ECOLOGY OF EUROPEAN PINE SAWFLY, *Neodiprion sertifer* (Geoff.) NUCLEAR POLYHEDROSIS VIRUS

The distribution and accumulation of viral inclusion bodies in forest soils

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ABSTRACT

Neodiprion sertifer nuclear polyhedrosis virus (NPV) was extracted from 2 cm deep soil core samples collected to determine the horizontal distribution of virus beneath lodgepole pine, *Pinus contorta*, trees and to assess the annual accumulation of this virus in forest soils in Great Britain. Virus was found in all soil samples collected beneath these trees, with more NPV found in soil cores collected near the tree trunk and at the canopy edges. Virus was also found in soil collected from drainage ditches, indicating that precipitation may spread NPV to disease-free areas.

Yearly accumulation of *N. sertifer* NPV in soil was studied in nine plots from three different locations throughout Great Britain. Average quantities recovered from the soil samples ranged from 5.0×10^5 to 4.3×10^6 viral inclusion bodies per core. The quantity of virus entering the soil was related to the presence and intensity of NPV epizootics in the sawfly population and sawfly population density. Generally, quantities of virus in soil decreased in the absence of epizootics as virus moved deeper into the soil. It was considered unlikely that NPV in the soil played a role in initiating annual epizootics of NPV, but soil as a reservoir for NPV is an important factor in European pine sawfly population dynamics on a long-term basis.

RÉSUMÉ

Le virus de la polyédrose nucléaire (VPN) qui infecte *Neodiprion sertifer* a été dénombré dans des d'échantillons de sol (carottes de 2 cm de longueur) afin de déterminer la distribution horizontale du virus sous les pins lodgepole (*Pinus contorta*) et d'évaluer l'accumulation annuelle de ce virus dans les sols forestiers en Grande-Bretagne. On a détecté le virus dans tous les échantillons prélevés sous les arbres. Cependant, les carottes de sol prises près du tronc des arbres et aux limites du couvert contenaient plus de VPN. On a également détecté le virus dans des échantillons provenant de fossés de drainage, ce qui indique que les précipitations peuvent propager le VPN dans des régions non touchées par la maladie.

On a étudié l'accumulation annuelle du VPN de *N. sertifer* dans le sol, dans 9 parcelles établies dans 3 régions différentes de la Grande-Bretagne. Le nombre moyen de corps d'inclusion virale présents dans les échantillons de sol a varié de $5{,}0 \times 10^5$ à $4{,}3 \times 10^6$ par carotte. On a établi un rapport entre, d'une part, le nombre de virus qui pénètrent dans le sol et, d'autre part, la présence d'épizooties de VPN touchant la population de diprions, l'intensité de ces épizooties et la densité de la population de diprions. En général, le nombre de virus présents dans le sol diminuait en l'absence d'épizooties, puisque le virus était enfoui plus profondément dans le sol. On a conclu qu'il est peu probable que la présence du VPN dans le sol joue un rôle dans le déclenchement d'épizooties annuelles de VPN, mais que le sol, en tant que réservoir de VPN, est un facteur important dans la dynamique à long terme des populations de diprions du pin sylvestre.

INTRODUCTION

Virus reaching the soil can provide a stable source of active inoculum between insect host generations. Studies involving both the nuclear polyhedrosis viruses (NPVs) and granulosis viruses (GVs) of insect pests of agricultural crops have shown that these occluded baculoviruses can persist and remain infective in soil for long periods of time and are often responsible for initiating disease epizootics (Kelsey 1958; Jacques 1964, 1969, 1985; David and Gardiner 1967) The contribution of soil-borne virus to the development of epizootics in a forest ecosystem is thought to be much diminished. The distance to the hosts, habitat and the constant accumulation of forest litter can make the occurrence of foliage contamination rare (Wellington 1962; Hukuhara and Namura 1972; Thompson and Scott 1979; Thompson *et al.* 1981). Investigations by Mohamed *et al.*(1982) and Olofsson (1988) have shown that viable European pine sawfly NPV can persist in soil for up to 21 months. However, the distribution of virus in forest soil has not been extensively studied, leading to speculation on its potential to initiate epizootics in host insect populations. Additional observations on the horizontal distribution and accumulation of European pine sawfly NPV in soil are reported here. These observations are part of a 3-year study conducted in Great Britain between 1978 and 1980 on the ecology of an NPV affecting this sawfly (Kaupp 1981; 1983a, b).

METHODS

Description of Study Areas.

Nine study plots were selected in 3 regions of Great Britain; Southwest Wales, North Yorkshire and Scotland. This provided an opportunity to study the various interactions within insect populations of different densities and disease histories.

Five study plots (W1 to W4, and W9) were established 11.0 km north of Glyn Neath, Wales in lodgepole pine, *Pinus contorta*, 1.2 m to 3.0 m in height planted in 1968 and 1970 and forming part of the Forestry Commission's Coed-y-Rhaiardr Forest (National Grid Reference SO 929 157) (Table 1). This part of the forest is on a south–facing, exposed hillside 117 m above sea level. Sitka spruce, *Picea sitchensis*, and larch, *Larix decidua*, were planted on the periphery of the forest. Ground cover was predominantly grass, *Eriophoretum vaginatum*, with some heather, *Calluna vulgaris*, also present. The ground had been ploughed to a depth of 0.3 m and the trees planted on the banks of the furrows, a common practice on wet sites. Trees were planted at 2.0 m intervals, but because some trees had died there was little consistency in spacing from one row to the next. The canopy remained open throughout the 3-year study period. This area had been heavily infested with sawfly in 1977. The observation of sawfly cadavers containing NPV still attached to the foliage confirmed the presence of virus disease in the larval population in 1977.

Two study plots (Y5 and Y6) were established in plantings of lodgepole pine located north of Pickering, North Yorkshire in part of Langdale Forest (National Grid Reference TA 908 938) (Table 1). These areas, planted in 1966 and 1968, included some sitka spruce and larch and were located in dry heathland. Heather was the dominant ground vegetation. The plots were 246 m above sea level. The ground had been deeply ploughed (1.0 m) and the trees planted in the furrows as is the practice in dry areas. The trees were originally planted 1.2 m apart and had developed in a regularly spaced pattern. These trees were uniform in growth, approximately 1.5 m tall, with no signs of major interference between trees. Throughout the project the canopy of both these study plots remained open. Both plots had been heavily infested with sawfly in 1977, the year previous to the initiation of this study. Egg

Table 1. Description of study plots

Study area & forest	Study plot number	Date planted	Number of trees in plot	Area of plot (m^2)	Approx. tree height in 1978	Presence of sawfly in 1977	Presence of virus in 1977
Coed-y-Raiadr	W1	1970	100	638	3.0m	yes	yes
Coed-y-Raiadr	W2	1970	100	352	3.0m	yes	yes
Coed-y-Raiadr	W3	1968	100	226	2.0m	yes	yes
Coed-y-Raiadr	W4	1970	50	341	1.2m	yes	yes
Coed-y-Raiadr	W9	1970	50	164	1.6m	*	*
Langdale	Y5	1966	100	252	1.5m	yes	yes
Langdale	Y6	1968	100	234	1.5m	yes	yes
Sneaton	Y7	1972	100	496	1.0m	yes	no
North Dalchork	S8	1971	25	82	2.0m	yes	unknown

**plot established in 1979*

had been heavily infested with sawfly in 1977, the year previous to the initiation of this study. Egg masses found in 1978 indicated that the sawfly was still present in the selected areas. Mortality of sawfly due to NPV in 1977 was confirmed in plots Y5 and Y6 by diagnosis of sawfly cadavers found on the foliage.

Another study plot (Y7) was established in Sneaton Forest, 3.2 km west of Whitby (National Grid Reference SE 879 021) at an elevation of 261 m (Table 1). This plot contained 1.0 m tall lodgepole pines that had been planted in 1972 at spacings of 1.5 m on the banks of furrows. The plot had a uniform arrangement of trees both within and between rows. The dominant ground vegetation was heather. The canopy remained open throughout the duration of the project. This plot suffered its first major sawfly infestation in 1978. Most trees showed some signs of defoliation, and contained large numbers of sawfly egg masses. Evidence of NPV disease was not detected at this site, although it was present in 1977 in adjacent forest blocks.

The final study area (S8), was located in a 6-year old planting of lodgepole pine in the North Dalchork block of the Forestry Commission's elevation of 200 m. The dominant ground cover was heather. The area was deeply ploughed, and the trees were planted on the furrow banks. The trees in the study area were regularly spaced (2.2 m apart). The spacing of trees between adjacent rows was the most consistent of all study plots. The trees were approximately 2.0 m in height and, at the onset of the study, the canopy of the plot was relatively open, but after 3-years some contact between trees occurred. This area had a long history of sawfly attack. Numerous egg masses were observed and the trees were

heavily defoliated in 1977. We could not determine if the resident sawfly population had suffered from NPV disease in 1977, but in 1978 NPV was observed in the insect population.

Layout Of Study Plots

Study plots were established in areas where tree height was uniform. Most of the plots consisted of a block of 10 adjacent rows of trees within which 10 consecutive trees were selected from each row starting from a common base-line. This produced a compact block of 100 trees. Three sides of each block were well-defined, while the fourth was irregular due to the spacing of trees within the rows. The trees within these plots were numbered and permanently labeled.

The study plot located in North Dalchork, Scotland, consisted of 25 trees arranged as 5 trees selected from 5 adjacent rows. Two study plots in Coed-y-Rhaiardr, Wales consisted of only 50 trees each because it was not possible to select a greater number and still remain within a uniform forest type. In these two plots, 10 consecutive trees were selected from 5 adjacent rows. Each plot was given a specific identification number (Table 1).

Population Assessment

The number of *N. sertifer* egg masses present in each plot was assessed in April. After eclosion, the absolute number of larval sawfly colonies was determined by inspection. Healthy colonies were counted on all trees, a healthy colony being defined as a group of two or more feeding larvae showing no overt signs of virus infection. The effects of various mortality factors on larval population density was determined from the difference between the initial colony count and later weekly estimates, when only healthy colonies were recorded (Table 2).

Table 2. Intital colony density and percentage of colonies killed by virus disease in the study plots

Plot	Plot area (m2)	Number of trees	1978		1979		1980	
			Number of colonies per plot	% colony mortality	Number of colonies per plot	% colony mortality	Number of colonies per plot	% colony mortality
W1	638	100	2,638	0.03	151	0	224	0
W2	352	100	2,007	1.5	196	0	478	0
W3	226	100	144	5.0	23	40.0	13	0
W4	341	50	494	0.4	69	0	132	0
Y5	252	100	32	87.5	8	75.0	2	0
Y6	234	100	238	99.9	56	99.9	24	25
Y7	496	100	1,862	98.5	55	99.9	12	41.6
S8	82	25	1,629	96.8	891	99.8	109	99.1
W9	164	50	–	–	186	0	543	0

Estimating Concentrations of Viral Inclusion Bodies in the Soil.

A 5-cm diameter core sampler was used to collect soil samples from study plots. These samples were placed individually in labeled plastic bags, transported to the laboratory and stored at 4° C. Care was taken to keep these cores intact. To investigate the horizontal distribution of *N. sertifer* NPV in soil, core samples were collected at 15 cm intervals along two transects extending between several tree trunks in plots Y7 and S8 in 1978. To assess the quantities of viral inclusion bodies in the forest soil from one year to the next, core samples were collected from the study plots just prior to sawfly hatch and at the end of the larval period in 1978, 1979 and in 1980. Two samples from opposite sides of the tree canopy were collected from an area 0.3 m inside the canopy edge beneath 10 randomly selected trees in each plot. In plot S8, weekly samples were collected beneath 5 sample trees during the 1978 larval season.

All virus extractions were carried out using a procedure adapted from one described by Evans *et al.* (1980). The top 2 cm of each soil sample, including the litter layer, was placed in a 500 ml beaker, mixed with 50 ml of phosphate buffered saline containing 0.1% sodium dodecyl sulphate (PBS-SDS) and sonicated with continuous stirring for 5 min. at 4° C in a 200-W ultrasonicater. The aqueous portion containing the viral inclusion bodies was decanted through a single layer of cheesecloth into a 100 ml centrifuge tube and the extraction procedure was repeated with an additional 40 ml PBS-SDS added to the residue in the beakers. To sediment any large debris, the combined washings were centrifuged at 180 g for 5 min. The supernatant was then centrifuged at 2975 g for 20 min. The pellet was resuspended in a known volume of deionised water and the concentration of viral inclusion bodies present in the soil extract estimated by a dry-counting technique (Wigley, 1980).

To determine the efficiency of this method, known quantities of purified *N. sertifer* NPV viral inclusion bodies were mixed with 25 g samples of soil collected near plots in Wales and North Yorkshire. These samples were stored in plastic pots for 4 months at 4° C to mimic the storage of field-collected samples.

Table 3. Recovery of viral inclusion bodies in soil samples

Initial virus concentrations (in 25 g soil)	Soil Origin	Number of replicates	Percentage recovery (+S.D.)
1×10^9	[a] Wales	3	18.5 + 1.9
1×10^9	Wales	2	22.9 + 3.2
1×10^7	[a] Wales	2	29.9 + 1.9
1×10^7	[a] Wales	2	25.8 + 9.4
1×10^9	[a] North Yorkshire	2	28.8 + 6.8
1×10^8	[a] North Yorkshire	2	28.8 + 1.7
Mean percentage extraction efficiency			25.7 + 4.4

[a] No significant difference at the 95% confidence level between mean recoveries from soil collected from Wales and North Yorkshire (Snedecor and Cochrane 1967).

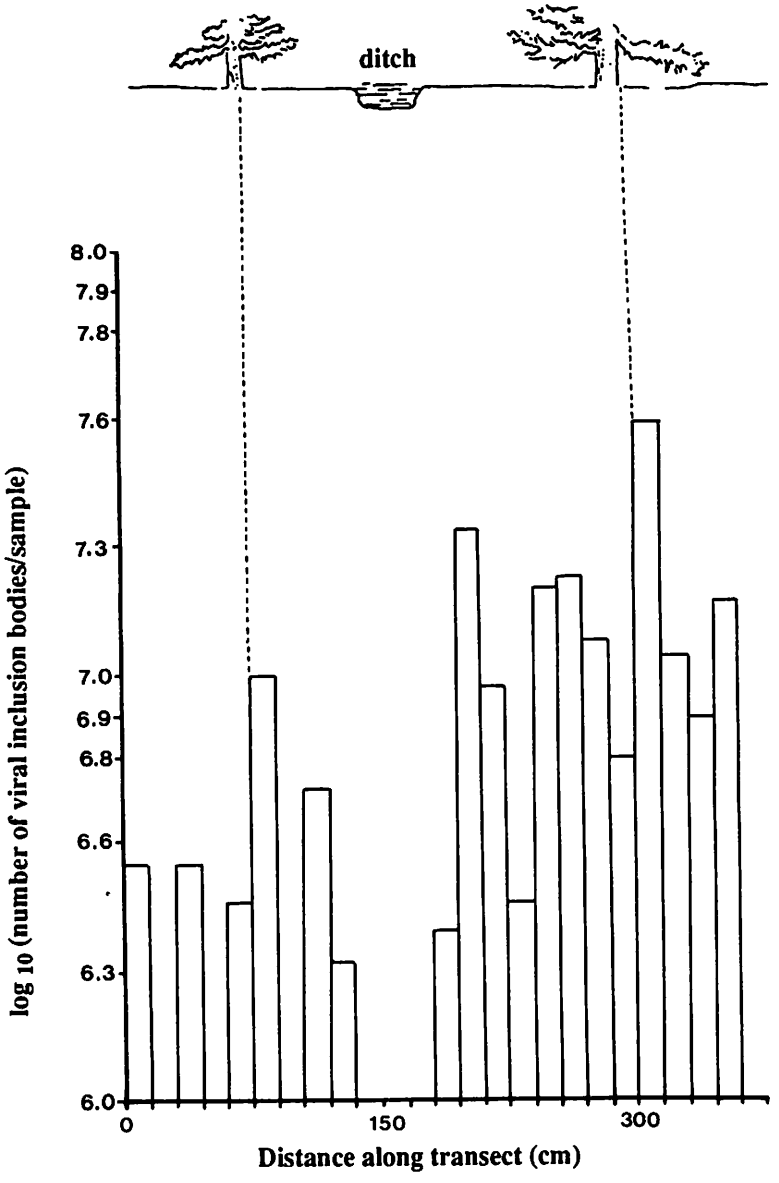

Figure 1. Distribution of *N. sertifer* NPV inclusion bodies in soil cores collected along a transect between two trees in plot Y7.

Using the above procedure 25.7±4.4% of the viral inclusion bodies could be recovered from the soil, with no significant difference ($p = 0.05$) in the amount extracted from soil from either location (Table 3). This figure, similar to that reported by Mohamed *et al.* (1982), was used as a correction factor to assess the number of viral inclusion bodies present in field-collected samples.

Statistical Analysis:

Significant differences between the means of the numbers of viral inclusion bodies extracted from soil samples were assessed using Students' t-test at the 95% confidence level (Snedecor and Cochrane, 1967).

RESULTS AND DISCUSSION

Horizontal Distribution:

The quantity of viral inclusion bodies extracted from 2-cm deep soil core samples collected along a transect established between trees in two plots (Y7 and S8) is shown in Figs. 1 and 2. Viral inclusion bodies produced in epizootics tended to accumulate in the soil beneath the tree, particularly at the base of the tree trunk and near the edges of the tree canopy. These accumulations were most likely caused by virus being carried along with precipitation, which is deposited in these areas according to the through-fall profile of the tree canopy (Lee 1980). A tendency for viral inclusion bodies to be dispersed beyond the crown boundaries was observed and can probably be attributed to the combined effect of wind and rainfall. As the trees become taller and more exposed, and as their canopies begin to close, viral inclusion bodies produced from successive epizootics may be dispersed beyond the crown and eventually cover the entire forest floor. In plot S8 dispersal of viral inclusion bodies away from the canopy appears to be directional, occurring more on one side of the tree than the other and could result from virus being driven from the canopy by the prevailing wind during rainstorms (Fig. 2).

Large numbers of viral inclusion bodies were recovered from samples collected from drainage ditches in plot S8 (Fig 2). However no viral inclusion bodies were recovered from the ditch-sides as would be expected if dispersal had occurred in a uniform pattern away from the canopy. Most likely these viral

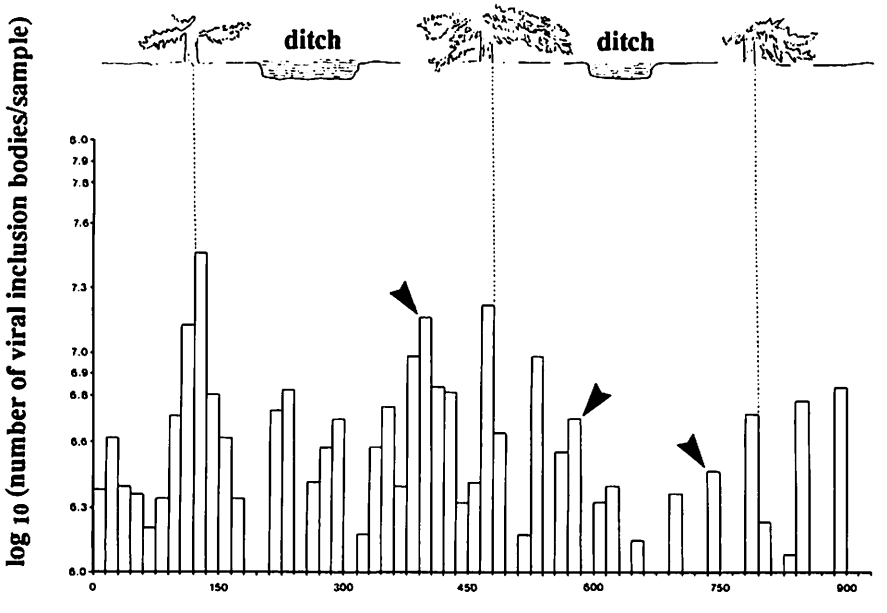

Figure 2. Distribution of *N. sertifer* NPV inclusion bodies in soil cores collected along a transect between three trees in plot S8. Arrows indicate accumulation of viral inclusion bodies at canopy edges.

inclusion bodies that had accumulated in the ditches were carried there by drainage water. Subsequent transport of viral inclusion bodies in drainage ditches may disperse this virus to disease-free localities.

The accumulation of quantities of viral inclusion bodies at the trunk base due to the influence of stemflow may indicate two mechanisms by which virus persisting on the tree may initiate epizootics. In these circumstances, viral inclusion bodies may be deposited in bark crevices on the stem where they could persist, constituting an effective inoculum capable of causing infection. At high population densities, sawfly larvae feed on the bark while migrating from tree to tree. The large quantities of *N. sertifer* viral inclusion bodies observed accumulated at the stem base may also be a source of infection at high larval densities as migrating larvae move from tree to tree.

Yearly Accumulation of Viral Inclusion Bodies in Soil.

The average number of viral inclusion bodies in the 10 soil samples collected from plots W1, W3, and W9 on each sampling date showed no significant change throughout the 3-year study period (Fig. 3). Averages of $1.2 \times 10^6 \pm 1.9 \times 10^5$, $1.2 \times 10^6 \pm 6.6 \times 10^5$, and $5.0 \times 10^5 \pm 1.4 \times 10^5$ viral inclusion bodies per soil core were recovered for each of these plots respectively over the study period. The quantity of virus detected is probably an expression of the long-term persistence and stability of *N. sertifer* NPV in the soil.

In plot W4, significant increases of virus in soil samples were observed over the 1978 winter period and the 1980 larval period (Fig. 4). There was also a decrease in quantities recovered for the 1979 larval period. The quantity of viral inclusion bodies in the final samples was significantly greater than that in the initial sample collected in June 1978. Similar fluctuations were observed for samples collected from plot W2; significant decreases over the 1979 larval period were followed by an increase in the content of viral inclusion bodies in soil in 1980, which differed significantly from the content of the initial sample (Fig. 4). The low incidence of virus disease in 1978 in these two plots did not explain these fluctuations (Table 2). However, both of these study plots were more open and exposed to the effects of rainfall than other plots, which may have allowed viral inclusion bodies to be transported deeper into the soil in 1979 causing a decline in virus found in the top 2 cm. The increase in levels of viral inclusion bodies in 1980 may also have been caused by rainfall leaching virus from the canopy in all plots, but more so in plots W2 and W4. On average $1.7 \times 10^6 \pm 5.6 \times 10^5$ and $1.9 \times 10^6 \pm 7.2 \times 10^5$ viral inclusion bodies per soil core were recovered from plots W2 and W4 respectively throughout the study.

Quantities of viral inclusion bodies in soil samples collected from all study plots during the 1978 larval period showed an increase concurrent with viral epizootics in the sawfly populations when the larvae were in early-instars (Fig. 5, Table 2). However, only in plots Y5 and Y7 did pre-and post-epizootics levels differ significantly. Quantities of viral inclusion bodies found in samples from plot Y6 remained statistically unchanged throughout the study period; $3.6 \times 10^6 \pm 5.2 \times 10^5$ viral inclusion bodies were recovered on average from this plot over the entire study period.

Following the initial increase in 1978 that was caused by a virus epizootic in the sawfly population, levels of viral inclusion bodies recorded in plot Y5 remained unchanged, except for a significant decrease over the 1979-80 winter period, possibly due to a loss of viral inclusion bodies deeper into the soil. These levels tended to increase in 1980 to levels comparable to those from 1978, which may be due to an augmentation of levels in the soil by virus leaving the canopy. On average $1.1 \times 10^6 \pm 2.9 \times 10^5$

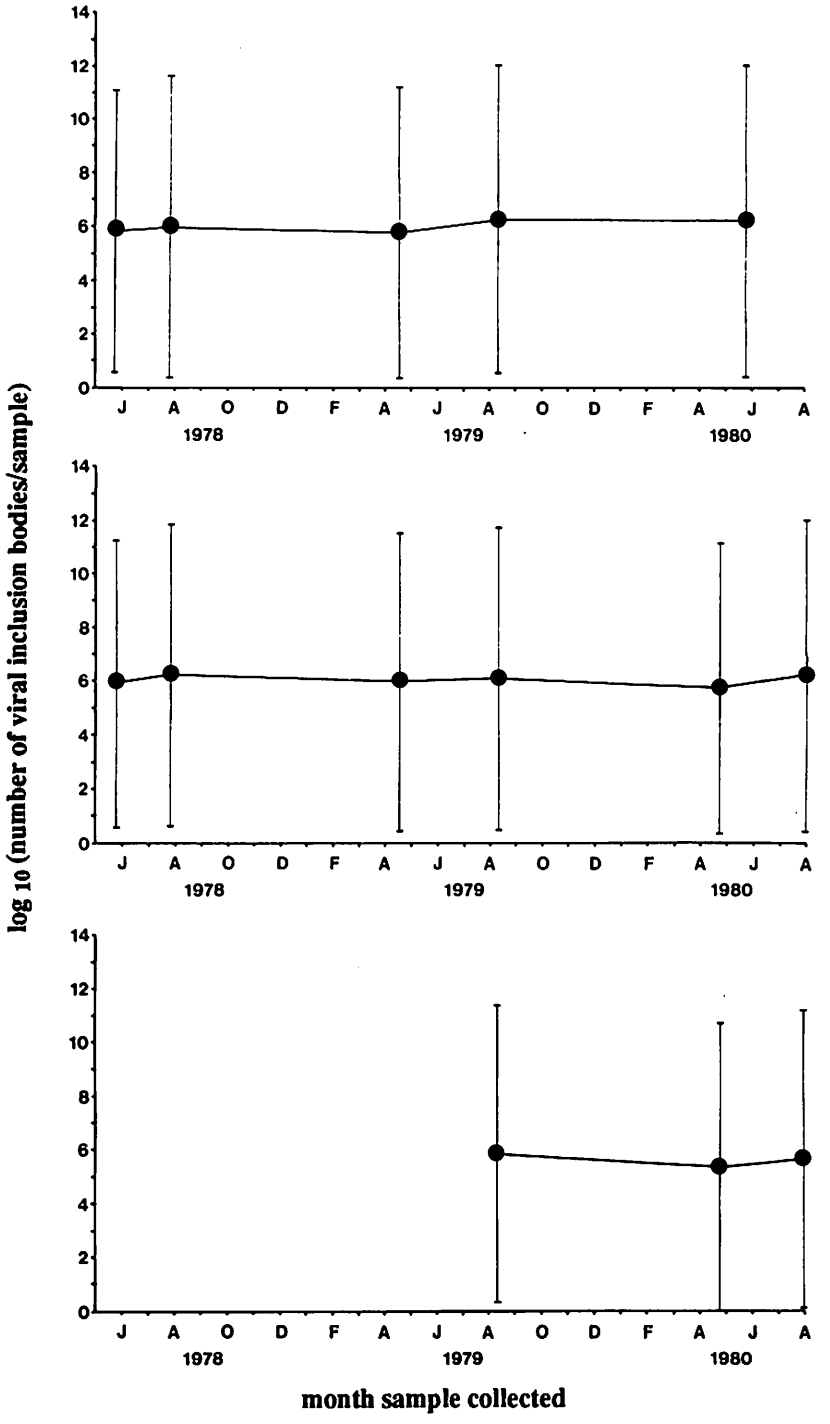

Figure 3. Quantity of *N. sertifer* NPV inclusion bodies in soil core samples collected periodically from plots W1, W3 and W9 over the 3-year study. Standard errors are represented by vertical bars.

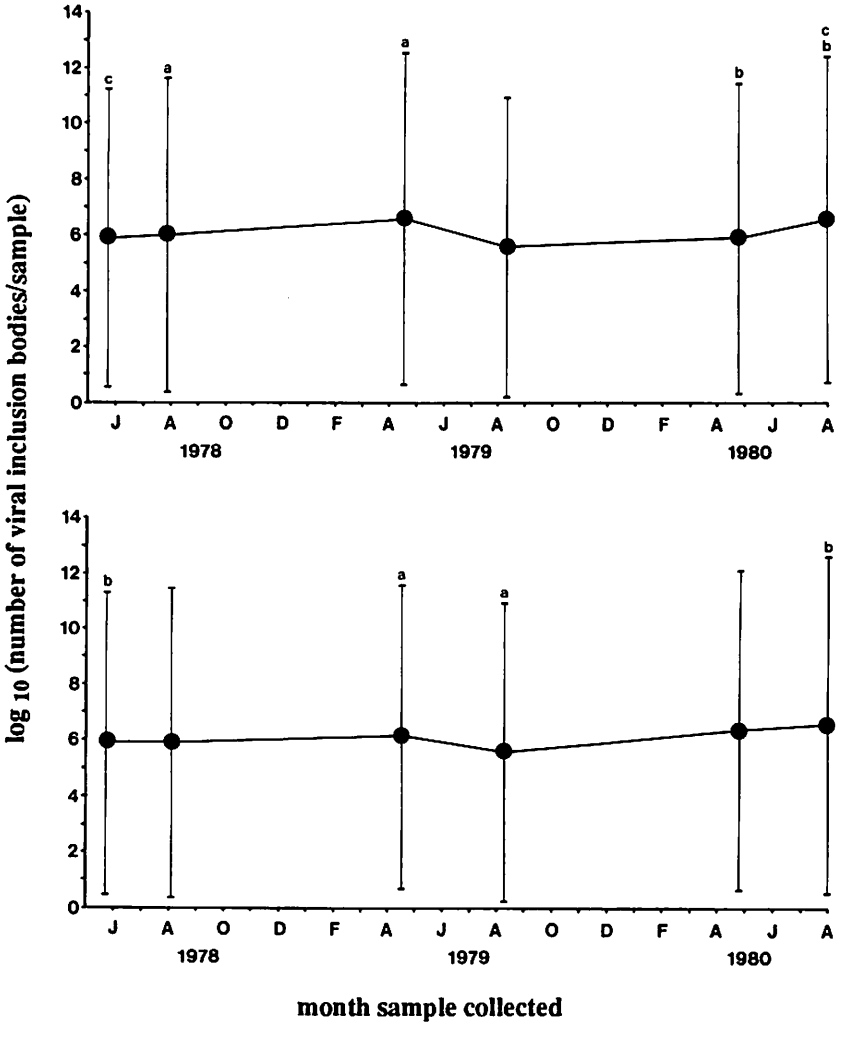

Figure 4. Quantity of *N. sertifer* NPV inclusion bodies in soil core samples collected periodically from plots W4 and W2 over the 3-year study. The same letters indicate significant difference between samples at 95% confidence level. Standard errors are represented by vertical bars.

viral inclusion bodies per soil core were recovered in this plot. In plot Y7 there was also a significant decline in the quantities of viral inclusion bodies observed in the samples collected after the winter. Levels remained unchanged for the rest of the study with an average of $4.3 \times 10^6 \pm 2.7 \times 10^6$ viral inclusion bodies per soil core being recovered.

For the first two larval periods studied, the quantities of viral inclusion bodies in soil samples from S8 increased significantly from the pre-to post-larval period concurrent with a virus disease epizootic in

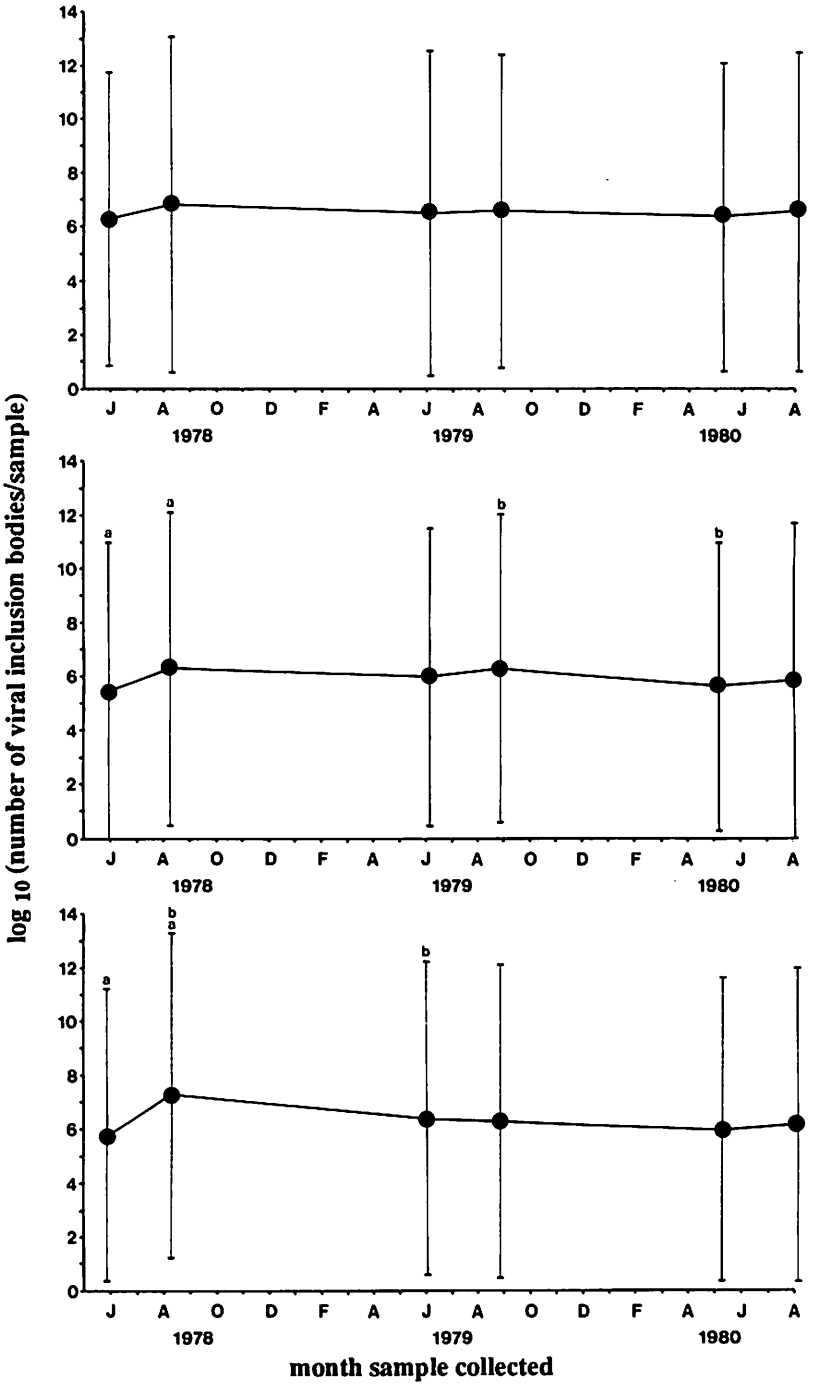

Figure 5. Quantity of *N. sertifer* NPV inclusion bodies in soil core samples collected periodically from plots Y5, Y6 and Y7 over the 3-year study. The same letters indicate significant difference between samples at 95% confidence level. Standard errors are represented by vertical bars.

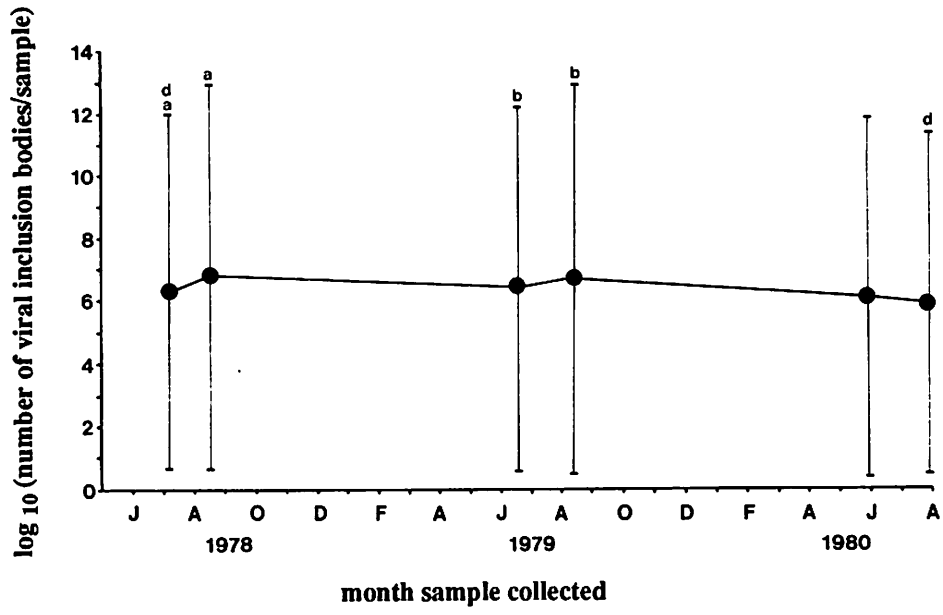

Figure 6. Quantity of *N. sertifer* inclusion bodies in soil core samples collected periodically from plot S8 over the 3-year study. The same letters indicate significant differences between the samples at 95% confidence level. Standard errors are represented by vertical bars.

the insect population (Fig. 6). In the final year, there was no noticeable increase, the result of a low sawfly population in the plot. Levels tended to decline over the winter period, significantly so only in 1980, the result of viral inclusion bodies moving deeper into the soil. Over the 3 years, an average of 2.8 x $10^6 \pm 7.0 \times 10^6$ viral inclusion bodies per soil core was recovered. Intensive soil sampling of this plot during 1978 revealed significant increases in the number of viral inclusion bodies found in samples coincided with the collapse of the sawfly population from virus disease. The accumulation continued for a short time thereafter, demonstrating that much of the virus produced in epizootics is released onto the forest floor (Fig. 7).

In general, changes in the quantities of viral inclusion bodies detected by sequential sampling in most plots could be attributed to accumulation in the soil following death of the current sawfly population. Viral inclusion bodies were found to be present in the top 2 cm of soil at detectable levels in most plots throughout this study, although actual quantities were related to the epizootic history of the sawfly population. Seasonal changes in the quantity of viral inclusion bodies found in soil samples were attributed to augmentation from virus persisting in the canopy and to depletion due to movement deeper into the soil caused by precipitation.

CONCLUSIONS

The quantities of *N. sertifer* NPV inclusion bodies in the forest canopy are gradually reduced as viral inclusion bodies are leached from the tree to the soil. There is a period of marked increase in viral inclusion bodies accumulating in soil during, and shortly, after the larval period. The quantity of viral

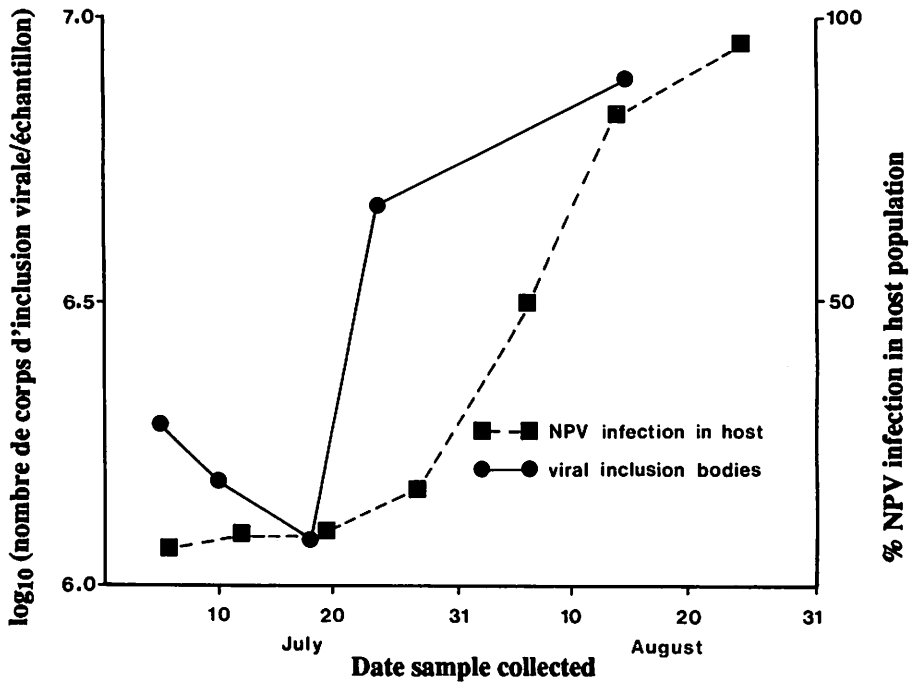

Figure 7. Development of the NPV epizootic compared to quantities of viral inclusion bodies recovered from soil core samples collected from plot S8 in 1978.

inclusion bodies entering the soil during both the larval and non-larval period are related to the character of the originating epizootic (i.e. density of the larval population, intensity of the epizootic and timing of infection). Reflecting these criteria, the quantity of viral inclusion bodies found in soil on plots affected by virus disease (Y6, Y7, S8) were greater than the levels of viral inclusion bodies found in disease-free study plots (W1-W4, W9).

Available evidence suggests that the return of *N. sertifer* NPV inclusion bodies from the soil to the tree occurs at a very low frequency. In the Welsh plots, where active *N. sertifer* viral inclusion bodies were found leaching from the trees, and were assessed at appreciable levels in the top 2 cm of soil, no naturally occurring virus disease was observed in the larval sawfly populations in 1979 and 1980. Therefore, NPV in soil probably constitute a virus inoculum pool, which has a low level of short-term impact on viral epizootics. The role of the NPV in soil as a long-term reservoir, capable of initiating epizootics in disease-free *N. sertifer* populations remains unknown.

REFERENCES

David, W.A.L. and B.O.C. Gardiner. 1967. The persistence of a granulosis virus of *Pieris brassicae* in soil and in sand. J. Invertebr. Pathol. *9*: 342-347.

Evans, H.F., J.M. Bishop and E.A. Page. 1980. Methods for the quantitative assessment of nuclear polyhedrosis in soil. J. Invertebr. Pathol. *35*: 1-8.

Hukuhara, T. and H. Namura. 1972. Distribution of the nuclear polyhedrosis virus of the fall webworm, *Hyphantria cunea*, in soil. J. Invertebr. Pathol. *19*: 308-316.

Jacques, R.P. 1964. The persistence of a nuclear polyhedrosis virus in soil. J. Invertebr. Pathol. *6*: 251-254.

Jacques, R.P. 1969. The leaching of the nuclear polyhedrosis virus of *Trichoplusia ni* from soil. J. Invertebr. Pathol. *13*: 256-263.

Jacques, R.P. 1985. Stability of insect viruses in the environment. Pages 285-360 *in* K. Maramorosch and K.E. Sherman (Eds.) *Viral Insecticides For Biological Control.* Academic Press, Fl. USA.

Kelsey, R.P. 1958. Control of *Pieris rapae* by granulosis virus. N.Z. J. agric. Res. *7*: 778-782.

Kaupp, W.J. 1981. Studies on the ecology of the nuclear polyhedrosis virus of the European pine sawfly, *Neodiprion sertifer* (Geoff.). D.Phil. Thesis. Univ. of Oxford. Oxford, U.K. 363 pp.

Kaupp, W.J. 1983a. Persistence of *Neodiprion sertifer* (Hymenoptera:Diprionidae) nuclear polyhedrosis virus on *Pinus contorta* foliage. Can. Entomol. *115*: 869-873.

Kaupp, W.J. 1983b. Estimation of nuclear polyhedrosis virus produced in field populations of the European pine sawfly, *Neodiprion sertifer* (Geoff.) (Hymenoptera:Diprionidae). Can. J. Zool. *61*: 1857-1861.

Lee, R. 1980. *Forest Hydrology.* Columbia University Press, New York, USA.

Mohamed, M.A., H.C. Coppel and J.D. Podgwaite. 1982. Persistence in soil and on foliage of nucleopolyhedrosis virus of the European pine sawfly, *Neodiprion sertifer* (Hymenoptera:Diprionidae). Environ. Entomol. *11*: 1116-1118.

Olofsson, E. 1988. Environmental persistence of the nuclear polyhedrosis virus of the European pine sawfly in relation to epizootics in Swedish Scots pine forests. J. Invertebr. Pathol. *52*: 119-129.

Snedecor, G.W. and G.C. Cochrane. 1967. *Statistical Methods.* Iowa State University Press. 593pp.

Thompson, C.G. and D.W. Scott. 1979. Production and persistence of the nuclear polyhedrosis virus of the Douglas-fir tussock moth *Orgyia pseudotsugata* (Lepidoptera:Lymantriidae) in the forest ecosystem. J. Invertebr. Pathol. *33*: 57-65.

Thompson, C.G., D.W. Scott and B.E. Wickman. 1981. Long-term persistence of the nuclear polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera:Lymantriidae) in forest soil. Environ. Entomol. *10*: 254-305.

Wellington, W.G. 1962. Population quality and the maintenance of nuclear polyhedrosis between outbreaks of *Malacosoma pluviale* (Dyar). J. Invertebr. Pathol. *4*: 285-305.

Wigley, P.G. 1980. Counting micro-organisms. Pages 29-34 *in* J. Kalmakoff and J.F. Longworth (Eds.) *Microbial Control of Insect Pests.* N.Z. DSIR Bull. 228.

RÉFÉRENCES BIBLIOGRAPHIQUES

David, W.A.L.; Gardiner, B.O.C. 1967. The persistence of a granulosis virus of *Pieris brassicae* in soil and in sand. J. Invertebr. Pathol. 9:342-347.

Evans, H.F.; Bishop, J.M.; Page, E.A. 1980. Methods for the quantitative assessment of nuclear polyhedrosis in soil. J. Invertebr. Pathol. 35:1-8.

Hukuhara, T.; Namura, H. 1972. Distribution of the nuclear polyhedrosis virus of the fall webworm, *Hyphantria cunea*, in soil. J. Invertebr. Pathol. 19:308-316.

Jacques, R.P. 1964. The persistence of a nuclear polyhedrosis virus in soil. J. Invertebr. Pathol. 6:251-254.

Jacques, R.P. 1969. The leaching of the nuclear polyhedrosis virus of *Trichoplusia ni* from soil. J. Invertebr. Pathol. 13:256-263.

Jacques, R.P. 1985. Stability of insect viruses in the environment. Pages 285-360 in K. Maramorosch and K.E. Sherman (Eds.) Viral Insecticides for Biological Control. Academic Press, Fl. U.S.A.

Kelsey, R.P. 1958. Control of *Pieris rapae* by granulosis virus. N.Z. J. agric. Res. 7:778-782.

Kaupp, W.J. 1981. Studies on the ecology of the nuclear polyhedrosis virus of the European pine sawfly, *Neodiprion sertifer* (Geoff.) D. Phil. Thesis. Univ. of Oxford, Oxford, U.K. 363 pp.

Kaupp, W.J. 1983a. Persistence of *Neodiprion sertifer* (Hymenoptera:Diprionidae) nuclear polyhedrosis virus on Pinus contorta foliage. Can. Entomol. 115:869-873.

Kaupp, W.J. 1983b. Estimation of nuclear polyhedrosis virus produced in field populations of the European pine sawfly, *Neodiprion sertifer* (Geoff). (Hymenoptera:Diprionidae). Can. J. Zool. 61:1857-1861.

Lee, R. 1980. Forest Hydrology. Columbia University Press, New York, U.S.A.

Mohamed, M.A.; Coppel, H.C.; Podgwaite, J.D. 1982. Persistence in soil and on foliage of nucleopolyhedrosis virus of the European pine sawfly, *Neodiprion sertifer* (Hymenoptera: Diprionidae). Environ. Entomol. 11:1116-1118.

Olofsson, E. 1988. Environmental persistence of the nuclear polyhedrosis virus of the European pine sawfly in relation to epizootics in Swedish Scots pine forests. J. Invertebr. Pathol. 52:119-129.

Snedecor, G.W.; Cochrane, G.C. 1967. Statistical Methods. Iowa State University Press. 593 pp.

Thompson, C.G.; Scott, D.W. 1979. Production and persistence of the nuclear polyhedrosis virus of the Douglas-fir tussock moth *Orgyia pseudotsugata* (Lepidoptera:Lymantriidae) in the forest ecosystem. J. Invertebr. Pathol. 33:57-65.

Thompson, C.G.; Scott, D.W.; Wickman, B.E. 1981. Long–term persistence of the nuclear polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae) in forest soil. Environ. Entomol. 10:254-305.

Wellington, W.G. 1962. Population quality and the maintenance of nuclear polyhedrosis between outbreaks of *Malacosoma pluviale* (Dyar). J. Invertebr. Pathol. 4:285-305.

Wigley, P.G. 1980. Counting micro-organisms. Pages 29-34 in J. Kalmakoff and J.F. Longworth (Eds.) Microbial Control of Insect Pests. N.Z. DSIR Bull. 228.