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Foraging in a pathogen reservoir can lead to local host population extinction: a case study of a Lepidoptera-virus interaction

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Abstract In 1990, natural infestations of the polyphagous vapourer moth, *Orgyia antiqua* (Lepidoptera: Lymantriidae) in lodgepole pine plantations in northern Scotland, were studied to ascertain the role of host foraging behaviour on the prevalence of nucleopolyhedrovirus (NPV; Baculoviridae) infection in the population. Aerial dispersal of early instar larvae (L1–L3) from the tree canopy onto heather foliage at the forest understorey, with subsequent relocation back onto the tree as late-instar larvae (L4–L6) appeared to play a significant role in the development of a widespread virus epizootic in which approximately 80% of L4–L6 individuals succumbed to disease. Bioassays of foliage 1 year later showed that the distribution of NPV followed a pronounced vertical gradient through the forest canopy culminating in high concentrations of virus in the forest understorey. Experimental systems comprising potted pine trees positioned above heather bases showed that NPV infections could be acquired by early stage larvae following dispersal from the tree and feeding on the undercanopy vegetation, then translocated to the tree component for secondary transmission to susceptible tree-feeding individuals. Behavioural studies indicated that the tendency for first-, second- and third-instar larvae to disperse to the understorey was probably not influenced by larval density on the tree but was strongly dependent on larval instar. In contrast, the tendency for larvae to relocate from the understorey heather to the tree was affected by both larval density and larval instar, suggesting that both these factors may significantly affect virus acquisition, translocation and transmission in the host population. In the present study, the heather understorey appeared to act as a pathogen reservoir in which virus could persist between host generations. Spatial heterogeneity in virus distribution combined with host foraging behaviour (dispersal and feeding) resulted in the pathogen playing a major role in host population dynamics over an extended time period (3 years). The reservoir theory is supported by the observation that similar dynamics were not observed in *O. antiqua* populations at neighbouring sites which lacked understorey food plants.

Key words Nucleopolyhedrovirus · Dispersal · Foraging · Transmission · Pathogen reservoir

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Introduction

The role of insect dispersal and feeding behaviours on the transmission and spread of entomopathogens is considered a major factor influencing disease dynamics in insect populations (Onstad 1988; Dwyer 1991, 1992). Typically, the pattern of disease spread has been modelled as a travelling wave, the velocity and magnitude of which depend on pathogen transmission rate, pathogen virulence, productivity and inactivation, and host density (Dwyer 1992). Dwyer and Elkinton (1995) assessed the combined impact of long-distance aerial dispersal and short-range crawling by *Lymantria dispar* (Lepidoptera Lymantriidae) larvae, on the direction and rate of horizontal spread of baculovirus infections from artificially induced foci. In this study, early larval ballooning was a good predictor of early spread of infection but subsequent spread could not be accounted for by either ballooning or short-range larval dispersal.

Baculoviruses are capable of persisting outside the host in a viable state for long periods of time and accomplish this by forming a viral occlusion body in which the infectious virus particles are shielded from the abiotic environment (Tanada and Fuxa 1987). In localities where insect pathogens are protected from sunlight e.g. the soil environment, baculoviruses can remain infectious for many years (e.g. Thompson et al. 1981). Nevertheless, in positions where baculovirus occlusion bodies are exposed to solar radiation, virus is rapidly inactivated (e.g. Jones et al. 1993).

Hochberg (1989) suggested that existing insect-pathogen population models did not adequately describe the infection dynamics of some species because they took no account of spatial heterogeneity in the pathogen population (Anderson and May 1981). Hochberg divided the pathogen population into two habitat classes: one where transmission to hosts was possible but where pathogen persistence was low (i.e. feeding sites on plant surfaces), and another where persistence was extended but transmission could not occur (e.g. the soil environment below the food plant). The outcome of simulation models was stable limit cycles or stable host-pathogen population equilibria, depending on the rate of pathogen translocation between the two habitats. The role a virus reservoir appears to play in the *Wiseana*-baculovirus association in New Zealand pasture (Crawford and Kalmakoff 1977) was presented by Hochberg as support for the importance of pathogen reservoirs and translocation processes in the infection dynamics of some insect-pathogen systems.

In habitats where the food plant (transmission site) is short-lived, e.g. annual crops, the soil is probably the most important habitat for the virus reservoir. In these cases, translocation from the soil pathogen reservoir to the transmission site may be achieved by seedlings growing through the soil, by rainsplash or by biotic vectors such as grazing animals (e.g. Jacques 1970; Crawford and Kalmakoff 1977; Fuxa and Geaghan 1983). In habitats where the host food plant is long-lived and/or vertically stratified (e.g. forests) pathogen decay by UV irradiation is reduced at the lower canopy feeding sites by upper canopy shading (Olofsson 1988; Woods et al. 1990). This can lead to spatial heterogeneity in pathogen density between canopy levels. The action of rainfall washing infective material from upper to lower branches and eventually onto the soil will augment this effect (D'Amico and Elkington 1995). In such cases, the probability of disease transmission to healthy insects will be highly dependent on host movement and feeding behaviours between canopy levels.

This study aimed to ascertain whether short-range host dispersal and feeding behaviours (collectively termed here as "foraging") can influence virus transmission, translocation and transmission in a vertically stratified forest habitat, where the shaded understorey is both a pathogen reservoir and a food plant. The system is represented by the vapourer moth, *Orgyia antiqua* and a nucleopolyhedrovirus (NPV) in a forest habitat comprising lodgepole pine (*Pinus contorta*) and an understorey of heather (*Calluna vulgaris*).

Materials and methods

Phenology of *O. antiqua* on lodgepole pine in northern Scotland

In the far north of Britain, the vapourer moth, *O. antiqua*, is univoltine. After mating, the flightless female lays 100–300 eggs in a single, conspicuous mass on the pupal cocoon. The eggs overwinter and larval emergence occurs during the period April to June, depending on temperature, to coincide with the production of new foliage in the spring. Following egg eclosion, neonate larvae consume most of the egg chorion then congregate at the ends of pine branches. Larvae represent the specialised stage for dispersal. Larvae spin silken threads from which they dangle in the air awaiting wind currents to balloon them to new sites. Larval development is completed by the end of summer (August to September) and the pupal period takes 2–4 weeks. Winged adult males fly to the wingless females resident upon the pupal cocoon and after mating, the next generation of eggs is laid.

Description of field site

In May 1990, a population of *O. antiqua* was located at Braehour forest (Ordnance Survey reference: N582505, E033300) in the Caithness region of northern Scotland. The forest comprised pure stands of lodgepole pine, sitka spruce (*Picea* spp.) and larch (*Larix* spp.). A study plot was established in a 9-year-old block of lodgepole pine with trees measuring 1.5–3.0 m in height, planted 2 m apart. The soil type was a deep, unflushed peat. Tree canopies did not overlap and the understorey vegetation of heather (*C. vulgaris*) was estimated by quadrat sampling as covering >75% of the forest understorey. A sampling grid of 10×10 points, with 10 m between each point, was established by tagging and coding groups of four trees. The 1-ha sampling grid (100 points, 400 trees) was used for the duration of the 3-year-study period (1990–1993).

Determining *O. antiqua* distribution and abundance

Immediately prior to egg eclosion in May 1990, the four trees comprising each grid point were each measured for height and each tree canopy divided equally into three height levels termed upper, middle and lower. The number of egg masses at each height level was recorded for each of the 400 trees. Egg mass counts were also conducted in the forest understorey using one 0.25-m^2 quadrat positioned at the epicentre of the four trees comprising each grid point. On 19 June, when >90% of viable egg masses had shown signs of eclosion, larvae were sampled from a single tree at each of ten randomly selected grid points. This sample was repeated for different grid points every 14 days until pupation in late September. Larval sampling was conducted by cutting off two branches from the mid-point of each canopy height level, followed by beating over a canvas sheet. In addition, a 0.25-m^2 quadrat, positioned at the centre of the four trees comprising each sampling grid point was used to make larval counts in the forest understorey. Sampling of larvae was conducted between 1000–1400 hours. The number and instar of larvae were recorded in the field; larval instar was determined using a combination of size, coloration and development of abdominal hair tufts. Sub-samples of larvae were later checked in the laboratory to confirm instar by measurement of head capsule width.

Estimating the level of virus infection in the insect population

Field-sampled larvae were held individually inside sterile plastic containers for transport to the field laboratory, and were reared separately on the type of foliage on which they had been found, i.e. pine or heather. Foliage was collected from the field site and surface decontaminated by immersion in 0.1 M Na_2CO_3 for 30 min, then rinsed thoroughly in several changes of clean tap water. Sprigs of current-year pine foliage and heather were cut to 5-cm lengths and maintained in transparent plastic containers with sprig stems fed by a water supply. Foliage was replaced every 7 days and larvae reared until death or adult emergence. Cadavers were stored at −20°C until examination. NPV infection was diagnosed by Giemsa staining and microscopic examination for the presence of viral occlusion bodies (Wigley 1976).

Measuring the distribution of virus in the forest canopy in 1991

Samples of pine and heather foliage were collected from the Braehour study area in 1991 and bioassayed using a clean laboratory culture of *O. antiqua* larvae to determine the distribution of NPV inoculum in the forest canopy 1 year after the first field observations. To synchronise foliage collection with larval emergence, foliage was sampled when approximately 50% of pre-tagged egg masses had shown signs of hatching (mid-June 1991). A sprig of pine comprising both current-year and 1-year-old growth was removed from the mid-point of upper, middle and lower canopy height positions, from a single tree, located at each of 40 randomly selected grid points. A sprig of heather from the forest understorey was removed from each grid point concurrently. This procedure was conducted on a weekly basis for a period of 5 weeks.

In the laboratory, sprigs were trimmed from the cut end to give 5 cm of 1-year-old growth and approximately 10 cm of new shoot growth. Care was taken when handling foliage to minimise the risk of cross-contaminating samples, particularly those from different canopy height levels. Pine sprigs that had been surface decontaminated by immersion in 0.1 M Na_2CO_3 for 30 min, then rinsed in several changes of clean tap water, were included as a control. Each of the 40 sprigs of heather foliage sampled from the forest understorey were sub-divided into two, with one set of sprigs surface-decontaminated and the other set of sprigs left untreated.

Insect material used in foliage bioassays in 1991

Larvae used in bioassays were hatched from approximately 50 field-collected egg masses from a separate population located some 50 miles from Braehour forest, at North Dalchork forest, in May 1991. Egg masses were first surface-decontaminated by immersion in 10% formalin solution for 30 min then rinsed in several changes of fresh water. After hatching, larvae were maintained on a sterile insect diet at ambient temperature in a mobile laboratory. Sprigs of foliage were individually placed into plastic containers which were enclosed with a single first-instar larva (48–72 h old) using a piece of damp muslin to prevent dessication. Foliage was replaced weekly and larvae checked for mortality at that time. Cadavers were stored at −20°C to await confirmation of NPV infection as previously described.

Larval-foraging studies

The aim of this study was to test whether the tendency for *O. antiqua* larvae to disperse between pine and heather understorey positions was influenced by larval instar and/or larval density. Experimental units were constructed using lodgepole pine trees with trays of heather positioned around the base of the tree trunk to provide an area of understorey foliage. Lodgepole pine trees and turfs of heather used in this experiment were removed from South Dalchork forest in May 1992. Trees were selected for similar height (approx. 2 m) and growth form then pruned to a standard pattern so that each tree comprised three main whorls of branches, one each to represent the lower, middle and upper canopy height levels. Each canopy height level (whorl) consisted of four main branches. To minimise the risk of exposing test larvae to naturally occurring NPV, each pine tree and heather turf was completely immersed in a large volume of 0.1 M sodium carbonate solution for 30 min and rinsed thoroughly in two changes of clean tap water and then allowed to air dry.

Each tree was planted in a bucket containing a 1:1 soil-peat mixture. Turfs of heather were planted in shallow plastic trays measuring 0.125 m^2 containing the peat compost only. Two of these trays of heather were fastened at the base of each tree, one either side of the tree trunk and supported by the rim of the bucket, to give a total area of understorey heather of 0.25 m^2 per pine-heather unit. Each experimental unit was then surrounded by a catchment funnel made of polythene sheeting coated with Fluon, spanning outwards and upwards from the perimeter of the trays of heather. This structure allowed for the aerial movement of larvae between tree and heather while minimising the number of larvae lost from the tree-heather units. Experimental units were placed inside windowless observation rooms fitted with overhead strip lights set to a 16:8 h light:dark cycle and maintained at 20°C ± 3°C. Each observation room contained three pine-heather units.

All larvae used in the study had been hatched from egg masses collected from North Dalchork forest in May 1992. Egg masses were surface-decontaminated by immersion in 10% formalin solution for 30 min. Prior to use, larvae were maintained on a semi-synthetic diet at 20°C in an incubator on a 16:8 h light:dark cycle. Immediately before releasing larvae into the experimental units, the abdominal hairs of all larvae were lightly brushed with coloured fluorescent dusts. This served two purposes: first, insects could be located during dark hours by visualising larvae with a hand-held UV illuminator and, second, this allowed larvae to be colour coded with respect to their position in the unit (tree or heather) at the time of release. Larvae were released at the mid-point of the tree canopy

Table 1 Stage and density specifications for the *Orgyia antiqua* larval dispersal study

Larval instar	Density class	Number of larvae released/tree	Mean number and range of larvae observed on tree	Number of larvae released/0.25 m^2 heather	Mean number and range of larvae observed on heather
First	Low	50	34 (33–35)	–	–
	Medium	100	68 (64–71)	–	–
	High	200	155 (154–166)	–	–
Second	Low	20	14 (13–16)	15	10 (8–11)
	Medium	40	28 (26–30)	25	18 (15–21)
	High	60	45 (39–53)	40	29 (28–30)
Third	Low	20	14 (12–15)	15	9 (7–11)
	Medium	30	21.7 (18–24)	25	19 (18–20)
	High	50	37 (35–40)	50	38 (36–45)

and to the centre of each of the two trays of understorey heather at the densities given in Table 1. Each instar was tested separately. For each instar, nine replicate trees were used. The measurement of dispersal was taken to be the percentage of individuals which had changed position during the entire study period. The first count was conducted 24 h following release, and counting was repeated every 4 h for a total of ten successive time points. The number of larvae which had dispersed divided by the total number of larvae observed, averaged for all time points, was used in the calculation of mean percent dispersal for 'tree larvae' and for 'heather larvae'.

Virus transmission and translocation

The aim of the virus translocation study was to determine whether larvae could acquire a lethal NPV infection from the understorey heather following dispersal off the tree, then translocate secondary inoculum from the heather understorey to the pine by relocating back onto the tree. This was done by comparing percentage virus mortality in larvae sampled from the tree component only for each of two treatments: one in which the heather understorey was surface contaminated with virus and the other in which the heather understorey was treated with water only. Both treatments were replicated five times. A dose of 2×10^7 polyhedral inclusion bodies (PIBs) in 100 ml of water was applied to each 0.25-m^2 area of heather using a household hand-sprayer and allowed to dry before attaching the heather trays to the tree bases. These experiments were performed in Oxford, during the period June to July 1992.

The pine-heather units used in this experiment were placed in one of two 2.5-m-high wind-break structures constructed out of green polythene sheeting. The roof of each structure was covered by a fine mesh to prevent predation of larvae by birds. All larvae used in the study were hatched from surface-sterilised egg masses collected from North Dalchork forest in May 1992. Hatching larvae were maintained on the semi-synthetic diet described previously. At the second-instar stage, 200 larvae were released from paper tubs into the tree canopy of each experimental unit and left to redistribute. Larvae were only released onto the tree component of each unit. At 8, 10, 11, 15, 27 and 28 days post-release, all larvae present on each tree were removed and reared individually in sterile pots containing a semi-synthetic diet at 20°C, under a 16:8 h light:dark cycle, until death or adult emergence. Larvae were not collected from the heather at any time and this was the only source of virus inoculum in the experiment. Virus mortality was diagnosed as previously described.

Data analysis

Data from studies on the distribution of *O. antiqua* at Braehour forest, stage- and density-related insect dispersal, and virus transmission and translocation were analysed using the Generalized Interactive Modelling (GLIM) programme (Royal Statistical Society 1985). The probability associated with any change in model deviance, after fitting the various explanatory variables and interactions, was examined by calculating the χ^2 statistic with binomial errors, where the degree of over-dispersion was within acceptable limits (residual deviance divided by the residual degrees of freedom was <3). In such cases, a dispersion parameter was calculated and used to adjust the scale parameter (given in text). When scaling was >3, data were arc-sine transformed and *F*-values presented.

Between-tree egg mass frequency distributions were examined using a procedure computer programme devised by Dr. R. Hails of the IVEM, Oxford, which calculated the exponent k (a dispersion parameter) of the negative binomial, and tested agreement with this distribution by comparing expected against observed frequency counts using a χ^2 goodness-of-fit test. The distribution of virus in the forest canopy in 1991 was examined from bioassays of foliage sampled from different canopy height levels. Multiple pairwise comparisons of bioassay data were made using a χ^2 goodness-of-fit test with probability values corrected using a sequential Bonferroni procedure (Rice 1989).

Results

Distribution of *O. antiqua* in the forest canopy

Egg masses

The distribution of *O. antiqua* egg masses on lodgepole pine at Braehour forest, in May 1990, showed close agreement with the negative binomial distribution ($\chi^2 = 14.35$; $df = 20$, $P > 0.8$). The fractional value of the dispersion parameter k (0.809) indicated that within the 1-ha sampling area, the population was very highly clumped with almost 70% of the total egg masses observed ($n = 999$) present on <25% of the available trees ($n = 400$). No egg masses were ever observed on the understorey heather in the 1-ha sampling area in 1990, or in subsequent years (1991 and 1992). In contrast, in open tracts of forest, e.g. forest rides, egg masses were frequently observed in this position. Mean egg mass density in 1990 was 2.50 egg masses/tree (± 0.16 SE).

Data on the within-tree distribution of *O. antiqua* egg masses showed that a significantly higher percentage of the population were located in the central third of the tree canopy ($\chi^2 = 143.80$, $df = 2$, $P < 0.0001$; scale parameter = 1.49; Fig. 1a). Following egg eclosion in the spring of 1990, the spatial dispersion of the *O. antiqua* population was characterised by dense aggregations of larvae on a small proportion of available food resources.

Larvae and pupae

In contrast to the egg stage, the position of early larvae (L1–L3 combined) within the tree canopy indicated that the population had largely redistributed from the middle canopy to upper and lower tree canopy positions ($\chi^2 = 29.10$, $df = 2$, $P < 0.0001$; scale parameter = 1.95; Fig. 1b). Furthermore, by the second larval collection (4 July 1990), a large proportion of the larval population, comprising mostly second- and third-instar individuals, was observed feeding in the forest understorey on the heather foliage. By 19 June 1990, the majority of the larval population were represented as fourth-instar individuals and were observed on the understorey heather and in the tree canopies with similar frequency. Conversely, fifth- and sixth-instar larvae were nearly always found in trees. In the tree canopy, late-instar larvae (L4–L6 combined) showed no preference for any particular height level ($\chi^2 = 2.75$, $df = 2$, $P > 0.25$; scale parameter = 1.76; Fig. 1c). Within-tree distributions of pupae mirrored that of egg masses with a numerical bias toward the middle third of the tree compared to upper and lower canopy levels, although in this case the difference was not significant ($\chi^2 = 2.97$, $df = 2$, $P > 0.22$; scale parameter = 1.19; Fig. 1d). No pupae were observed on the heather foliage inside the 1-ha sampling grid in 1990 or in subsequent years.

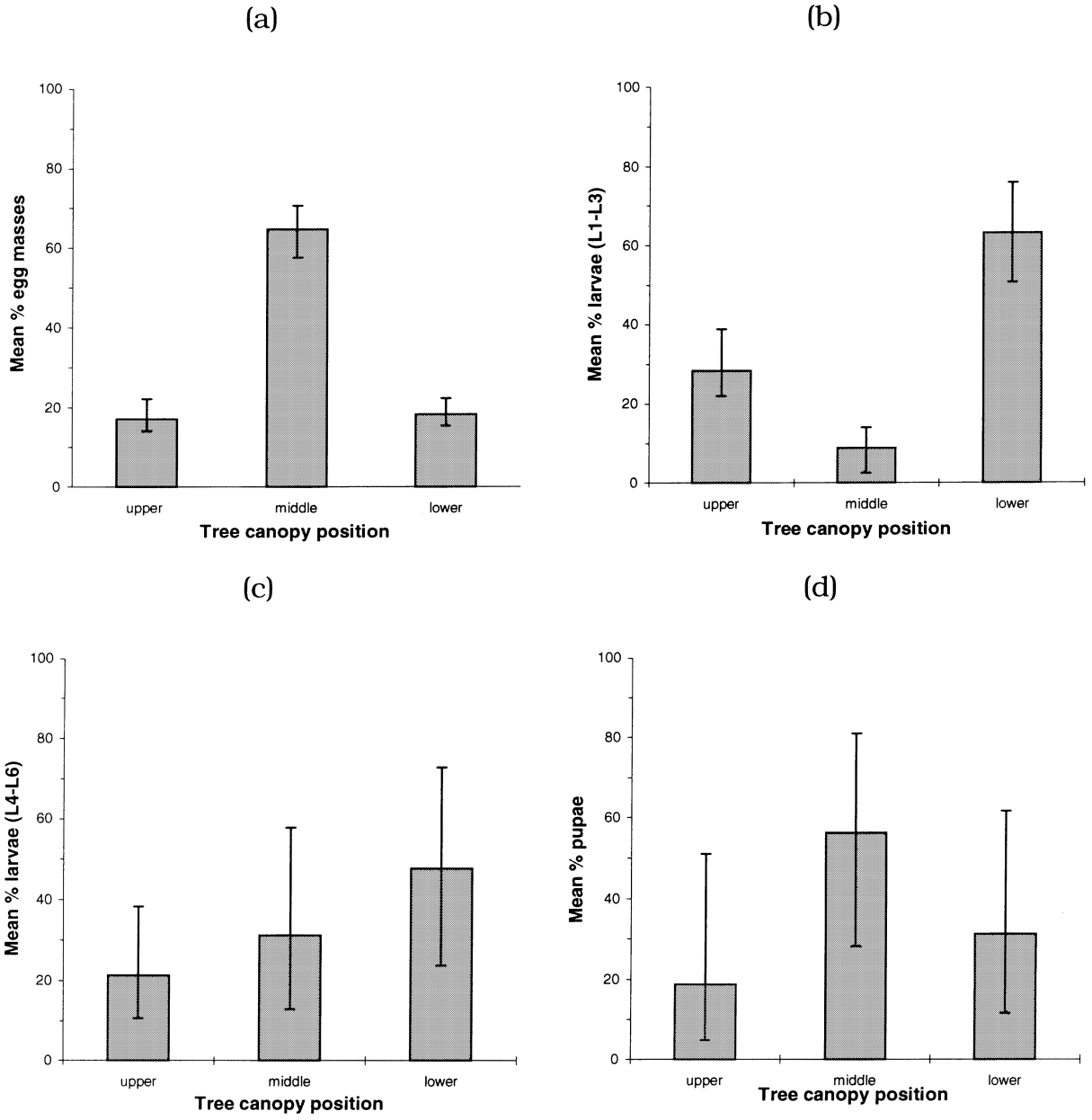

Fig. 1 Within-tree canopy distribution of *Orgyia antiqua* at various life-stages: egg masses (**a**), early instar larvae (L1–L3) (**b**), late-instar larvae (L4–L6) (**c**) and pupae (**d**). *Error bars* represent the ±95% confidence interval

Incidence of virus infection in 1990

Table 2 shows the incidence of NPV infection in early stage (instars L1–L3) and late-stage (instars L4–L6) *O. antiqua* larvae sampled from the tree canopy and understorey heather at Braehour forest in 1990. Percent NPV infection in early stage larvae sampled from the tree and heather components (12% and 53.9%, respectively) indicated the understorey to be an important source of virus inoculum. Virus mortality had dramatically increased during the late larval stages (L4–L6 larvae) to 79.5% for tree and understorey larvae combined. The implication from these data that an epizootic was developing at Braehour forest was confirmed at the end of August both within the 1-ha sampling area and a large area (at least 1 km radius) outside the sample grid. Large numbers of infected cadavers were observed, in the vast majority of cases in the tree canopy rather than on the understorey heather. Pupal samples taken on 14 and 28 September 1990 (total $n = 86$) showed the level of NPV mortality was 38.4% with additional mortalities arising from hymenopteran parasitoids (28%), and unknown factors (2.3%). A χ^2 goodness-of-fit test indicated that of the 31.3% pupal survivors to the adult stage, the sex ratio was heavily biased toward males (70.9%) ($\chi^2 = 4.48$, $df = 1$, $P < 0.05$).

Table 2 Larval age-structure and incidence of nucleopolyhedrovirus (*NPV*) infection in a population of *O. antiqua* at Braehour forest in May 1990

Sample period	Larval instar	Tree canopy		Understorey heather	
		Larval stage structure (%)	NPV mortality (%)	Larval stage structure (%)	NPV mortality (%)
19 June 1990 to 6 August 1990	L1	81.8	L1–L3 12% ($n=159$)	0	L1–L3: 54% ($n=87$)
	L2	11.3		16.5	
	L3	6.9		83.5	
19 July 1990 to 14 September 1990	L4	61.4	L4–L6: 63.2% ($n=57$)	90	L4–L6: 95% ($n=60$)
	L5	21.1		8.3	
	L6	17.5		1.7	

Abundance of *O. antiqua* over the study period

Meticulous searches for egg masses in 50 whole-tree canopies and routine observational searches of several hundred trees inside the 1-ha sampling area at Braehour forest in May 1991 and May 1992 revealed that not a single *O. antiqua* egg mass had been laid in the autumn of 1990 or 1991, even though the remains of egg masses from preceding years were frequently encountered. Moreover, this pattern appeared to have been repeated over an extensive area (5–10 km^2 searched) where egg masses were conspicuous in 1990. While the possibility exists that the *O. antiqua* population had been reduced to a level too low to be detected by the sampling programme, the fact that less rigorous procedures were used to routinely monitor very low density populations at other forest sites of similar growth characteristics (data not shown) lends some weight to the contention that *O. antiqua* had become extinct at Braehour forest in the autumn of 1990 following the NPV epizootic.

Distribution of virus in the forest canopy in 1991

The persistence and distribution of virus inoculum in the canopy at Braehour forest in 1991, 1 year after the devastating epizootic, was examined using foliage bioassays. A clear pattern emerged in terms of percentage virus mortality of test larvae reared on foliage sampled from different height levels in the Braehour forest canopy (Fig. 2) indicating a marked gradient of increasing NPV concentration with decreasing canopy height, culminating in a significantly higher concentration of virus in the forest understorey ($\chi^2 = 10.94$, $df = 1$, $P < 0.01$).

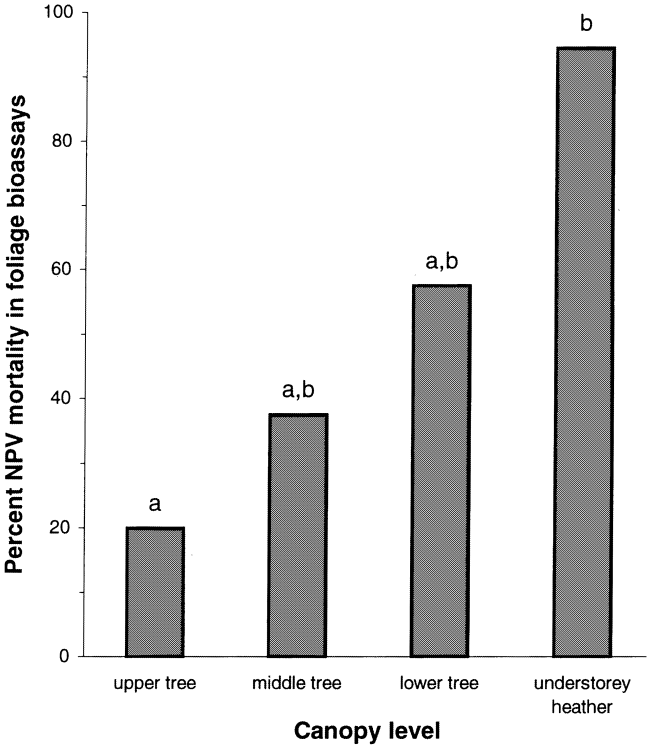

Fig. 2 Percent NPV larval infection in bioassays of foliage sampled through decreasing heights in the forest canopy, Braehour forest, June to July 1991. Data were compared using a χ^2 goodness-of-fit test and *P* values corrected for multiple comparisons. *Bars with different letters* indicate significant differences

Stage- and density-related host dispersal behaviour

The effect of larval instar on mean percent tree-to-heather relocation by early instar (L1–L3) larvae was highly significant ($F_{2,24} = 23.87$, $P < 0.0001$; Fig. 3a). In contrast, larval density did not significantly effect the tendency of larvae to relocate from tree to heather ($F_{2,22} = 3.31$, $P = 0.06$). The effect of block (i.e. observation room) on tree-to-heather dispersal for L1–L3 larvae combined was not significant ($F_{2,24} = 0.43$, $P = 0.66$) nor were any of the possible interactions.

In contrast to downward movement, the effect of larval density on percent heather-to-tree relocation was significant for second- and third-instar larvae ($F_{2,14} = 6.50$, $P = 0.01$; Fig. 4). Analysis of the effect of larval instar on percent dispersal showed that the tendency for larvae to relocate to the tree was also significantly different ($F_{1,16} = 6.62$, $P = 0.02$; Fig. 3b), increasing from a mean of 10% for second-instar larvae to almost 50% for third-instar larvae, although the dispersal response by the two instars to increasing density was very similar ($F_{2,12} = 0.54$, $P = 0.60$). The effect of block design on heather-to-tree dispersal for L2 and L3 larvae combined was not significant ($F_{2,15} = 0.20$, $P = 0.82$).

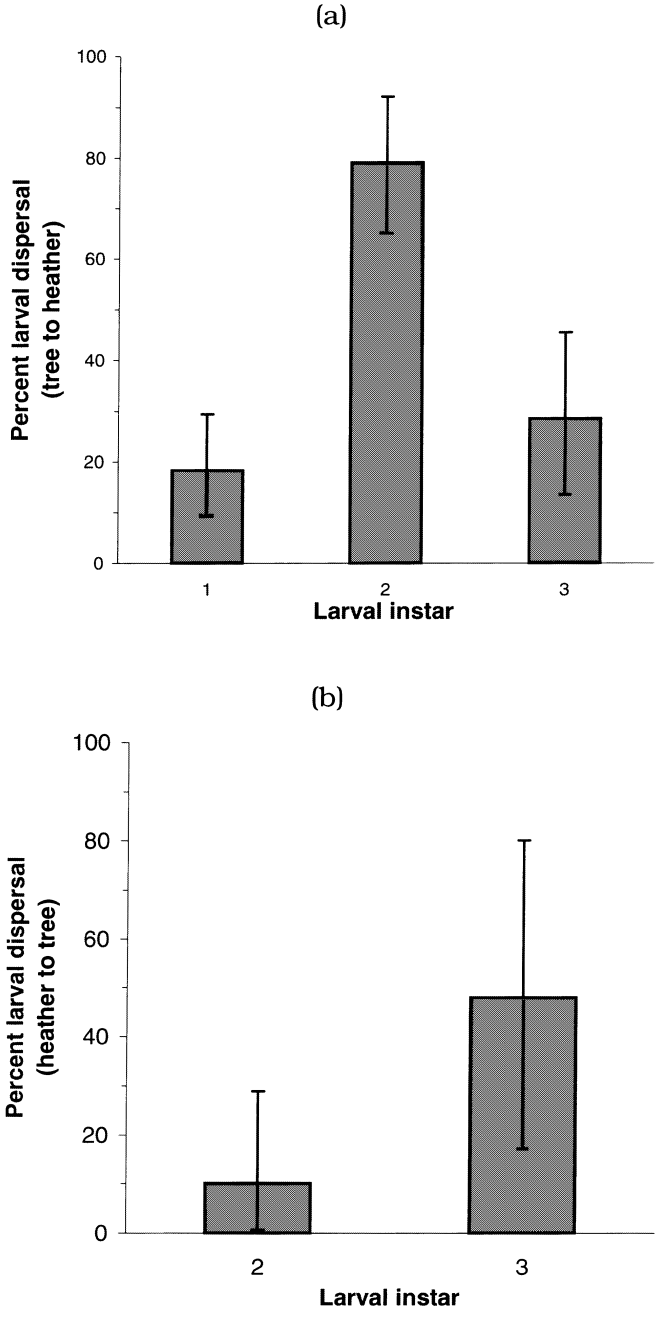

Fig. 3 Stage-dependent dispersal of *O. antiqua* larvae from tree canopy to heather understorey (**a**) and heather understorey to tree canopy (**b**). *Error bars* represent the ±95% confidence interval

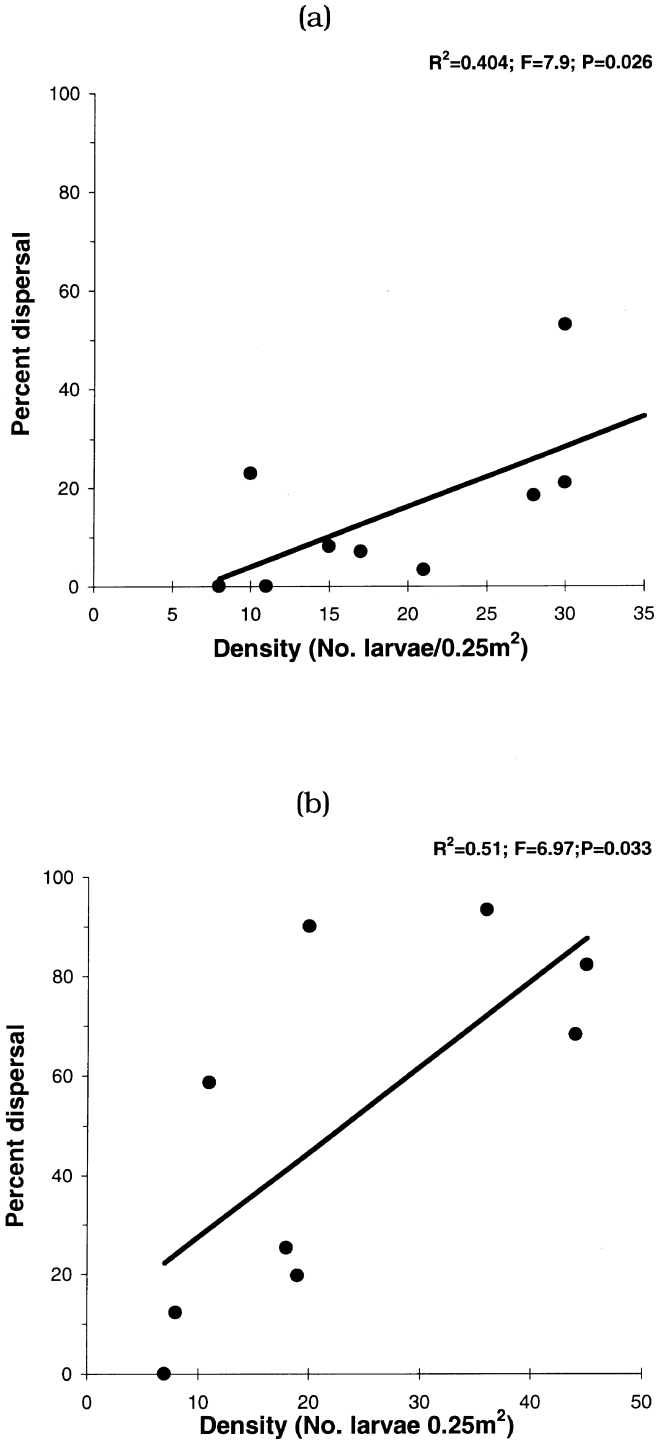

Fig. 4 Density-dependent dispersal from heather understorey to tree canopy for second-instar (**a**) and third-instar (**b**) *O. antiqua* larvae

Virus transmission and translocation

The difference in the cumulative percentage of total larvae recovered from the tree canopies above virus-treated and control understorey heather, over the 28-day study period, was highly significant ($F_{13,56} = 25.85$; $P < 0.0001$; Fig. 5a). At its most extreme, the rate of recovery of tree larvae from virus-treated compared to control tree-heather units differed by a factor of more than 3 at day 7 (51.3% and 16.8%, respectively) indicating a strong modifying effect of NPV infection on host orientation.

Mean percent virus infection in larvae sampled from the tree component only sited above treated heather bases is shown graphically in Fig. 5b. Mean NPV infection was lowest in larvae recovered in the first sample (day 7, mean: 61.9%; range: 41.7–82.6%) rising to >90% infection by day 11 and peaking at 98.3% by day 15 and remaining near this level for the duration

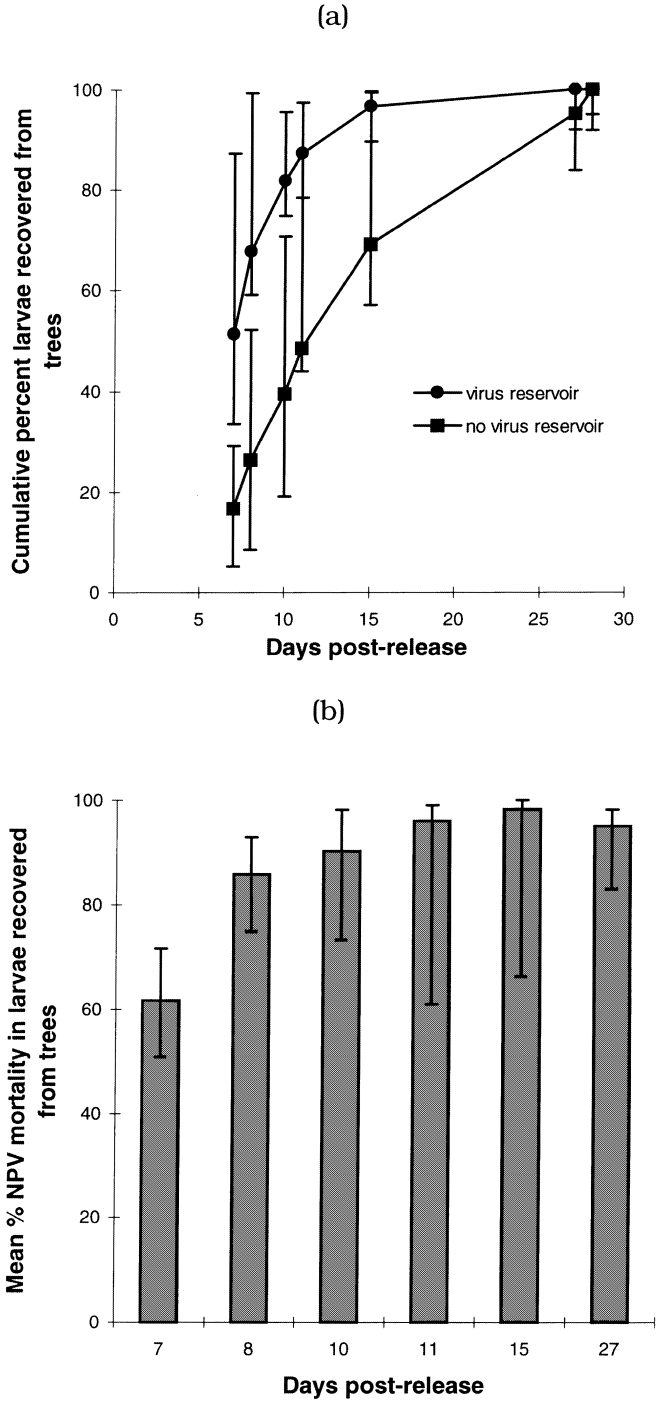

Fig. 5 Cumulative recovery of *O. antiqua* larvae from trees with and without an understorey virus reservoir (**a**), and mean percent virus infection in larvae recovered from tree canopies sited above an understorey virus reservoir of heather (**b**). *Error bars* represent the ±95% confidence interval

of the study. Only one larva (infected) was retrieved from the treated plots at day 28 and was not included in the analysis. The experiment was terminated at this point. The lower percent infection in the first larval sample was probably due to the presence of a proportion of non-dispersing individuals which did not have sufficient time for acquisition of secondary inoculum translocated onto the tree by lethally infected larvae relocating from the heather-virus understorey. This interpretation is supported by the fact that virus-infected cadavers were observed in tree canopies only after the fourth time point at day 11. The overall effect of sample date on percent virus infection, however, was not significant ($\chi^2 = 3.91$, $df = 5$, $P = 0.56$, scale parameter = 1.72). The presence or absence of an understorey virus reservoir on mean percentage virus infection in *O. antiqua* larvae removed from trees sited above treated and control heather bases (73.2% and 1.9%, respectively) was very highly significant ($F_{1,8} = 77.18$, $P < 0.0001$).

Discussion

The spatial distribution of the *O. antiqua* population in Braehour forest at egg eclosion in 1990 was highly aggregated, with nearly 70% of individuals on less than 25% of the available pine trees. Within-tree patterns were also highly aggregated with over two-thirds of individuals located in the middle third portion of the tree canopy. The spatial structure of the insect population changed markedly following large-scale aerial dispersal of early instar larvae, resulting in a pronounced redistribution to the heather foliage in the forest understorey. Relocation back onto the tree by third- and fourth-instar larvae was followed by a widespread virus epizootic, and egg mass searches covering an extensive area of Braehour forest in the subsequent 2 years indicated that this population had become extinct.

Foliage bioassays in 1991 revealed that the distribution of virus particles followed a well-defined vertical gradient through the tree canopy culminating in a high concentration of virus on the understorey heather foliage. This pattern presumably arose from the downward movement of virus due to rainfall and the fact that virus on the heather was protected from solar inactivation by tree canopy shading. Similar spatial gradients have been reported for other NPVs in forest habitats (Olofsson 1988; Killick and Warden 1991) and other plants which show pronounced vertical stratification (Young and Yearian 1989).

Examination of larval distribution patterns indicated a marked, age-specific tendency for *O. antiqua* larvae to disperse to and feed on the understorey heather before returning to tree feeding. When the heather was deliberately contaminated by virus, this behaviour dramatically increased the probability of virus transmission in those larvae undertaking downward dispersal and resulted in the translocation of virus inoculum by infected larvae returning to the tree prior to death. We therefore predict that heather-to-tree movement of infected individuals will increase the likelihood of secondary cycling of virus through transmission to conspecifics showing

solely tree-feeding behaviour. Very few virus infections were observed during the same period in *O. antiqua* populations in other forests of similar growth and age characteristics but which lacked understorey food plant species (data not shown), indicating that polyphagous feeding behaviour and the presence of a virus reservoir were key factors in the development of a virus epizootic in the Braehour forest population. Dispersal of primary-infected larvae giving rise to a second infection wave later in the larval period has been observed in populations of other lymantriid species although under circumstances different to those observed in the present study (Woods and Elkinton 1987; Sterling et al. 1988; Woods et al. 1989; Murray and Elkinton 1990).

Polyphagy in insect species has been suggested as a mechanism for predator and pathogen avoidance (Rossiter 1987; Bernays and Graham 1988), and Foster et al. (1992) related host plant exploitation by *L. dispar* larvae to the risks associated with infection by its NPV. They suggested it was advantageous for *L. dispar* larvae to exploit host plant foliage with high concentrations of allelochemicals (e.g. phenols and tannins) known to significantly reduce the susceptibility of larvae to infection (Keating and Yendol 1987; Richter et al. 1987; Duffey et al. 1995) because this would probably compensate for any costs such as lower fecundity associated with their intake. The authors also suggested that unstable insect-pathogen population dynamics are likely to result from the effect of plant canopy shading on the pathogen population. Heathers are generally high in tannins (W. Wint, personal communication) but in our study, the protective effect of secondary plant compounds in heather may have been insufficient to overcome high virus densities on the shaded understorey vegetation.

Results from this study also provided tentative evidence that the tendency for larvae to move up onto the trees from the understorey heather increased with host density. Leonard (1970, 1974) suggested that density-dependent dispersal behaviour in *L. dispar* larvae may arise through increased interactions among conspecifics. Lance et al. (1986) questioned this interpretation and offered evidence that intense defoliation resulted in the production of plant defence compounds which also triggered dispersal behaviour. Hochberg (1991) has proposed that an age-related breakdown in gregarious feeding may be an adaptive response by some lepidopteran species to reduce the probability of local transmission of pathogens. While the actual trigger of *O. antiqua* dispersal remains unknown, density-driven host dispersal from heather to tree would seem a plausible behavioural response by a susceptible insect to minimise its probability of viral infection in a habitat favouring vertical distribution of the virus population.

Another interesting finding from this study was the effect of virus infection on the rate of *O. antiqua* larval relocation to the tree canopy. Indeed, virus-modified host behaviours were some of the earliest recorded symptoms of NPV-infected larvae, e.g. *L. monacha* (Wahl 1909). Since that time, increased movement and a tendency to adopt positions high up on the food plant by NPV-infected larvae has been shown e.g. for *L. dispar* larvae in forest habitats (Murray and Elkinton 1992) and NPV-infected *Mamestra brassicae* larvae on cabbage (Goulson 1997). The epizootiological significance of virus-modified host behaviours lies in the possibility that transmission will be maximised in the host population (Kaya 1987) and data on the spatial distribution of infected larvae and redistribution of progeny virus, following the action of wind and/or rain, provide clear support for such a case (D'Amico and Elkinton 1995; Gouslon 1997). Pathogen translocation between reservoir and transmissible habitats has now been examined in several systems (see e.g. Crawford and Kalmakoff 1977; Fuxa and Geaghan 1983; Murray and Elkinton 1990) with a recent investigation quantifying pathogen loss from the reservoir as well as translocation to the food plant (R.S. Hails, J.S. Cory, M.L. Hirst, M.R. Speight, D. Goulson, T. Williams, unpublished data).

The present study demonstrated how host behaviour and heterogeneity in pathogen spatial distribution can interact to markedly affect the population dynamics of an insect-entomopathogen association. Hochberg (1989) raised ecological awareness to the importance of pathogen spatial heterogeneity by showing in population models how a non-transmissible pathogen reservoir can influence host population behaviour. The system described in this study is not strictly equivalent to Hochberg's reservoir model in that the virus in the reservoir habitat (heather) was directly transmissible in the host population to a proportion of individuals showing foraging (dispersal and subsequent feeding) behaviours. Nevertheless, the role of the heather as a functional virus reservoir and source of viral infection appeared to be pivotal to the dynamics of *O. antiqua* and gives an example of how the pathogen reservoir and the transmissible habitat can be one and the same. As such, this example may give clues as to how dual-canopy cropping systems which display temporal stability (e.g. agroforests) can be manipulated for long-term regulation of polyphagous insect pest populations (Richards et al. 1993).

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