1991 followed by cold-moist weather in 1992 had a depressing effect on a nontarget Lepidoptera population in West Virginia.

With only one reference plot and just a single treatment plot, it is impossible to state with certainty if the population reduction of the sparsely distributed species in the treatment plot was coincidental with a natural population reduction, or if it was a carry-over effect of the *Btk* spray (see Stewart-Oaten and Murdoch 1986). However, the occurrence of an even greater decline in the reference plot suggests that this decline was unrelated to the *Btk* treatment.

Limitations, Logistical Constraints, and Sources of Error. The statistical demonstration that a change has occurred after a *Btk* treatment merely indicates the scale or direction of an effect; it does not provide information about the ecological importance of the disturbance (Aldridge and Carter 1992, Grieg-Smith 1992, Thacker and Jepson 1993). Because we did not know the ecological contributions of the affected species, we could not determine the ecological importance of the observed reductions of nontarget Lepidoptera caused by the *Btk* application. Instead, we attempted to determine whether or not the nontarget Lepidoptera populations recovered within 2 yr of being treated with *Btk*, under the assumption that a full recovery indicates less environmental damage than a partial recovery or no recovery.

By physically covering the plants within the treatment plot, we were able to sample with sufficient replication in the year of application. The covers that were placed over *R. cereum* plants were effective at excluding *Btk* spray deposits and, thus, they provided true controls. Covering plants like this might have had some negative effects, but these were probably minimal because the plants were only covered for ≈2 h. Covering plants is only useful for assessing impacts on univoltine species in the year that pesticide is applied. All the insects we have reared are univoltine.

Comparisons between insect populations are valid only if each population is measured at the same phenological stage. This can be difficult to achieve because weather conditions, and hence phenology, can vary drastically among sites, elevation, and between years (Speight and Wainhouse 1989, Johansen 1997). Therefore, it is important to collect samples over many dates. Data sets with too few collection dates are difficult to align because the date of peak abundance cannot be estimated precisely. When a single insect species is monitored over multiple years, it is possible to time the collection dates so that the samples from different years contain insects at similar stages of development. This was achieved for *G. ribesella* and *E. gracilariella*, but it may not have been achieved for each of the sparsely distributed species. Therefore, the between-year comparisons are more accurate for *G. ribesella* and *E. gracilariella* than for among the sparsely distributed species group.

To obtain a complete understanding of the nature and extent of *Btk* side effects on leaf-feeding Lepidoptera as a whole, all nontarget Lepidoptera species, and all of their host plants, would need to be examined. However, such exhaustive studies are usually impossible given the wide range of host plants that occur in forest habitats. Moreover, the majority of forest Lepidoptera occur at very low densities (Magurran 1988) so vast amounts of forest land would have to be treated and sampled to provide sufficient data for each species. For these reasons, research on forest Lepidoptera is often restricted to a single insect species or a group of insects from just one or a few host plants. In the current study, our option was to focus on the foliage-feeding guild (see Simberloff and Dayan 1991) of immature Lepidoptera on *R. cereum.* We believe that our results, even though they were based on the leaf-feeding guild of a single host plant, are representative of how other nontarget Lepidoptera feeding on other host plants, may respond to *Btk* treatment. Our results support the pattern that has emerged from other studies. *Btk* treatment reduces nontarget Lepidoptera in the treated stands. However, most but the rare species will recover in about 2 yr and rebound to their pretreatment population levels.

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