

VIRUSES TO CONTROL WINTER MOTH, *OPEROPHTERA BRUMATA* (LEPIDOPTERA: GEOMETRIDAE)

J. C. CUNNINGHAM¹, N. V. TONKS² AND W. J. KAUPP¹

¹Forest Pest Management Institute
Canadian Forestry Service
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7

and
²Agriculture Canada
Saanichton Research Station
Sidney, B.C.
V8L 1H3

ABSTRACT

An abandoned apple orchard in Victoria, British Columbia, was used to test winter moth nuclear polyhedrosis virus (NPV) in 1979. Concentrations of 10^8 , 10^7 and 10^6 polyhedral inclusion bodies (PIB)/ml were applied at the rate of 1 l/tree using a backpack mistblower, soon after the larvae hatched when buds were pre-pink and 8 days later when the buds were full pink. Each treatment was replicated on 6 trees; 6 trees were untreated checks. Best results were with 10^8 PIB/ml on pre-pink buds which caused 46% population reduction, a statistically significant saving of foliage and high levels of larval infection with both NPV and cytoplasmic polyhedrosis virus (CPV). Both viruses were found in larvae on the check trees and this was attributed to spray drift. The source of the CPV was investigated and found to be a contaminant of the NPV suspension in which the ratio of NPV:CPV PIB was 161:1. Despite the low level of CPV applied, up to 65% of the larvae were infected. In 1980, a survey to determine levels of infection in winter moth larvae showed no viruses in 5 untreated sites and only 1% NPV and 5% CPV in the treated orchard. The virus treatment did not initiate a continuing epizootic and the effective concentration of 10^8 PIB/ml was too costly to produce as a biocontrol agent having an impact only in the season of application.

INTRODUCTION

The winter moth, *Operophtera brumata* (L.), is found in Europe, Asia and Africa and was accidentally introduced into Nova Scotia sometime before 1930. Its distribution in eastern North America is limited to Nova Scotia, S.E. New Brunswick, and Prince Edward Island (Embree and Cuming, 1967). Prior to 1949, it was not recognized as winter moth and was confused with fall cankerworm, *Alsophila pometaria* Harris, and spring cankerworm, *Paleacrita vernata* Peck (Hawboldt and Cuming, 1950). There were reports in 1972 of damage by Bruce spanworm, *Operophtera bruceata* (Hulst), around Victoria on Vancouver Island and further investigations in 1977 revealed that winter moth was also present (Gillespie *et al.* 1978). Its distribution in western Canada was thought to be restricted to this area (Morris and Wood, 1978), but a U.S.D.A. Forest Service report indicates that winter moth was present in Washington State and Oregon prior to its discovery in British Columbia (Ferguson, 1978).

Three types of inclusion body viruses have been isolated from winter moth larvae. A nuclear polyhedrosis virus (NPV) was reported

in a bibliography of insect viruses (Martignoni and Langston, 1960), but Wigley (1976) pointed out that this citation was in error as the work referred to, by Smith (1956), concerned a cytoplasmic polyhedrosis virus (CPV). The first documented report of NPV in winter moth refers to naturally occurring infection of larvae in Nova Scotia (Neilson, 1965). NPVs are classified as *Baculoviruses*, subgroup A (Matthews, 1979) and it has been shown that winter moth NPV is a singly-embedded (unicapsid) type (Wigley, 1976). A naturally occurring entomopoxvirus was found in winter moth larvae from Moravia in Europe (Weiser and Vago, 1966). Naturally occurring CPV infections have not been recorded, but a CPV was isolated from winter moth following infection with NPV from the painted lady butterfly, *Vanessa cardui* (L.), by Smith (1954). In the light of recent investigations, it seems probable that the NPV from *V. cardui* was contaminated with CPV. Later, the CPV obtained in Smith's experiment was sent to Canada and tested on 18 species of Lepidoptera and 2 of Hymenoptera. Infection resulted in 11 species of Lepidoptera, including the winter moth (Neilson 1964).

During a study of the epizootiology of winter moth NPV in England, Wigley (1976) calculated the LD₅₀ for different larval instars in terms of polyhedral inclusion bodies (PIB) per larva. For first instar the LD₅₀ was 2.4, for second instar 15, for third instar 156, for small fourth instar 295 and for large fourth instar 1,813. He could not calculate an LD₅₀ for fifth instar larvae because only 3/233 larvae in his test became infected after ingesting up to 720,000 PIB. If NPV is to be an effective control agent, it should be applied on early instar larvae. However, at this time of year, buds are in a relatively early stage of development and there is little target area for a spray deposit. Later, when the buds are further developed, a good virus deposit can be obtained on the leaf surfaces, but the larvae by then are larger and less susceptible to infection.

The use of winter moth NPV as a biocontrol agent had not been investigated and the present 2-year study was undertaken to evaluate NPV for regulating winter moth populations and to determine its epizootic potential in subsequent generations.

MATERIALS AND METHODS

Virus Production

A sample of winter moth NPV, obtained in England, was propagated in winter moth larvae at the Forest Pest Management Institute. Larvae were reared in 15 ml plastic cups, half filled with artificial diet (McMorran, 1965)¹. When larvae reached fourth instar, they were transferred to fresh cups of diet which were

surface-treated with 0.3 ml of a suspension containing 10⁷ PIB/ml. Mortality began about 8 days post-infection and dead larvae were harvested daily thereafter and placed in beakers of water. They were homogenized in a Waring blender and the suspension filtered through 4 layers of cheesecloth to remove the larger particles of insect debris. The virus was purified further by differential centrifugation. A total of 5,500 fourth instar larvae was infected with NPV and harvested. These produced 2 x 10¹² PIB making the average yield/larva 3.64 x 10⁸ PIB.

Virus Application

The trials were conducted in a heavily infested, neglected orchard of dwarf apple trees (mostly var. Spartan) on University of Victoria property at 2,400 Cedar Hill Cross Road. The aqueous NPV suspensions were applied in a formulation containing 25% animal-feed grade molasses, 50 g/l Sandoz Shade[®] (a UV screening agent) and 1% Chevron[®] sticker. Three concentrations of virus, viz. 10⁶, 10⁷ and 10⁸ PIB/ml, were applied to 6 replicates of 1 tree each on April 19, 1979 between 0900 and 1100 hr using a Solo Junior 410[®] mistblower. Application rates ranged from 0.75 l to 1.5 l/tree and averaged 1.3 l. At the time of application, there was scattered cloud with sunny periods, wind of 2 to 4 km/hr and temperature of 8° to 10° C. All the winter moth eggs had hatched and the larvae were in the first and second instars but buds on the trees were still in the pre-pink stage.

The same treatments were applied to an additional 18 trees on April 27 between 0900 and 1100 hr. About 1 l of spray was applied per

¹This diet is unsuitable for continuous laboratory rearing of winter moth because pupae are deformed and no adults emerge.

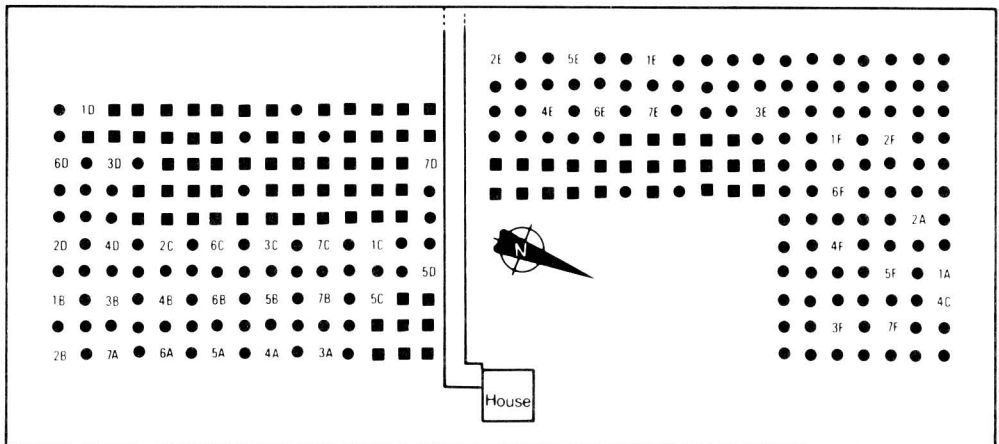


Fig. 1. Plan of the University of Victoria apple orchard where virus spray trials for winter moth control were conducted in 1979. Treated trees are marked with numbers, 1A, 1B, etc., denoting treatment 1 and A to F for the 6 replicates of that treatment (see Table 1 for treatments), ■ indicates trees used in a pesticide trial and ● indicates missing or unused trees.

TABLE 1. Treatments of nuclear polyhedrosis virus on winter moth larvae on apple trees in Victoria, B.C. in 1979.

Treatment Number	Application date	PIB/ ml	ℓ/tree	PIB/ tree	No. of NPV-infected larvae required to treat 1 tree
1	April 19	10 ⁶	1.3	1.3 x 10 ⁹	3.6
2	April 19	10 ⁷	1.3	1.3 x 10 ¹⁰	35.6
3	April 19	10 ⁸	1.3	1.3 x 10 ¹¹	357.5
4	April 27	10 ⁶	1.0	10 ⁹	2.8
5	April 27	10 ⁷	1.0	10 ¹⁰	27.5
6	April 27	10 ⁸	1.0	10 ¹¹	275
7 (check)	None	-	-	-	-

*Based on 1978/79 production figures at the Forest Pest Management Institute.

tree, the weather was clear, there was no wind and the temperature was 12°C. Buds on the apple trees had reached full pink and the larvae were mainly in the third instar.

Besides the 36 treated trees, 6 untreated trees were designated as checks. The treatments are listed in Table 1, and the orchard is mapped in Fig. 1. Some of the trees in this orchard were used for a pesticide trial, making it necessary to scatter the test trees over a wide area. However, the basic plot layout can be considered as a randomized complete block design.

Assessing Impact of the Treatment

Three methods were used to assess the impact of the 6 treatments: 1) population estimates were made by comparing pre- and post-spray counts of living larvae; 2) defoliation estimates were made visually on treated and untreated trees; 3) levels of virus infection were

determined by microscopic examination of samples of larvae.

1) For population estimates, pre-treatment early spray samples consisted of 15 leaf clusters per tree, collected at random at chest height from treated and check trees on April 18. On April 26, 10 leaf clusters per tree were taken from the late-spray treatment trees and the check trees were re-sampled. Post-spray samples of 10 leaf clusters per tree were collected May 11 from all the trees in all the treatments and the check. The larvae on each leaf cluster were counted and the mean number of larvae per leaf cluster per tree was calculated. The mean number of larvae per leaf cluster per treatment was then established and the percentage population reduction due to treatment calculated using a modified Abbott's formula (Abbott, 1925) as follows:

$$\text{Percent population reduction due to treatment} = 1 - \left(\frac{\text{Post-spray density in treatment}}{\text{Pre-spray density in treatment}} \right) \times \left(\frac{\text{Pre-spray density in check}}{\text{Post-spray density in check}} \right) \times 100$$

2) Defoliation estimates were made on June 5. All treated and check trees were rated visually for extent of winter moth defoliation using a scale of 0 (no defoliation) to 10 (complete defoliation).

3) Larval samples, to determine the incidence of pathogens in the population, were collected and shipped to the Forest Pest Management Institute on April 30, May 7 and 14. From each sample, squash preparations of gut and fat

tissue were made from about 50 individual larvae from each treatment and from the check. These preparations were examined using phase contrast optics; presence of inclusion body viruses and other pathogens was recorded.

Determination of CPV in the NPV Preparation

After a fairly high incidence of CPV was noticed in larvae collected from the treated and check trees, we examined the purity of the NPV propagated at the Forest Pest Management Institute. The inclusion bodies were counted by smearing a known volume of suspension on a measured area of a microscope slide (Wigley, 1976; Evans, Bishop and Page, 1980), and staining them with giemsa instead

of naphthalene black (Wigley, 1976). CPV inclusion bodies stained blue and those of NPV remained unstained against a pink background. The concentration of both viruses could be determined.

Surveys Conducted in 1980

The orchard which was treated in 1979 was re-sampled in 1980 along with 5 other sites which had no previous history of treatment with virus or pesticide. Random samples were collected on May 13 when larvae were mainly in the fifth instar. They were shipped to the Forest Pest Management Institute where 100 larvae per site were examined for pathogens as described above. The 6 sites are located in

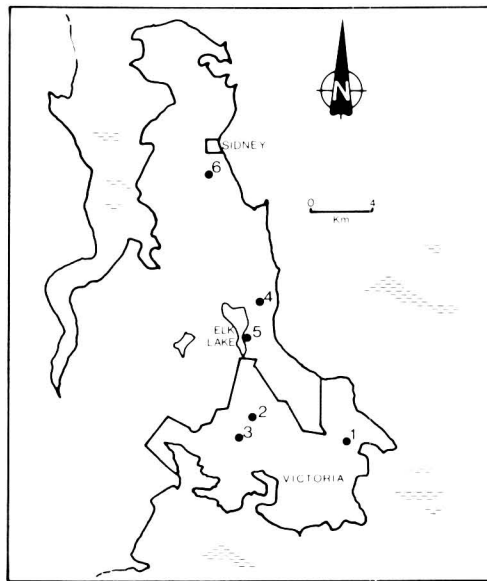


Fig. 2. Map of the Saanich Peninsula showing sites where collections of winter moth larvae were made in 1980. Site no. 1 was

treated with viruses in 1979 but sites 2 to 6 had no history of virus or pesticide treatments.

Fig. 2 and described below:

1. University of Victoria apple orchard: the site of virus and pesticide spray trials in 1979;
2. Glanford Avenue at Agnes Street: an abandoned orchard with apple and pear trees;
3. Interurban Road at Dumeresq Crescent: includes heavily defoliated maple, willow and hawthorn. It was here that the winter moth was first observed on Vancouver Island. This area had been heavily infested with geometrid larvae for at least the past 9 years;
4. Hunt Road at Fowler Road: an old, abandoned orchard with a few apple and pear trees;

5. Patricia Bay Highway at Jennings Lane (Elk Lake): an old, abandoned cherry, pear and apple orchard;
6. East Saanich Road at Bengordon Road: roadside apple and pear trees are on this site.

RESULTS

Assessment of 1979 Spray Trial

Table 2 shows the pre- and post-spray counts of larvae, percent population reductions due to treatment and visual defoliation estimates. The virus concentration which had the greatest impact was 10^6 PIB/ml, applied on April 19. It gave the highest population reduction of 46% and was the only one in which defoliation estimates differed significantly from the check

TABLE 2. Pre-spray and post-spray counts of winter moth larvae and visual defoliation ratings of apple trees sprayed with virus preparations and untreated trees.

Treatment number	Treatment date	Concentration of NPV (PIB/ml)	Mean number of larvae/leaf cluster			Percent population reduction due to treatment	Defoliation rating (\pm S.D.) June 5
			Pre-spray April 18	Pre-spray April 26	Post-spray May 11		
1	April 19	10 ⁶	5.44	--	2.08	21	9.0 \pm 1.5
2	April 19	10 ⁷	6.57	--	3.10	3	6.2 \pm 2.5
3	April 19	10 ⁸	6.39	--	1.66	46	2.6 \pm 1.7*
4	April 27	10 ⁶	--	8.08	2.43	14	9.8 \pm 0.4
5	April 27	10 ⁷	--	8.23	2.16	26	8.7 \pm 0.5
6	April 27	10 ⁸	--	8.45	2.31	22	5.3 \pm 3.2
7	Check	-	5.18	--	2.50	-	9.6 \pm 0.7
7	Check	-	--	7.15	2.50	-	9.6 \pm 0.7

*Significantly different from the check at the 95% confidence level



Fig. 3. Typical winter moth defoliation on apple trees in Victoria, B.C. A) untreated, B) sprayed with viruses at 10⁸ PIB/ml on April 19, 1979. Photographs taken on June 5, 1979.

trees. A typical tree which received this treatment and a typical untreated check tree are shown in Fig. 3.

Table 3 shows the incidence of virus infection in larvae examined microscopically. The only pathogens detected were NPV and CPV and the levels of CPV were, in several samples, higher than those of NPV. Infections with both viruses present were common. The occurrence of CPV was unexpected and merited further investigation.

Level of CPV Contamination in the NPV Preparation

We determined that the ratio of NPV:CPV PIB in the preparation used in these trials was 161:1.

Surveys Conducted in 1980

From 100 larvae examined from each of the 5 untreated sites, no virus was found. Even in site no. 1, the orchard treated in 1979, the incidence of viruses was low, with only 1.0% NPV and 5.0% CPV being recorded.

DISCUSSION

The 1979 trial with NPV to control winter moth larvae raised two interesting points which

were resolved by further investigation. Firstly, the detection of virus in larvae on untreated, check trees suggested that there was a natural virus epizootic in the winter moth population. The survey conducted in 1980 indicated that this was not the case. The plan of the orchard in Fig. 1 shows that there was only one buffer tree between trees sprayed with virus using a mistblower and untreated check trees. We concluded that spray drift caused the virus infection in larvae on the check trees. This low deposit, however, did little to control the insect, and it was not until 33 days after the early treatment that any marked effect was observed in samples of larvae which were examined microscopically. These check trees were then almost totally defoliated. Virus infection of larvae on check trees can obviously influence population studies and appear to diminish the effectiveness of the treatments. However, the post-spray count was made on May 11; on May 7, microscopic examination showed 98.0% of the check larvae free from virus infection and on May 14th, 90.0% were free from infection. Hence, within the scope of this trial, population reduction estimates can be regarded as reasonably unaffected by the spray drift.

TABLE 3. Incidence of viruses in winter moth larvae collected from apple trees sprayed with viruses in 1979.

Plot No.	Treatment date	Dosage of NPV (PIB/ml)	Sample date	No. of larvae examined	Percent virus infection			Percent showing no disease
					NPV	CPV	NPV + CPV ¹	
1	April 19	10 ⁶	April 30	50	18.0	4.0	2.0	80.0
			May 7	50	10.0	0	0	90.0
			May 14	50	22.0	8.0	0	70.0
2	April 19	10 ⁷	April 30	50	18.0	0	0	82.0
			May 7	50	14.0	16.0	0	70.0
			May 14	50	28.0	16.0	4.0	60.0
3	April 19	10 ⁸	April 30	55	32.7	5.5	1.8	63.6
			May 7	50	32.0	8.0	0	60.0
			May 14	49	36.7	42.8	22.5	42.9
4	April 27	10 ⁶	May 7	50	8.0	8.0	2.0	86.0
			May 14	59	6.8	16.9	1.7	77.9
5	April 27	10 ⁷	May 7	50	20.0	18.0	4.0	66.0
			May 14	50	28.0	20.0	4.0	56.0
6	April 27	10 ⁸	May 7	49	30.6	65.3	26.5	30.6
			May 14	50	24.0	56.0	10.0	30.0
7	None (check)	-	April 30	50	2.0	2.0	0	96.0
			May 7	50	2.0	2.0	2.0	98.0
			May 14	50	6.0	8.0	4.0	90.0

¹Double infections with NPV and CPV together were recorded separately and also in the NPV + CPV column. Hence, total infection cannot be calculated by adding these 3 columns.

Secondly, the occurrence of CPV is particularly interesting. On further investigation, we found it was a contaminant of the NPV preparation which was applied. The source of the CPV is unknown. It might have been a very low-level contaminant in the sample received from England; it might have been present in the stock of winter moth larvae used for virus production; or it might have been spruce budworm CPV which was being studied in the Sault Ste. Marie laboratory at the same time as the winter moth NPV was being produced. In general, CPVs have a much wider host range than NPVs (Neilson, 1964) and detailed biochemical studies are required to establish the identity of this particular CPV.

With the small quantity of CPV in the preparation, it is surprising that such high levels of infection were found in larvae following its application, up to 65.3% in one instance. The highest level of NPV recorded was 36.7%. A similar situation was reported when spruce budworm NPV, contaminated with CPV, was applied to spruce budworm (Cunningham, in press) on two occasions. In the first instance, the ratio of NPV:CPV was 400:1 and in the second was 178:1. In both cases, a high level of CPV infection resulted. It is possible that there is a synergistic effect between these two viruses, but exhaustive studies would be needed to establish this hypothesis.

A significant level of foliage protection was obtained only from the early treatment with 10^8 PIB/ml. Foliage protection was quite evident from early treatment with 10^7 PIB/ml and from late treatment with 10^8 PIB/ml. Because there were considerable variations between the 6

replicates in these treatments, the results were not statistically significant. To produce a dosage of 10^8 PIB/ml applied at 1 l/tree, it is necessary to infect and harvest 275 winter moth larvae to treat one tree. This is obviously unacceptable in economic terms. Similar situations exist with spruce budworm NPV (Cunningham *et al.*, 1978) and Bruce spanworm NPV (Ives and Cunningham, 1980).

We hoped that the application of winter moth NPV in 1979 would result in a virus epizootic in the treated orchard in 1980, but this did not occur. Population studies were not needed in 1980 because a high winter moth population was obvious and defoliation was severe throughout the entire orchard. Microscopic examination of samples of larvae revealed that NPV and CPV were both present in the population, but at levels which were too low to have any regulating effect. This preliminary trial indicates that, at present, neither NPV or CPV appears to show promise as a biocontrol agent for winter moth.

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ANOTHER OLETHREUTINE, *PHANETA LATENS* (LEPIDOPTERA: TORTRICIDAE), ATTRACTED TO THE SEX PHEROMONE OF THE CODLING MOTH¹

DARRELL O. HATHAWAY AND GEORGE TAMAKI
Yakima Agricultural Research Laboratory, Agricultural Research,
Science and Education Administration, USDA
Yakima, WA 98902

ABSTRACT

The olethreutine, *Phaneta latens* (Heinrich), was attracted to the pheromone of the codling moth *Cydia pomonella* (L.), (*E,E*)-8,10-dodecadien-1-01. Field trapping records indicated that flights of *P. latens* occurred between May 12 and June 20, 1978, with the greatest trap catches in late May. In 1979 flights occurred from May 15 to July 2, and the peak catches dispersed throughout May and June. Responses of *P. latens* to the pheromone was significantly less at concentrations of 0.25 mg/trap than at 1.0 and 2.0 mg/trap; the latter two were equally attractive.

The attraction of extracts from female codlingmoths, *Cydia pomonella* (L.), to male codling moths was first shown at Yakima, Wash., in July 1963 (Butt and Hathaway 1966). Later, Roelofs et al. (1971) identified the sex attractant of codling moth to be (*E,E*) 8,10-dodecadien-1-01. We found another olethreutine, *Phaneta latens* (Heinrich), to be attracted to this pheromone.

In 1929, Heinrich reported that the adult tortricid, *P. latens*, was known only from the type locality in Tulare County, Calif. The food plant was listed as unknown. Dr. Thomas D. Eichlin of the Insect Taxonomy Laboratory, Sacramento, Calif., identified *P. latens* for us strictly by comparing the male genitalia with illustrations of the species in Heinrich (1929).

¹Mention of a commercial product does not constitute a recommendation for use by the U.S. Department of Agriculture.

Dr. Eichlin said that information about *P. latens* was limited and that specific determinations were very difficult (pers. comm.).

In this paper we describe the effects of the sex attractant of the codling moth on *P. latens*, including the seasonal flight activity and variations in the attractiveness of different dosage rates of the attractant.

METHODS AND MATERIALS

The seasonal activity of the moth was determined with two pheromone traps that were set out in peach trees from May 1 to Sept. 28 in 1978, 1979, and 1980 at Moxee, Wash. In 1980, two additional pheromone traps were placed in apricot trees in Moxee from May 20 to June 12 and in pear trees in Ellensburg, Wash., from June 10 to July 7. The Sectar 1[®] traps were baited with red rubber-sleeve stoppers (septa)