AERIAL APPLICATION OF HIGH DOSAGES *of* NUCLEAR POLYHEDROSIS VIRUS TO EARLY INSTAR SPRUCE BUDWORM, *Choristoneura fumiferana (Clem).*

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ABSTRACT

Two 20 ha plots located in the Gaspé region of Quebec were treated with spruce budworm, *Choristoneura fumiferana* (Clem.), nuclear polyhedrosis virus (NPV). Plot 1 received a single application of 1.15×10^{12} polyhedral inclusion bodies (PIBs)/ha in an emitted volume of 4.5 L/ha when second instar larvae were emerging from hibernacula. Plot 2 received two applications of NPV. The first treatment of 3.44×10^{12} PIBs/ha was applied as second instar larvae were emerging from hibernacula; the second treatment of 2.3×10^{12} PIBs/ha was applied 26 days later, when balsam fir buds had flushed and 94% on the larvae were in their third instar. Both treatments were applied in an emitted volume of 4.5 L/ha.

Assessment of the population reduction due to treatment and defoliation showed no significant differences ($p = 0.05$) between the two treated plots and an untreated check plot. However, incidence of NPV infection, confirmed by microscopic diagnosis of smeared larvae, reached $22.9 \pm 18.5\%$ of larvae infected in plot 2, which was significantly higher than either plot 1 ($10.3 \pm 13.7\%$) or the check plot ($6.9 \pm 14.4\%$). Incidence of infection with the microsporidian parasite, *Nosema fumiferanae*, remained relatively stable in all three areas with approximately 60% of the larvae infected. NPV infection in the check plot was attributed to a low level, persistent, naturally occurring virus.

These dosages of NPV were unsuccessful in protecting foliage or achieving insect control by initiating an epizootic. Early application of NPV does not appear to be a viable control strategy. Even higher dosages of NPV may be required to initiate epizootics in spruce budworm populations.

RÉSUMÉ

2 parcelles de 20 ha situées dans la région de Gaspé au Québec ont été traitées a l'aide de virus de la polyédrose nucléaire (VPN) pour combattre la tordeuse des bourgeons de l'épinette (*Choristoneura fumiferana* [Clem.]). La parcelle 1 a été soumise a une seule pulvérisation de $1{,}15 \times 10^{12}$ corps d'inclusion polyédrique (CIP)/ha à raison de 4,5 L/ha, au moment ou les larves au deuxième stade émergeaient de leur hibernacle. La parcelle 2 a été soumise a deux pulvérisations de virus; la première, de $3{,}44 \times 10^{12}$ CIP/ha, a été également effectuée au moment ou les larves au deuxième stade émergeaient de l'hibernacle et la deuxième, de $2{,}3 \times 10^{12}$, 26 jours plus tard, après l'eclosion des bourgeons de sapin baumier au moment ou 94% des larves en étaient a leur troisième stade. Les deux traitements ont été appliques à raison de 4,5 L/ha.

Après évaluation de la diminution de la population attribuable au traitement et de la défoliation, il est apparu qu'il n'y avait aucune différence significative ($p = 0{,}05$) entre les deux parcelles traitées et une parcelle-témoin non traitée. Toutefois, l'infection par les virus, confirmée par un examen des larves (frottis) au microscope, atteignait $22{,}9 \pm 18{,}5$ % des larves dans la parcelle 2, ce qui est beaucoup plus que dans la parcelle 1 ($10{,}3 \pm 13.7$ %) ou dans la parcelle-témoin ($6.9 \pm 14{,}4$ %). L'incidence de l'infection par le parasite microsporidien *Nosema fumiferanae* est demeurée relativement stable dans les trois parcelles, ou environ 60 % des larves ont été infectées. L'infection par les VPN dans la parcelle-témoin a été attribuée a de faibles concentrations naturelles et persistantes des virus.

Ces doses de virus n'ont pas réussi a protéger le feuillage ou à éliminer les insectes en déclenchant une épizootie. La pulvérisation de virus aux premiers stades larvaires de la tordeuse ne semble donc pas constituer une stratégie de lutte viable. Il se pourrait qu'il faille utiliser des doses de virus plus fortes encore pour provoquer des épizooties dans les populations de tordeuses des bourgeons de l'épinette.

INTRODUCTION

Nuclear polyhedrosis virus, (NPV) was applied to populations of spruce budworm, *Choristoneura fumiferana* (Clem.), between 1959 and 1981 to evaluate such parameters as timing of application, dosage and composition of formulation (Cunningham and Howse 1984; Cunningham 1985). This research indicated that NPV offers no simple solution to the spruce budworm problem in Canada. Evidence suggests that although spruce budworm NPV becomes established in the treated population, it neither spreads outside the research area by way of epizootic nor does it persist from one year to the next to cause sufficient insect mortality. Studies of naturally occurring NPV epizootics in some sawflies indicate that very large quantities of virus are released into the environment and this affects both the epizootic potential and the persistence of the virus from one year to the next (Kaupp 1983). No NPV epizootic has been observed in spruce budworm populations and, presumably, any NPV present persists at low levels. Hence, we decided to treat early instar spruce budworm with substantial dosages of NPV at emergence and again at budflush in an attempt to create a disease epizootic to provide some degree of population reduction and foliage protection.

MATERIALS AND METHODS

The Virus

The spruce budworm NPV was produced at FPMI. Fourth instar spruce budworm larvae were infected with NPV by surface treatment of artificial diet (McMorran 1965), and reared until moribund (Otvos *et al.* 1989). Infected larvae were harvested, frozen, lyophilized and ground to a fine powder using a commercial blender. This powder, containing spruce budworm NPV, was the active ingredient used in this spray operation.

Some of the virus was produced by infecting western spruce budworm, *Choristoneura occidentalis*, with eastern spruce budworm NPV because of the large quantity of material required. This species is easier to handle because it produces less webbing in rearing containers. Virus harvested from these insects is identical to the spruce budworm NPV used for inoculation. All preparations were standardized by determining the number of polyhedral inclusion bodies per gram (PIBs/g) of dry material using a dry counting procedure (Wigley 1980).

The Study Area and Plot Layout

Suitable infestations of spruce budworm were located in the Gaspé region of the province of Québec. Two treated blocks (20 ha each) and one check area were located on forest access roads approximately 15 km south east of St. Fabian, Que. (Fig. 1). The treated blocks were approximately 5 km from provincial spray blocks No. 109, 601 and 201.

Fifteen balsam fir 8-12 m tall, were located on each of three sample lines running perpendicular to proposed flight lines in each plot (Fig. 2). Kromecote cards were placed along cleared sample lines, and near each sample tree, to monitor spray deposit. During spray applications, plot boundaries and spray swaths were marked with large red weather balloons. A check area consisting of 45 balsam fir trees, was established nearby.

Larval Development and Tree Phenology

Larval development was assessed by determining the instar of all larvae found on 5 branches collected periodically from each plot. Time of emergence of larvae onto the branches was determined by visual

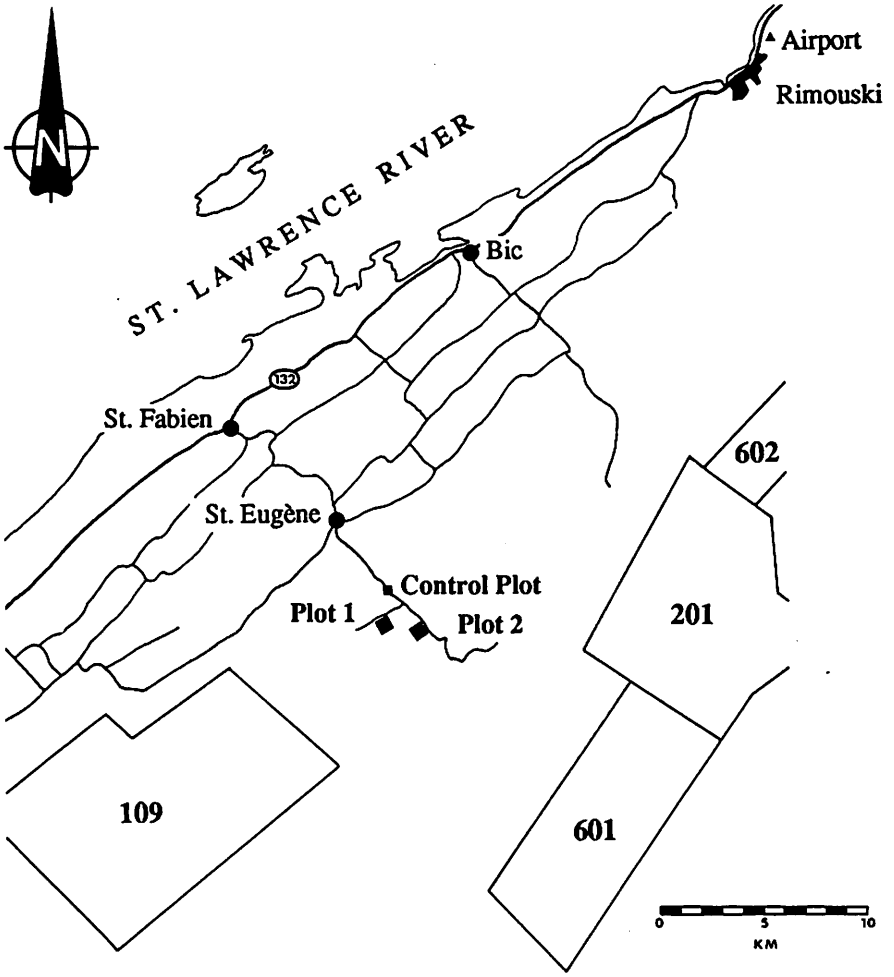

Figure 1. Location of the two treated plots and single check plot.

inspection of foliage throughout the plot. Branches were also examined for budflush, defined as the time at which 90% of the bud caps had dropped from the new shoots.

Formulation and Spray Application

A dosage of 1.0×10^{12} PIB/ha, the highest dosage ever applied to budworm populations, was selected as a treatment for plot 1. It was presumed that the budworm emerging from the hibernacula were more susceptible to NPV than older larvae, and would easily contract infection when mining NPV-contaminated needles. Death of these larvae would provide inoculum to initiate an NPV epizootic in the budworm population. It was intended to treat plot 2 with three times this dosage (3.0×10^{12} PIB/ha) as larvae were emerging and again with twice this amount (2.0×10^{12} PIB/ha) at budflush in an attempt to create an epizootic by inundating the environment with NPV.

The powder was applied in a 25% emulsifiable oil formulation. The lyophilized insect material was mixed with Dipel 88 blank gelled oil carrier (Abbott Laboratories) using a Kalish turbo homogenizer,

and then strained through a 40 mesh sieve. Prior to spray application the oil-virus mixture was added to an appropriate quantity of water and pumped into the aircraft hopper. Erio Acid Red XB 400 was added at 1 g/L as a marker for deposit analysis. Tank samples were collected to confirm the PIB concentration of each formulation.

A Cessna 188B Agtruck equipped with 4 AU 3000 Micronair rotary atomizers was used for the applications. Variable restrictor units were set at 4 and blade angle was set at 30° to achieve an application rate of 4.5 L/ha. The aircraft flew at a height of 18-20 m above tree top level; the swath width was 30 m. Meteorological data were collected during the spray operation.

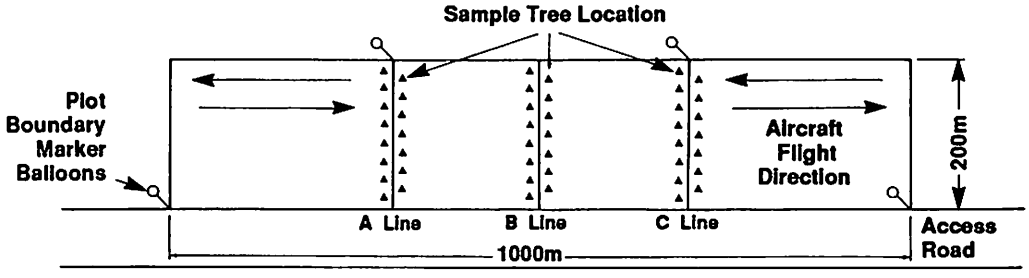

Figure 2. The 20 ha plots showing the location of 3 sample lines with 15 trees/line; sample lines were perpendicular to aircraft flight lines.

Application Conditions

Plot 2 received the first application of NPV between 1832 and 1836 h on May 14th when second instar budworm were observed wandering over the foliage in low numbers. Wind speed averaged 9 km/h, with 63% RH and a ground temperature of 17°C. Analysis of tank samples confirmed application of 3.44 x 10^{12} PIBs/ha. Plot 1 was sprayed with a dosage of 1.15 x 10^{12} PIBs/ha that same evening between 1921 and 1926 h, with 76% RH and a wind speed of 2 km/h. On June 9, 26 days later, plot 2 received a second application of 2.3 x 10^{12} PIBs/ha when balsam-fir buds had flushed and 94% of the larvae were in third instar. Virus was applied between 2035 and 2041 h with 62% RH and a windspeed of 3 km/h. The ground temperature was 10°C.

Deposit Sampling

Kromekote cards and glass slides on aluminum backings, were placed on stakes beside each sample tree (Randall 1980). After allowing 30 minutes for spray droplets to impact, these sampling devices were collected from the spray blocks. The Kromekote cards were analyzed to characterize the spray cloud, while deposition on the glass slides was analyzed to confirm the application rate (Retnakaran *et al.* 1973).

Assessment of Spray Efficacy: Population Reduction

A pre-spray sample, collected May 14, and 4 post-spray samples, collected June 5, 16, 23 and 30 respectively, were used to determine population reduction due to treatment. To determine the number of early instar spruce budworm present, two 90-cm branches were removed from the mid-crown of

Table 1. Percentage spruce budworm larvae found to be infected with NPV in the pre-spray and four post-spray collections. Numbers in parenthesis indicate sample size.

	May 14 (pre-spray)	June 5	June 16	June 23	June 30
Plot 1	1.38 ± 1.33* (7880)	5.5 ± 5.24 (1637)	5.5 ± 5.4 (952)	4.3 ± 4.9 (755)	10.3 ± 13.7 (647)
Plot 2	1.09 ± 1.29 (7022)	4.13 ± 3.61 (9198)	2.7 ± 4.5 (2872)	15.1 ± 16.2 (1430)	22.9 ± 18.5 (1142)
Check	1.74 ± 1.66 (7498)	1.53 ± 1.42 (8608)	0.41 ± 0.87 (2219)	1.9 ± 3.9 (1884)	6.86 ± 14.4 (1246)

* represents standard deviation.

each sample tree for the pre-spray and first post-spray samples, and placed in emergence boxes (Randall *et al* 1981). The number of second instar budworm trapped in these boxes represented the number of budworm expected to move to the outer limits of the branches later in the season. These were assessed by subsequent samples.

The last three post-spray samples consisted of two 45-cm branches taken from each tree and threshed inside cardboard drums to remove budworm larvae (Martineau and Benoit 1973). Total number of larvae obtained from each branch examined in the pre- and post-spray samples was recorded and averaged to give a population estimate for each tree. Treated plot and check plot population densities were calculated by averaging data from the 45 sample trees.

Incidence of NPV was assessed by the examination of stained insect smears for the presence of PIBs. Larvae collected from both treated plots and the check area were aseptically smeared on glass slides using toothpicks, and the smears were allowed to air dry. These smears were stained in 1.5% w/w solution of Naphthalene Black 12B in 40% glacial acetic acid at 40-45°C. After 5 minutes the slides were removed, rinsed in tap water and air dried. Each smear was examined using oil immersion optics. Smears containing the microsporidian parasite, *Nosema fumiferanae*, were also recorded.

All larvae found in samples from plot 2 and the check area were diagnosed for NPV infection and the presence of the microsporida. In plot 1, all larvae found in the pre-spray sample, and approximately 20 larvae from each sample tree in the post-spray collections were examined. All infection results were expressed as averages for the plot along with the standard deviations.

Assessment of Spray Efficacy: Defoliation

Two branches were collected from each tree in the treated plots and the check area. Defoliation due to spruce budworm was estimated using a modification of the Fettes' method (Cadogan *et al* 1984).

Statistical Analysis

Tests of significance ($p = 0.05$) were conducted using the pooled variance method described by Snedecor and Cochran (1967).

RESULTS

Deposit Analysis

Examination of the Kromekote cards indicated that plot 1 received an average of 34±16 drops cm^2, with a VMD of 57mm and an NMD of 18mm. Of the two sprays applied to plot 2, the first application was best providing 9±9 drops/cm^2 with a VMD and NMD of 67mm and 20mm, respectively. The second application provided 4±4 drops/cm^2 with a VMD of 69mm and NMD of 51mm. Spectrophotometric analysis of the deposit on the glass slides indicated that relatively low deposits were obtained on both plots, with plot 1 receiving 0.54±0.35 L/ha and plot 2 receiving 0.16±0.23 L/ha for the first and 0.16±0.14 L/ha for the second application, respectively.

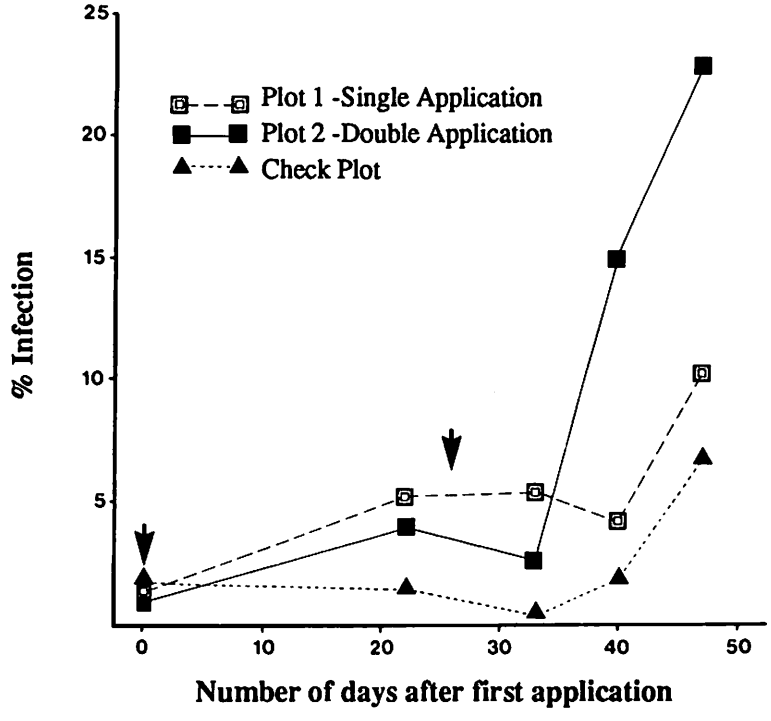

Figure 3. Incidence of NPV infection observed in spruce budworm larval samples collected from plot 1, plot 2 and the check plot. Arrows indicate spray applications.

Incidence of Infection

A low incidence of naturally occurring NPV was found in the pre-spray samples of larvae collected from the two treated plots and the single check plot. In plots 1 and 2, 1.38±1.33% and 1.09±1.29% of the emerging second instar larvae were found to be infected with NPV respectively, while 1.74±1.66% infection was observed in larvae collected from the check plot (Table 1, Fig. 3). These values are not significantly different. Naturally occurring NPV continued to be observed in the check area during three consecutive post-spray samples at significantly lower levels than found in the treated plots. In the

final sample, collected 47 days post-spray, 6.86±14.4% of the insects in the check area were infected with naturally occurring NPV.

Incidence of NPV infection in the treated plots was found to be significantly higher than in the untreated check area, with the exception of the last two samples in plot 1. The incidence of infection in the single and double applications was only significantly different in the last two samples (Fig. 3).

Table 2. Percentage spruce budworm larvae found to be infected with *Nosema fumiferanae* in the pre-spray and four post-spray collection.

	May 14 (pre-spray)	June 5	June 16	June 23	June 30
Plot 1	48.49 ± 7.3*	47.6 ± 10.5	22.3 ± 8.9	50.0 ± 10.6	72.9 ± 12.1
Plot 2	51.59 ± 8.36	66.7 ± 10.4	58.6 ± 12.4	60.9 ± 11.7	67.0 ± 12.5
Check	48.9 ± 8.2	69.6 ± 10.3	62.1 ± 12.2	66.3 ± 17.0	65.6 ± 15.3

* represents standard deviation.

Incidence of microsporidian infection remained relatively static in all treated plots and the check plot, with no significant difference observed between the pre- and final post spray percentages (Table 2, Fig.4). However, a significant drop in the incidence of the parasite was observed in plot 1 at the second post–spray sample. Pre- and post-spray samples were not significantly different from each other.

Population Reduction

There was no significant difference in the number of second and third instar larvae recovered from emergence boxes holding branches collected for the pre–spray and first post-spray samples (Table 3), or between larval density per 45-cm branches in the final three post-spray samples, except for an unexplained and significant drop in the budworm population in plot 2 as compared to plot 1 on June 23.

Defoliation

Examination of balsam fir branch samples indicated no significant difference in the percentage of defoliation between plot 1 (83.9±19.7%), plot 2 (84.0±16.9%) and the check plot (85.9±17.1%).

CONCLUSION AND DISCUSSION

Application of large quantities of spruce budworm NPV either in a single treatment at the emergence of second instar, or in two applications, with the second at budflush, did not significantly reduce the insect population or protect foliage. Obviously, a major disease epizootic was not initiated even after introducing 5.74 x 10^{12} PIBs/ha into a dense population of spruce budworm. Failure to achieve any

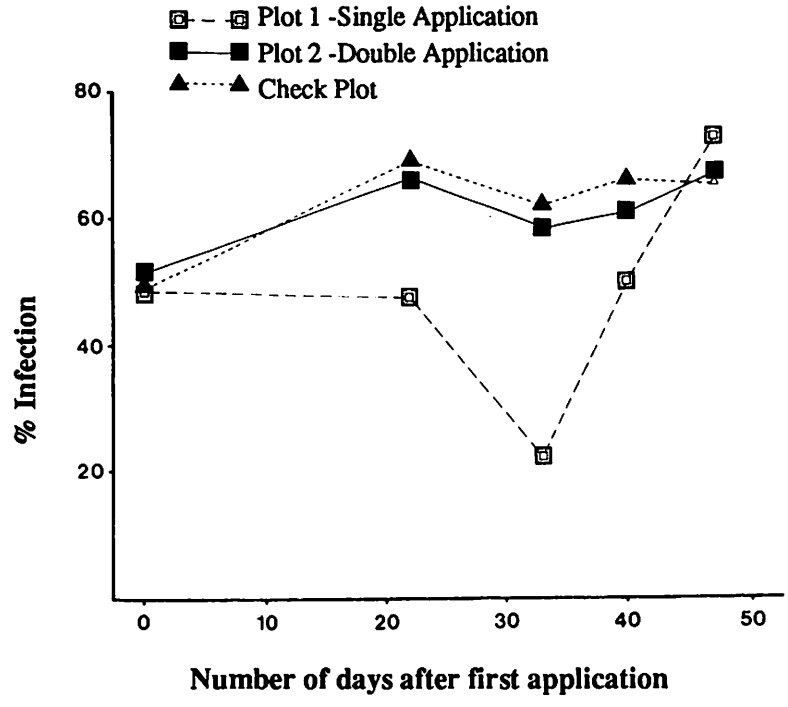

Figure 4. Incidence of infection with microsporidia, *Nosema fumiferanae*, observed in spruce budworm samples collected from plot 1, plot 2, and the check plot.

appreciable protection may be the result of several factors; the spruce budworm population density was too high for this type of efficacy trial, application strategies used in this research were not suitable for disseminating NPV, or not enough NPV was introduced to initiate a NPV disease epizootic.

NPV epizootics are a density-dependent phenomenon and are usually the result of a combination of inter-related factors (Entwistle 1986). Although NPV epizootics have been recorded in insect populations at outbreak levels, there is speculation that with a dense host population, conditions are unsuitable for the NPV to become the key factor in population reduction (Kaupp 1981). This probably occurs because the virus is not available as infectious inoculum long enough to initiate the primary infection cycle.

Application of NPV at second instar emergence gave poor results. Any pesticide applied primarily to affect early instar budworm must be accurately timed if it is to be effective. With viruses, this timing is even more critical because the insect is accessible for only a short period prior to needle mining; the virus remains active on the foliage for only three days (Kaupp, unpublished). Early application of NPV should be considered as a viable strategy only after we have acquired a better understanding of how weather influences emergence and dispersal of second instar budworm.

It is impossible to tell if a higher dosage of virus would create an epizootic. From the results of this research trial, it is apparent that a higher dosage of virus did significantly increase the incidence of infection in the spruce budworm population. It is generally accepted that a greater concentration of

Table 3. Number of spruce budworm larvae per blasam fir branch on May 14 and June 5, and collected from 45-cm branch-tips on June 16, June 23 and June 30.

	May 14 (pre-spray)	June 5	June 16	June 23	June 30
Plot 1	110.4 ± 8.2*	93.52 ± 7.5	33.8 ± 3.1	27.2 ± 3.3	11.5 ± 1.3
Plot 2	99.7 ± 8.8	112.4 ± 8.8	32.0 ± 2.6	13.0 ± 1.5	13.9 ± 1.2
Check	93.2 ± 6.7	104.2 ± 8.1	29.2 ± 2.5	19.3 ± 1.9	15.9 ± 1.4

* represents standard deviation.

NPV is more effective when used for insect control, but in dealing with a dense population of spruce budworm as in this case, defoliation will inevitably occur because of the 10-14 day incubation period of infection during which the larvae can feed before succumbing to NPV. Nevertheless, this spruce budworm population did have a naturally occurring NPV infection, which increased in incidence only when the larvae were about to pupate. The effect of this infection as a population limiting factor is unknown.

In conclusion, it is noted that little population reduction or foliage protection was achieved after 5.74 x 10^{12} PIBs/ha was introduced into a spruce budworm population. Significant differences in incidence of NPV infection between treated areas were due to differences in the dosage of NPV applied. We recommend that future work be directed at investigating how the naturally occurring NPV infection can be more efficiently used for population reduction; initially by assessing the impact of natural infection on budworm populations and then by manipulating the persistence and infectious nature of the virus to increase its effectiveness as a biocontrol agent.

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