CONSERVATION OF SPRUCE BUDWORM PARASITOIDS FOLLOWING APPLICATION OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* BERLINER

V.G. NEALIS

Forestry Canada, Ontario Region, PO Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

K. VAN FRANKENHUYZEN and B.L. CADOGAN

Forest Pest Management Institute, PO Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

Abstract

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Field trials in northern Ontario in 1990 and 1991 confirmed that survival of spruce budworm larvae [*Choristoneura fumiferana* (Clemens)] (Lepidoptera: Tortricidae) parasitized by *Apanteles fumiferanae* Viereck (Hymenoptera: Braconidae) was greater than survival of nonparasitized budworm larvae following application of *Bacillus thuringiensis* var. *kurstaki* Berliner (*Bt*). Apparent parasitism by *A. fumiferanae* increased slightly when application of *Bt* was early (instar III or IV) but increased significantly when applications were later in the season (instars IV–VI). Delaying the timing of the spray conserved parasitoids while providing significant reductions in budworm densities and not compromising the primary objective of *Bt* application, i.e. the protection of current year foliage. The potential for long-term management of spruce budworm through the strategic integration of natural and imposed mortalities is discussed.

Résumé

Des expériences sur le terrain dans le nord de l'Ontario, en 1990 et 1991, ont confirmé que la survie des larves de la Tordeuse des bourgeons de l'épinette [*Choristoneura fumiferanae* (Clemens)] (Lepidoptera: Tortricidae) parasitées par *Apanteles fumiferanae* Viereck (Hymenoptera: Braconidae) est supérieure à la survie des larves non parasitées à la suite d'un traitement au *Bacillus thuringiensis* var. *kurstaki* (Berliner) (*Bt*). Le parasitisme des larves par l'hyménoptère semble augmenter légèrement lorsque l'application de *Bt* est hâtive (stade III ou IV), mais augmente significativement lorsque les applications sont faites plus tard dans la saison (stades IV–VI). En différant le moment de la vaporisation, il est possible de conserver les parasitoïdes tout en réduisant significativement la densité des tordeuses, donc sans compromettre l'objectif premier de l'application du bacille, soit la protection du feuillage de l'année en cours. La possibilité d'un contrôle à long terme de la Tordeuse des bourgeons de l'épinette par intégration stratégique des mortalités naturelles et imposées fait l'objet d'une discussion.

[Traduit par la rédaction]

Introduction

Understanding the interactions between insecticides and the natural enemies of a pest is necessary for the development of an integrated pest management program. For native forest defoliators such as the spruce budworm [*Choristoneura fumiferana* (Clemens)] and the jack pine budworm (*C. pinus pinus* Freeman) (Lepidoptera: Tortricidae), the natural enemy fauna is rich and has a major influence on the population level of the pest (Miller 1963; Nealis 1991). The use of insecticides for control of these pests could interfere with the beneficial action of the natural enemies. If imposed mortality from insecticides could be combined with the action of natural enemies, there would be a significant, additive impact of the imposed and natural mortalities on pest populations.

Spruce budworm larvae parasitized by *Apanteles fumiferanae* Viereck (Hymenoptera: Braconidae) are less likely to acquire a lethal dose of *Bacillus thuringiensis* var. *kurstaki* Berliner (*Bt*) because these parasitized larvae eat less foliage than do nonparasitized budworm (Nealis and van Frankenhuyzen 1990). In a laboratory population of parasitized and

nonparasitized budworm exposed to *Bt*, there was higher survival of parasitized budworm resulting in higher apparent percentage parasitism by *A. fumiferanae* following exposure to the spray. This effect was enhanced for older budworm larvae, with older parasitoids, because of the increased debilitation and commensurate decreased feeding rate of the budworm larvae with an advanced stage of parasitism.

In this paper, we report field confirmation of the laboratory results of Nealis and van Frankenhuyzen (1990). The results show that the mechanisms elucidated in the laboratory are observable in the field and identify simple modifications to existing control protocols which can be used to integrate imposed and natural mortalities in pest management programs.

Methods

Study Locations. Trials in 1990 were carried out in Nipigon District (49°10′N, 88°40′W), Ontario. In 1991, trials were replicated in Dryden District (50°10′N, 92°55′W), Ontario. In both areas, experimental plots were 60- to 80-ha stands dominated by balsam fir [*Abies balsamea* (L.) Mill.].

Spray Trials. In both years, application of *Bt* was carried out with a Cessna 188 Agtruck equipped with four AU4000 atomizers and flying at 160 km per hour. Each trial utilized three, 60- to 80-ha plots in close proximity. Two plots each received single applications while a third, unsprayed plot served as a control. In 1990, Dipel 352 (Abbott Industries) was applied at 30 BIU (0.9 L per ha) on 7 June (early spray) to one plot and on 16 June (late spray) to the second plot. In 1991, Foray 48B (Novo Nordisk) was applied at 30 BIU (2.36 L per ha) to one plot on 26 May (early spray) and on 5 June (late spray) to the second plot.

Estimation of Insect Density and Parasitism. Samples of overwintering budworm consisted of a single, 1-m branch from the mid-crowns of 10–15 co-dominant trees in each plot. Budworm were either washed off the branches in a caustic soda solution or forced from their hibernacula by incubating the branches at room temperature (Nealis and Lysyk 1988). Density of spruce budworm was expressed as the number of insects per kilogram of foliage. Percentage parasitism was determined by dissecting a random subsample of all budworm retrieved. The density of *A. fumiferanae* was calculated as the density of spruce budworm multiplied by the corresponding proportion of those budworm that were parasitized.

Samples of actively feeding budworm for estimating budworm density and parasitism were obtained by removing a single 45-cm branch tip from each of 20–50 trees per sample date (Nipigon) or two branches from each of 20–25 trees per sample date (Dryden). The specific number of branches collected on any particular sample date reflected the expected number of budworm that would be retrieved. As budworm densities declined through the season, more branches were needed to obtain enough budworm larvae to make a sufficient estimate of parasitism. From these branches, all budworm larvae, pupae, and parasitoid cocoons were picked, counted, and an age determination made. The entire population of collected budworm was dissected and the frequency of parasitized and nonparasitized budworm recorded. Spruce budworm pupae were considered nonparasitized budworm and *A. fumiferanae* cocoons were considered parasitized budworm. Age distribution for the budworm was expressed as the percentage of individuals in each age class (instar II to pupae) and summarized as an average instar (Hardy et al. 1977). Age distribution of the parasitoid was expressed as the percentage of the total parasitoids observed that were in the free-living cocoon stage.

One pre-spray and one post-spray sample were taken for each spray treatment in each year. Pre-spray samples were taken no more than 4 days before the spray except for the pre-spray estimate of parasitism for the early spray in 1990. In that year, budworm density

and parasitism were estimated from the sample of overwintering budworm. Estimates of parasitism by *A. fumiferanae* in overwintering budworm have been shown to be equivalent to those taken after the budworm have established feeding sites in the spring (Nealis and Régnière 1987; Nealis and Lysyk 1988). Samples for post-spray population estimates were taken at least 1 week following the spray to ensure adequate time for mortality from the *Bt* to be expressed in the field.

Bioassays. In the Nipigon trials, an additional collection of budworm larvae was made 24 h after the spray application in each plot for direct confirmation of ingestion of *Bt*. One mid-crown branch was taken from each of 20 trees in each plot. Five shoots were clipped from each of these branches. All budworm larvae were removed from the tips and placed in groups of five on artificial diet (McMorran 1965) in 20-mL creamer cups. The cups with budworm were incubated at 25°C for 3 days. This is sufficient time for full expression of an ingested lethal dose of *Bt* (van Frankenhuyzen and Nystrom 1987). A subsample of dead budworm larvae was smeared and examined with a phase contrast microscope to observe the presence or absence of *Bt* crystals.

Defoliation. Defoliation was estimated at the end of the season using the method of Fettes (1950) from 45-cm branch tips removed from the mid-crowns of 15 trees in each block. Data were analyzed by analysis of variance. Where the analysis indicated significant main effects, pair-wise comparisons using Bonferonni tests were used to separate significantly different means (Dixon 1983).

Analysis. Percentage reduction in the spruce budworm population in the treated plots was calculated using Abbott's formula (Abbott 1925). This calculation was adjusted to accommodate asynchronous sampling (Cadogan 1987). The budworm density estimates used in the calculation were based on an independent sample of budworm collected from 60, 45-cm branch tips per plot, per sample (unpublished data).

Frequencies of parasitized and nonparasitized budworm were analyzed by log-linear models using the log-likelihood ratio (G^2) as the test statistic (Bishop et al. 1975). Successive hierarchial models were compared and inferences were based on the simplest, best-fit model for which the null hypothesis of variables could not be rejected at the $p = 0.01$ level (Bishop et al. 1975). Treatments within trials (early versus late sprays) were analyzed separately. In each analysis, frequencies were classified according to year (1990 or 1991), treatment (spray or control), sample time (pre- or post-spray), and parasitism (yes or no) to form a four-way table. Subsequent lower dimension tables were analyzed to test specific hypotheses. Analyses used the TABLES module of SYSTAT (Wilkinson 1990). Results are illustrated as percentage parasitism by *A. fumiferanae*.

Results and Discussion

Insect Density and Age Distribution. The mean (± SE) densities of overwintering budworm larvae in Nipigon (1990) were 48.2 (3.3) insects per 45-cm branch tip in the control plot and 62.3 (3.0) and 59.8 (2.6) insects per 45-cm branch tip in the treated plots. No overwintering samples were taken from Dryden in 1991 but budworm densities in these trials were lower than in the plots used in 1990. Pre-spray density estimates for the early spray (22 May) were 31.7 (1.8) budworm per 45-cm branch tip in the spray block and 44.5 (2.8) in the control. Pre-spray density estimates for the late spray (4 June) were 21.2 (1.7) budworm per 45-cm branch tip in the spray block and 22.2 (1.5) in the control.

The age distribution of the budworm population and the percentage of the *A. fumiferanae* population which had emerged from their hosts and were represented by cocoons at the time of the sample are shown for each spray event in Table 1. The age-classes of budworm at the time of each spray were advanced compared with those used in the laboratory studies of Nealis and van Frankenhuyzen (1990). Despite this, there was

Table 1. Sample size and age distributions of spruce budworm and its parasitoid, *Apanteles fumiferanae*, in pre- and post-spray samples in treated (early and late sprays) and control (no spray) plots in (*a*) Nipigon (1990) and (*b*) Dryden (1991) districts, Ontario

Date	Plot	Sample size	Percentage budworm in each age class						Average instar	Percentage cocoons
			II	III	IV	V	VI	Pupae		
(*a*) Nipigon 1990										
4 June	Control	200	—	—	—	—	—	—	—	—
	Early (pre-spray)	200	12	37	38	12	1	0	3.5	0
15 June	Control	494	0	6	38	47	9	0	4.6	2
	Early (post-spray)	410	0	14	53	31	2	0	4.2	3
	Late (pre-spray)	254	0	7	35	52	6	0	4.6	4
22 June	Control	223	0	0	16	10	74	0	5.6	45
	Late (post-spray)	239	0	0	29	23	47	0	5.1	34
(*b*) Dryden 1991										
24 May	Control	690	1	25	48	26	0	0	4.0	0
	Early (pre-spray)	543	1	21	54	24	0	0	4.0	0
3 June	Control	318	0	4	18	10	67	0	5.4	25
	Early (post-spray)	77	0	8	49	24	19	0	4.5	37
	Late (pre-spray)	324	0	2	24	17	57	0	5.3	25
12 June	Control	103	0	0	7	8	61	24	6.0	77
	Late (post-spray)	47	0	0	6	4	60	30	6.1	97

still a definite separation in ages between the early sprays for which the majority of budworm larvae in the pre-spray sample were instar IV or earlier, and the late sprays for which the majority of budworm larvae in the pre-spray sample were instars V and VI (Table 1). Less than 5% of the *A. fumiferanae* population had already emerged from the host at the time of the early sprays whereas by the time of the late sprays, emergence of the parasitoid from the host was well under way (Table 1).

Bioassay. The presence of sporulating *Bt* cells in cadavers from the spray blocks demonstrated that a lethal dose of *Bt* was ingested by at least 50% of the budworm population in both spray blocks. No *Bt* cells were observed in budworm from the control (no spray) plots.

Parasitism. As expected, the observed percentage parasitism of spruce budworm larvae by *A. fumiferanae* in the control plots did not change greatly over the observation period in either trial [(Fig. 1) **1990**: $G^2 = 2.57$, 2 df, $p = 0.28$; **1991**: $G^2 = 7.33$, 2 df, $p = 0.03$]. The reliability of this estimate of parasitism in budworm populations during the budworm's larval period has been documented by others (Nealis and Régnière 1987; Nealis and Lysyk 1988).

Early Sprays. The four-way interaction among year, treatment, sample time, and parasitism was not significant ($G^2 = 0.01$, df = 1, $p = 0.906$); there were slight increases in parasitism after the early sprays in both 1990 and 1991 (Fig. 1, open symbols). Nor was the three-way interaction among treatment, sample time, and parasitism significant in either year (**1990**: $G^2 = 4.01$, df = 1, $p = 0.045$; **1991**: $G^2 = 0.95$, df = 1, $p = 0.33$). The three-way interaction among year, sample time, and parasitism, however, was significant ($G^2 = 14.05$, df = 1, $p < 0.01$). The increase in parasitism in the spray plot following the early spray in 1991 was greater than the increase in the spray plot in 1990. This greater increase in parasitism observed following the early spray in 1991 compared with 1990 may reflect the relatively more advanced age distribution of the host and its parasitoid at the time of the spray. The average instar of the budworm host at the time of the early spray in 1991 was 4.0 compared with an average instar of 3.5 for the corresponding estimate in
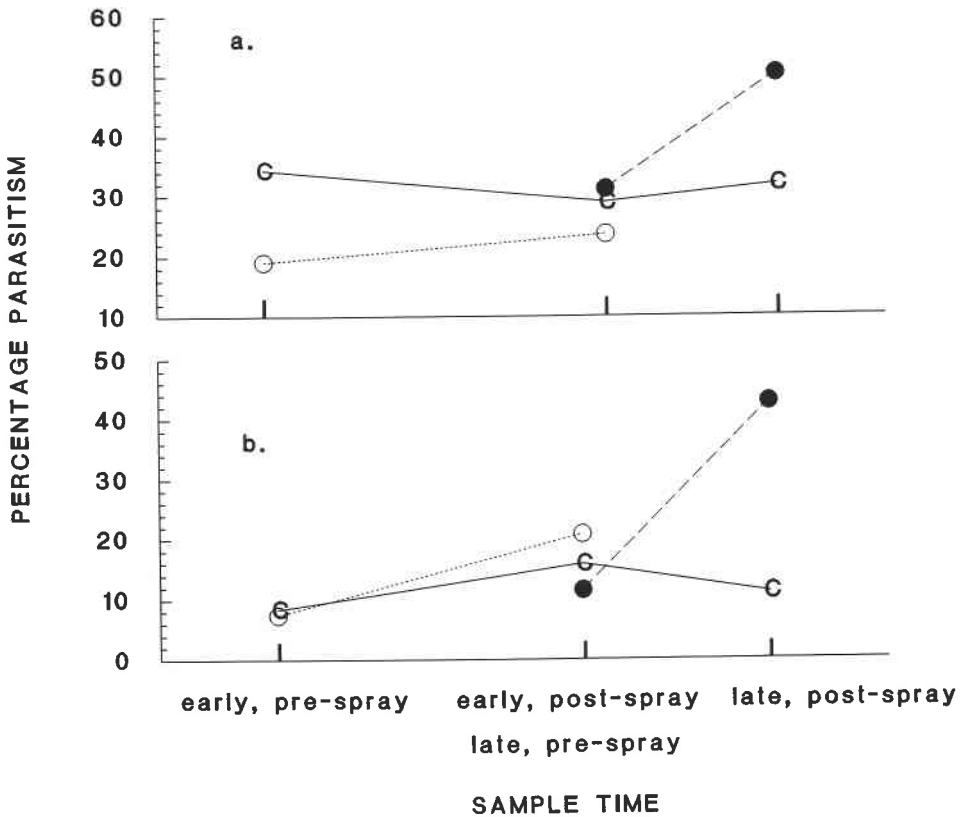
FIG. 1. Pre- and post-spray estimates of percentage parasitism of spruce budworm populations by *Apanteles fumiferanae* in experimental plots in (*a*) Nipigon (1990) and (*b*) Dryden (1991) districts. Open symbols are early spray treatments, closed symbols are late spray treatments, and C is the control. Sample sizes are in Table 1.

1990 (Table 1, pre-spray sample, early spray). As Nealis and van Frankenhuyzen (1990) have shown, the later the spray and the older the host larvae, the more advanced the parasitism and greater the differential response in survival.

Late Sprays. The prediction of increased survival of parasitized budworm following application of *Bt* was dramatically confirmed in the late spray plots in both years (Fig. 1, closed symbols). The four-way interaction among year, treatment, sample time, and parasitism was significant ($G^2 = 10.1$, df = 1, $p<0.01$) indicating that the increase in parasitism in the post-spray sample from the sprayed plot was different in each replicated trial. In fact, the increase was greater in 1991 than in 1990 (Fig. 1). As with the early spray treatment, the age distribution of the budworm at the time of the late spray treatment was advanced in 1991 relative to 1990. The average instar of spruce budworm at the time of the pre-spray sample for the late spray treatment was 5.3 in 1991 and only 4.6 in 1990 (Table 1). Once again, the enhanced effect of differential survival of parasitized budworm in 1991 was probably due to the relatively advanced age distribution, and therefore stage of the parasitism, at the time of the spray.

Parasitism of other budworm species following *Bt* treatment has shown no effect (Niwa et al. 1987) or a small effect (Hamel 1977) of the treatment. In those studies, parasitism was estimated by rearing budworm larvae and recording parasitoid emergence.

Table 2. Percentage reduction in budworm density in plots treated with *Bt* and percentage defoliation in control and treatment plots for both spray trials in 1990 and 1991. Estimates of percentage defoliation followed by different letters are different at the $p<0.01$ level within years

Treatment	Plot	Percentage reduction (Abbott's formula)	Percentage defoliation (Fettes' method)
(*a*) **Nipigon 1990**			
Early spray	*Bt*	34.0	87.5 a
	Control	—	87.5 a
Late spray	*Bt*	56.3	70.3 b
	Control	—	87.5 a
(*b*) **Dryden 1991**			
Early spray	*Bt*	74.3	18.7 a
	Control	—	42.2 b
Late spray	*Bt*	93.4	28.6 c
	Control	—	42.2 b

In our study, emphasis was placed on sampling the entire immature population of the host and the parasitoid. Because the proportion of the total parasitoid population that has already emerged from their hosts and is represented by cocoons can be quite high in post-spray samples (Table 1), our more comprehensive sampling method accounts for any sampling bias arising from the natural phenology of the parasitoid. The resulting estimate is more accurate and more consistent with respect to treatment.

Foliage Protection. A recommendation to delay spray application to conserve parasitoids raises the possibility of increased defoliation resulting from the delay. Table 2 shows that in both years, the percentage reduction in spruce budworm densities is actually greater in the late spray treatments than in the early spray treatments. These reductions in budworm densities resulted in lower levels of defoliation in the late spray plots relative to the controls (Table 2). In 1990, in fact, defoliation in the late spray plot was less than in the early spray plot. Thus, in our case, delayed application of *Bt* did not seriously compromise the objective of foliage protection. Of course, defoliation is a function of initial budworm density as well as mortality resulting from *Bt* and parasitoids. Higher overall densities of spruce budworm and lower reductions due to *Bt* resulted in higher levels of defoliation in treated and untreated plots in 1990 compared with 1991 (Table 2).

The greater reduction in budworm densities observed in the late spray plots may be explained by the same mechanism of differential survival invoked to explain conservation of parasitoids. Large budworm larvae and nonparasitized budworm larvae eat more foliage than either small or parasitized larvae. They are therefore more likely to encounter and ingest *Bt*. Additional, indirect evidence for this can be found in Table 1. Note that post-spray samples in early spray treatments in both years revealed retarded age distributions relative to the unsprayed blocks (control and late plots, pre-spray). This difference in age distribution following the spray could be explained by sublethal effects of *Bt* on the rate of development of exposed budworm (Fast and Régnière 1984; van Frankenhuyzen and Nystrom 1987). An alternative hypothesis is that the altered age distribution following *Bt* application is the result of proportionately higher mortality in the older, larger larvae. This hypothesis would not only explain the observed age distribution but, once again, would attribute it to the common mechanism of differential survival of those budworm that, either because of size (i.e. age) or parasitism, are eating little foliage at the time of the spray.

Carry-over Effects. By comparing the density of *A. fumiferanae* in the Nipigon plots in the winters before and after the 1990 spray program, we were able to determine any carry-over effect of the altered levels of parasitism to the year following the spray program. Table 3 provides estimates of the densities and percentage parasitism of *A. fumiferanae*

Table 3. Density (parasitoids per kg of foliage) and percentage parasitism of overwintering spruce budworm larvae by *Apanteles fumiferanae* in Nipigon before (1990) and after (1991) application of *Bt*

Plot	1990		1991	
	Parasitoids per kg foliage	Percentage parasitism	Parasitoids per kg foliage	Percentage parasitism
Early spray	94.5	19.0	7.6	5.3
Late spray	148.7	27.5	12.8	12.6
Control	145.1	34.3	29.1	14.4

in the study plots in 1990 and 1991. The density and percentage parasitism of *A. fumiferanae* decreased markedly in all plots between 1990 and 1991 for reasons that are unknown. This decline, however, was least in the control plot where there had been no imposed mortality on either the budworm or the parasitoid. The rank order of percentage parasitism was unchanged between years. The decline in density of *A. fumiferanae* in both spray plots, however, may have obscured the potential difference between spray treatments.

The possibility of a carry-over effect presumes that the net between-generation movement of both the parasitoid and its host is minimal, or that movement is at least confined to the scale on which the spray operation was carried out. Adult female *A. fumiferanae* exhibit limited movement once oviposition begins (Nealis 1988). In contrast, however, movement by the budworm moth can be extensive so that the probability of observing any carry-over effect is by no means certain. Critical examination of the effective scale for control methods is a necessary next step to optimize the long-term management possibilities of this, or any other, tactic.

Conclusions

These results confirm the predictions made from the laboratory studies of Nealis and van Frankenhuyzen (1990) and demonstrate the value of developing specific hypotheses for large-scale field experiments based on mechanisms observed in the laboratory. The results also show that the use of basic biological information can be readily converted into pest management programs with potentially little modification of existing practices and minimum compromise to existing objectives. Finally, the results suggest that there is an experimental basis for emphasizing the possibility of manipulating budworm populations over longer time periods than are currently considered.

Acknowledgments

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