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

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The development of nuclear polyhedrosis virus epizootics in sawfly populations

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ABSTRACT

The development of a nuclear polyhedrosis virus (NPV) epizootic in nine *Neodiprion sertifer* (Geoff.) populations was illustrated by the construction of sigmoid infection growth curves (IGCs). These ICGs indicated that epizootic development was influenced by inoculum production and NPV persistence acting in conjunction with sawfly population density. *N. sertifer* NPV epizootics are density-dependent.

The IGCs were normalized using the transformation $\log_{10}[(X)/(1-X)]$ to determine the rate of development of infection in each population. A low infection rate, under 0.02 infection units/day, was calculated for densities below 1 colony/tree, while rates above 0.05 infection units/day were recorded at higher densities. Similar rates were observed for NPV epizootics in populations of *N. lecontei*. There is a need for more precise measurement of epizootic development so that meaningful comparisons can be made between different insect-virus complexes.

RÉSUMÉ

On a illustré, au moyen de courbes sigmoïdes de croissance de l'infection (CCI), l'évolution d'une épizootie causée par un virus de la polyédrose nucléaire (VPN) dans 9 populations de *Neodiprion sertifer* (Geoff.). Ces courbes ont indiqué que l'évolution de l'épizootie l'était influencée par, d'une part, la production d'inoculum et la persistance du VPN et, d'autre part, la densité de la population de diprions. L'épizootie causée par le VPN de *N. sertifer* est un phénomène dépendant de la densité.

On a normalisé les CCI au moyen d'une transformation de type $\log_{10}[(X)/(1-X)]$ pour déterminer le taux d'évolution de l'infection dans chaque population. Un faible taux d'infection, inférieur à 0,02 unité infectieuse/jour, a été calculé pour des densités inférieures à une colonie par arbre, tandis que des taux supérieurs à 0,05 unité infectieuse/jour ont été observés pour de plus fortes densités de population. Des taux d'infection similaires ont été relevés pour des épizooties de VPN dans des populations de *N. lecontei*. Il faudrait effectuer des mesures plus précise de l'évolution des épizooties, de façon à établir des comparaisons significatives entre différents complexes insecte-virus.

INTRODUCTION

Several studies have been conducted on the development of nuclear polyhedrosis virus (NPV) disease epizootics in forest insect pests (Bird and Burke 1961; Stairs 1965; de Groot and Cunningham 1983; Woods and Elkinton 1987; Cunningham *et al.* 1988; Kaupp *et al.* 1989). All have illustrated the complexities in the relationship between the progress of infection, the host insect population and the environment. In a recent review, Entwistle (1986) made these relationships very evident and highlighted their importance in the successful use of viruses for insect control.

This report describes the development of NPV infection in several populations of the European pine sawfly, *Neodiprion sertifer*, and illustrates the effect of host population density, epizootic history, and weather on disease epizootics. It comprises part of a 3-year study conducted in Great Britain on the ecology of this NPV (Kaupp 1981; 1983a,b).

METHODS

A comprehensive description of the nine study plots is given in Kaupp *et al.* (1989). However, for completeness, this information is also provided in Table 1.

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*

Population Assessment.

The number of *N. sertifer* egg masses present in each plot was assessed in the spring (Table 2). After eclosion, the absolute number of sawfly colonies was determined. Healthy colonies were counted on all the trees. A healthy colony was defined as a group of two or more feeding larvae showing no overt

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 Number of colonies per plot	1978 % colony mortality	1979 Number of colonies per plot	1979 % colony mortality	1980 Number of colonies per plot	1980 % 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

signs of virus infection. The effects of various mortality factors on the larval population were determined from the difference between the initial colony count and subsequent, weekly, counts of healthy colonies

Sampling To Determine The Incidence Of Virus Infection In The Sawfly Populations.

Immediately after eclosion through to the time larvae disappeared from the trees, random samples of sawfly larvae were collected at weekly intervals from each plot. One larva from each colony sampled was collected, placed in a vial and stored at -20°C until diagnosed. Only colonies that appeared to be healthy were sampled.

Thin smears of each larva were made on glass microscope slides using disposable toothpicks to tease apart and spread the insect tissue. Use of toothpicks removed the possibility of cross-contamination between smears. The slides were stained with Giemsa stain (Wigley 1980). All smears were examined for viral inclusion bodies using oil immersion optics. Positive smears were assumed to have been collected from colonies infected with NPV. From these diagnostic results, in conjunction with colony counts, the proportion of sawfly colonies infected with NPV was estimated at weekly intervals. This data was used to develop infection growth curves (IGCs) for each sawfly population.

RESULTS AND DISCUSSION

Virus epizootics were observed in 5 of the 9 plots; no epizootics were observed in plots W1, W2, W4 and W9 (Table 2). In diseased sawfly populations, the proportion of larvae infected with NPV increased with larval development, producing a sigmoid infection growth curve (IGC).

Epizootics were observed in 1978 and 1979 in plot W3 (Fig. 1). NPV infection was detected in the sawfly population 28 days after egg hatch in 1978, and this caused 5% mortality. It was calculated that 1.06 X 10^{12} polyhedral inclusion bodies (PIBs)/ha were released into the forest ecosystem from the death of these larvae (Kaupp 1983a). These PIBs affected the 1979 epizootic, which began to cause insect mortality 20 days after egg hatch and affected 40% of the population. The quantity of inoculum

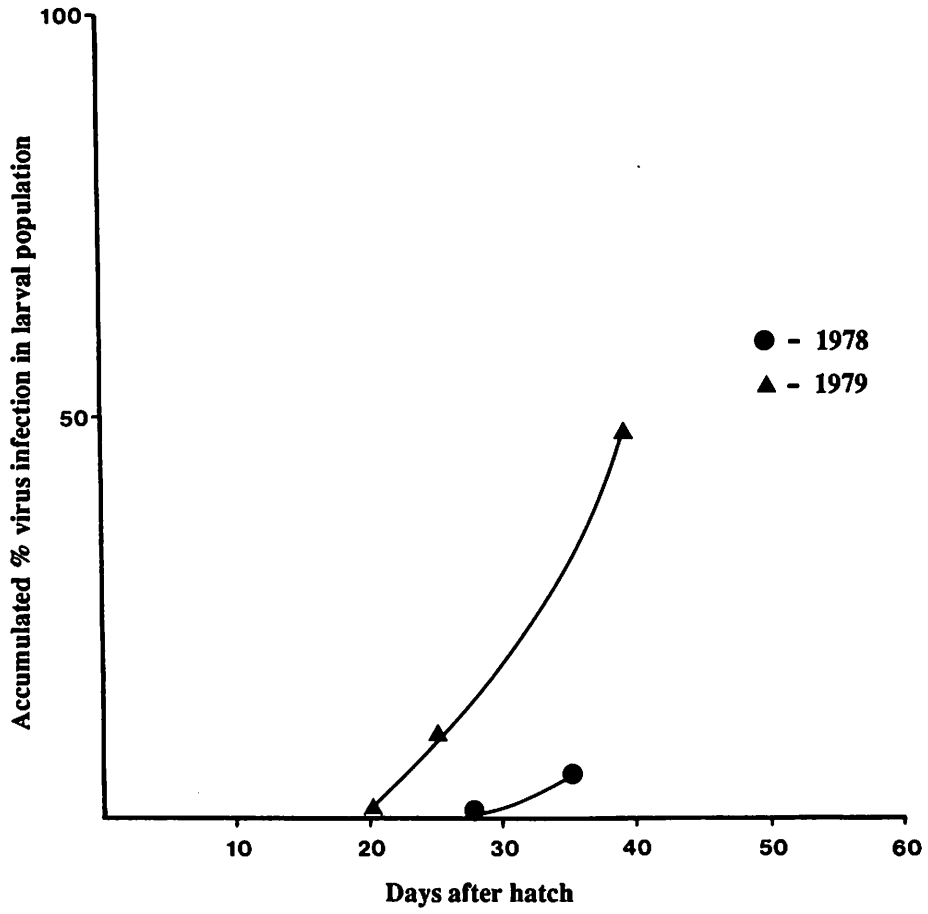

Figure 1. Infection growth curves (IGCs) of NPV epizootics observed in sawfly populations in plot W3 in 1978 and 1979; there was no epizootic in 1980.

released following the death of these larvae was calculated to be 4.5 X 10^{12}PIBs/ha (Kaupp 1983a). It is evident that inoculum produced during the epizootic in 1978 contributed to the development of an earlier epizootic in 1979. In 1980, NPV disease was not found in the 13 colonies that remained, indicating that population density is as important as the presence of inoculum for the development of an epizootic.

Dramatic epizootics were observed in plots Y5, Y6, and Y7 (Fig. 2). IGCs describing infection in these plots in 1978 were sigmoid, the first mortality due to NPV occurred 6 days after egg hatch and ended in 87.5%, 99.9% and 98.5% larval mortality in each plot, respectively. It was calculated that 2.8 X 10^{12}

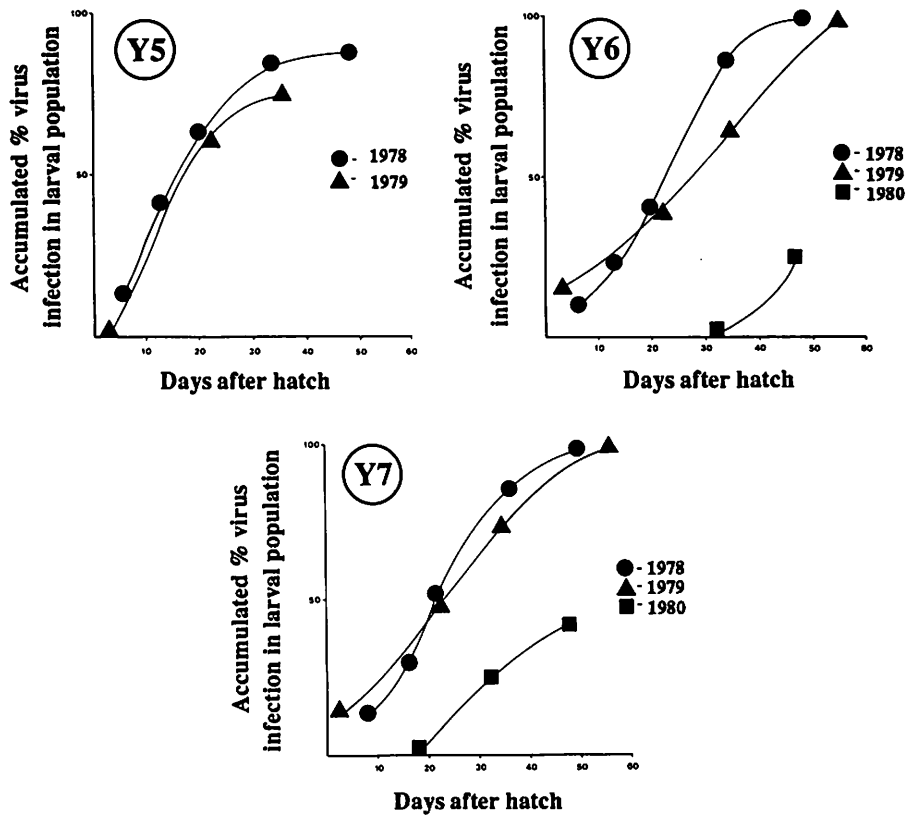

Figure 2. Infection growth curves (IGCs) of NPV epizootics occurring in sawfly populations in plots Y5, Y6 and Y7. Populations in plot Y were not affected by NPV epizootics in 1980.

3.7 X 10^{13} and 3.3 X 10^{14} PIBs/ha respectively, were released into the forest ecosystem from these plots due to death of larvae (Kaupp 1983a). In 1979, epizootics caused 75.0%, 99.9% and 99.9% mortality in plots Y5, Y6, and Y7 respectively, with disease observed 3 days earlier than in 1978. This event was in direct response to the large quantities of virus that were produced in 1978 and persisted on the trees. However, the quantity of inoculum produced in 1979, 5.5 X10^{11} PIBs/ha, 9.4 X 10^{12} PIBs/ha and 4.4 X 10^{12} PIBs/ha for plot Y5, Y6 and Y7, was less than the amount produced in 1978, which reflected the lower population levels (Kaupp 1983a). In 1980 no epizootic was observed in plot Y5. Levels of virus disease in plots Y6 and Y7 were much lower, with the first indication of infection observed at 20-30 days after egg hatch. Although virus was present in the environment, reduced population levels impeded the development of a major epizootic.

Epizootics in plot S8 occurred in much the same manner as in plots Y5, Y6 and Y7 (Fig. 3). In 1978, disease was first observed 6 days after hatch, with the IGC being sigmoid and reaching a level of 96.8% infection. This resulted in 2.3 X 10^{15} PIBs/ha being released into the forest ecosystem (Kaupp 1983a). As a result, the epizootic in 1979 occurred earlier than in 1978 and with more intensity, resulting in

99.8% insect mortality. As expected, only 3.9×10^{14} PIBs/ha was calculated to have been released into the forest ecosystem (Kaupp 1983a). In 1980, an epizootic was observed in the sawfly population probably due to the large quantities of NPV persisting in the canopy. However, infection was not detected until late in the season because of the reduced sawfly population. An abatement in the development of virus infection observed in the IGC can be attributed to a period of cold weather delaying both insect and pathogen development.

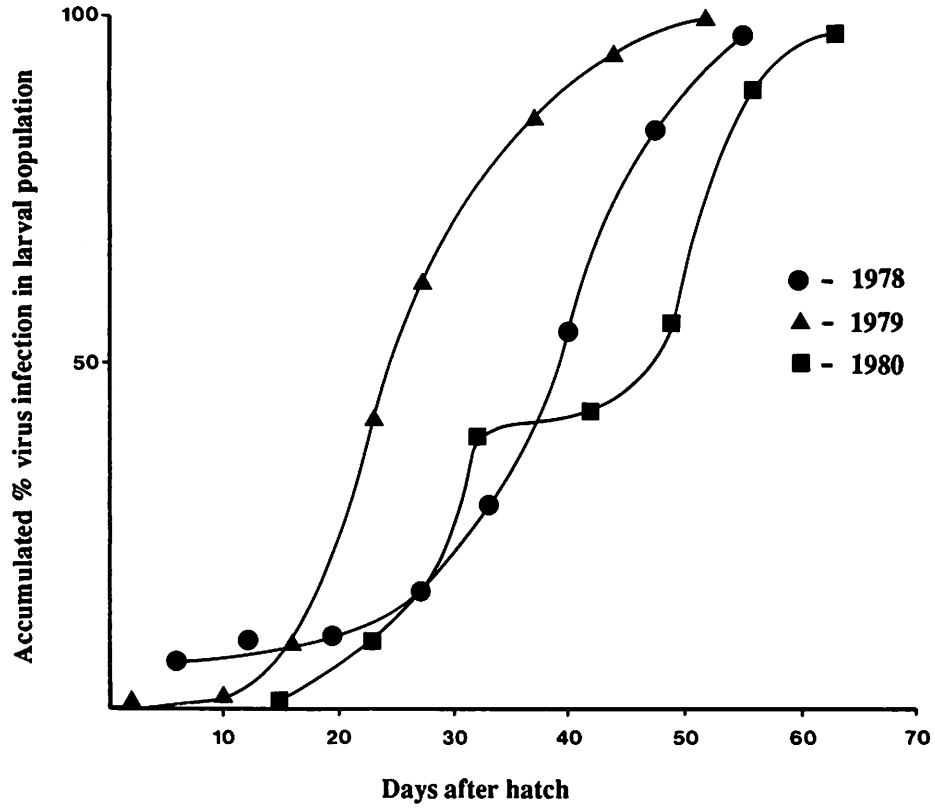

Figure 3. Infection growth curves (IGCs) of NPV epizootics in sawfly populations in plot S8.

To compare the rate of infection in the various populations, the IGCs were normalized by use of the transformation $\log_{10}[(X)/(1-X)]$, where "X" is the proportion of the population infected when sawfly densities are greater than 1 colony per tree. When sawfly population density was lower, the transformation $\log_{10}[(1)/(1-X)]$ was applied to infection data because disease development reflected only the impact of primary inoculum with no chance of multiple growth of infection resulting from infection and/or death of adjacent colonies (van der Plank 1963) (Fig. 4). Examination of the slopes of these normalized IGCs indicated that epizootics in sawfly populations greater than 1 colony per tree developed at much higher rates (0.05 - 0.09 infection units per day) than for populations with lower host densities (0.01 - 0.02 infection units per day) (Table 3). This difference can be attributed to

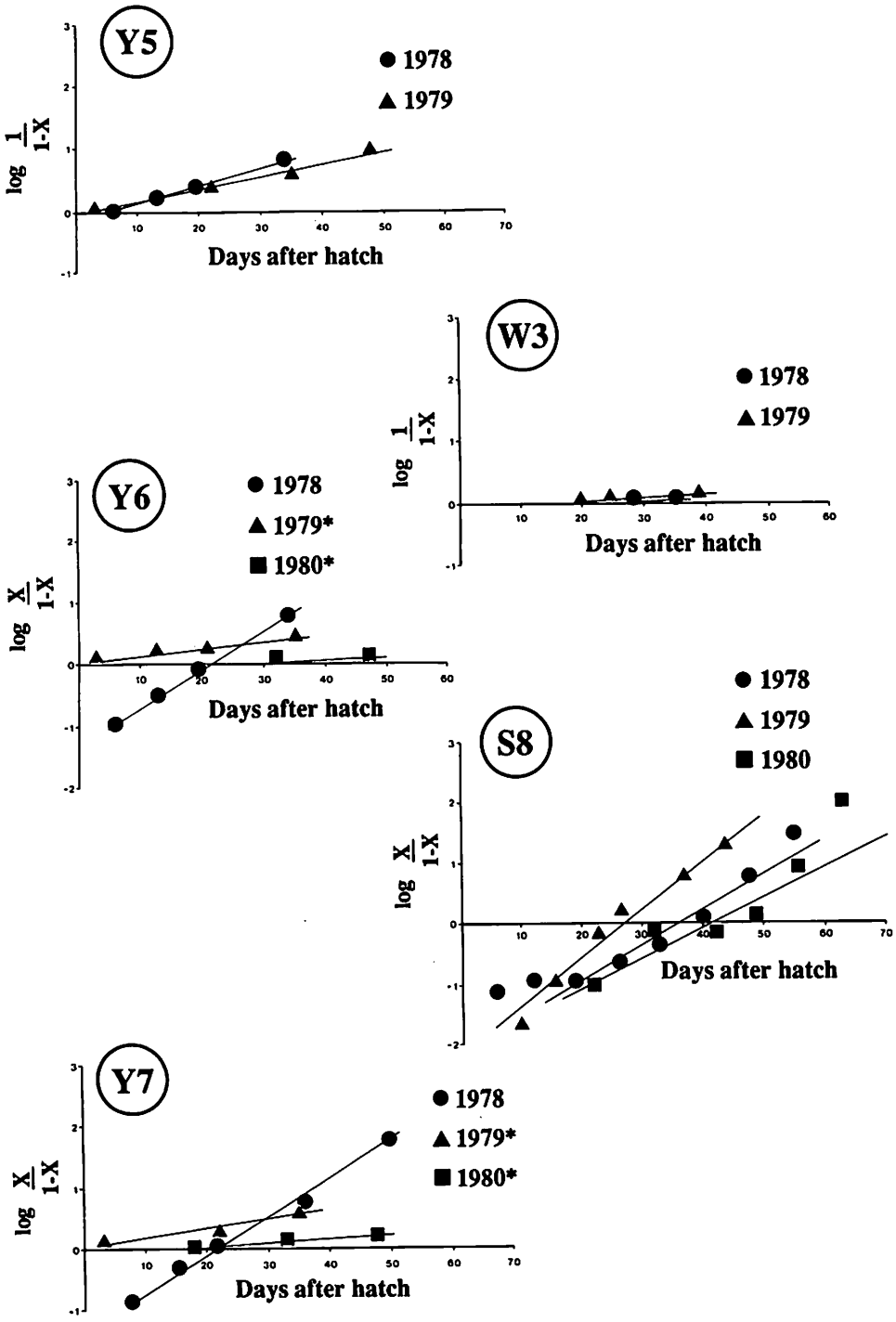

Figure 4. Normalized IGCs of all study plots. "*" denotes those years in which IGC was normalized by $\log_{10}$ [(1)/(1-X)].

interrelated dynamics involving disease spread, inoculum production and NPV persistence in changing densities of European pine sawfly. In this case, it reflects the density-dependence of *N. sertifer* NPV epizootics. Lower rates of infection reflect the reduced chance of infection of larvae with inoculum persisting in the canopy when population densities are low.

Table 3. Infection rates derived from the slopes of normalized infection growth curves

Plot	1978	1979	1980
S8	0.05	0.08	0.06
Y7	0.06	0.02	0.01
Y6	0.06	0.01	0.01
Y5	0.02	0.02	NV
W3	0.01	0.02	NV

"NV" no virus infection in population

CONCLUSIONS

The development of NPV disease epizootics in *N. sertifer* populations is density-dependent and infection usually develops in a typical sigmoid pattern. The nature of epizootics is also affected by weather and by the amount of inoculum produced in previous epizootics. Large amounts of inoculum tend to increase the persistence of NPV in the forest canopy, resulting in infection of the next sawfly generation at an earlier larval instar. This results in reduced inoculum production, which in turn contaminates the forest ecosystem. In conjunction with a reduction in sawfly numbers, the nature of the virus epizootic changes from dramatic to casual infection of the insect population.

The rate of epizootic development in high density European pine sawfly populations is similar to that observed in virus epizootics in redheaded pine sawfly, *Neodiprion lecontei* (Fitch), populations (de Groot and Cunningham 1983). Data available for other insects cannot be used to determine the rate of epizootic development because sampling methods were not designed to establish the proportion of the insect host population infected at any one point in time. Although percentage infection levels are often used to assess the effectiveness of viruses, there is a need for more precise measurements so that meaningful comparisons can be made between different insect-virus complexes.

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