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# *Wipfelkrankheit*: modification of host behaviour during baculoviral infection

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**Abstract** Infection with an endoparasite frequently alters host behaviour. This study provides the first quantification of larval behaviour in a baculovirus/Lepidoptera system, and attempts to assess the ecological consequences of behavioural modification during infection. Larvae of the moth *Mamestra brassicae* (Lepidoptera: Noctuidae) exhibited higher rates of dispersal in the laboratory and field when infected with *Mamestra brassicae* nuclear polyhedrosis virus (MbNPV) than did uninfected larvae. They adopted positions at death which were not characteristic of healthy larvae, climbing higher on the foodplant and onto the top and edge of leaves. The horizontal and vertical distribution of virus following larval lysis and the effects of rainfall on this distribution were assessed for comparison with the distributions of healthy and infected larvae. Exposure to rainfall increased the infectivity of vegetation in bioassays. Alternative explanations for the evolutionary origins of behavioural modification are considered. I suggest that the behavioural changes observed are most likely to benefit the virus. In particular, climbing prior to death is likely to result in contamination of more foliage with virus particles than would otherwise occur by increasing exposure of cadavers to rainfall. Thus it may profoundly influence horizontal transmission and the dynamics of the host-virus interaction.

**Key words** Baculovirus · Dispersal · *Mamestra brassicae* · Nuclear polyhedrosis virus · Transmission

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## Introduction

Manipulation of host behaviour by endoparasites and parasitoids is well documented, particularly for wasps, nematodes, acanthocephalans and fungi (Holmes and Bethel 1972; Curtis 1987; Brodeur and McNeil 1989; Moore and Gotelli 1990; Hechtel et al. 1993; Maitland 1994; Moore 1995). To my knowledge manipulation of host insect behaviour by pathogenic viruses has not been examined rigorously. However, observations of atypical climbing behaviour in infected larvae of the moth *Lymantria monacha* represent the first records of baculovirus epizootics in natural insect populations. At the time (1891) the causal agent of the disease was not recognised, but infected larvae could be distinguished from healthy larvae by behavioural differences. Infected larvae tended to migrate to, and die, in the tops of trees, giving rise to the term *Wipfelkrankheit* (tree-top disease) (Hofmann 1891). Baculovirus transmission occurs when an infected larva dies and lyzes, releasing large quantities of virus: *c*. $10^9$ polyhedral inclusion bodies (PIBs) in a late instar larva (Entwistle and Evans 1985). The particles drip onto the surrounding foliage, and may then be ingested by other larvae. The position in which a larva dies will thus affect the likelihood of virus transmission. Virus presumably drips downwards, and is likely to be washed downwards by rain (although rain splash may also raise PIBs). A larva dying at the top of the plant is likely to contaminate more foliage than one dying lower down. Hence the symptoms described as *Wipfelkrankheit* may increase transmission of the virus (although adaptive interpretations of changes in host behaviour are problematic: Moore and Gotelli 1990).

Despite the widespread use of baculoviruses as biocontrol agents during the last 50 years, knowledge of modification of host behaviour during infection has progressed little. To my knowledge one study has been carried out, using single (unreplicated) experimental plots in which dispersal rates of infected larvae

appeared to be greater than controls (Evans and Allaway 1983). More recently Goulson et al. (1995) report lower recapture rates of infected larvae of the moth, *Mamestra brassicae* (L.) in field plots, compared to controls (before virus-induced mortality occurred). It was suggested that this may have resulted from greater dispersal rates of infected larvae. No published data exist for the distribution of infected larvae at death, compared to healthy larvae of the same age. Yet changes in behaviour during infection which have been reported anecdotally are likely to be of fundamental importance to the ecology and dynamics of the host – virus interaction. Enhanced dispersal of infected larvae must influence the spatial structure of viral epizootics, and combined with climbing prior to death is likely to influence rates of horizontal transmission of the pathogen via influences on the distribution of viral bodies within the architecture of the host plant. Thus an understanding of the nature of behavioural modifications during infection may improve our ability to apply host-pathogen models to Lepidoptera-baculovirus systems (e.g. Anderson and May 1981, Dwyer 1991, 1994; Dwyer and Elkinton 1993; Goulson et al. 1995) and to manipulate these systems for biocontrol.

This study quantifies behavioural changes induced by infection in an insect/baculovirus system, and attempts to assess the ecological significance of these changes. In particular I examine whether behavioural changes during infection increase horizontal transmission to further hosts. Differences between healthy and infected larvae are quantified in terms of behaviour (dispersal) and position adopted on the plant, using both laboratory experiments and field plots. The vertical and horizontal distribution of virus in the field is then assessed, presumably dependent on larval behaviour prior to death. The cabbage moth, *M. brassicae*, and its nuclear polyhedrosis virus (MbNPV) were selected since this system has been one of the most intensively studied in terms of the ecology of the host/pathogen interaction, and is amenable to both laboratory and field study (Evans 1981, 1983; Evans and Allaway 1983; Doyle et al. 1990; Goulson and Cory 1995; Goulson et al. 1995).

## Methods

### General methods

The virus isolate used was obtained from A. Gröner (Biologische Bundesanstalt, Darmstadt, Germany) in 1976 and originated from an epizootic in a culture of *M. brassicae* in Darmstadt in 1973. It has since become known as the Oxford isolate, and has been characterised in terms of biological activity (Evans 1981, 1983), host range (Doyle et al. 1990) and biochemistry (Brown et al. 1981; Possee and Kelly 1988). The number of passages of this stock since isolation is not known. The *M. brassicae* culture used was initiated from the offspring of female moths trapped at light near Winchester, Hampshire in 1991, and since reared continuously in captivity for approximately 22 generations on artificial diet (Hunter et al. 1984). The culture has been shown to be free of latent infection of *M. brassicae* NPV (Hughes et al. 1993). Larvae were reared to the age required in groups of approximately 100 in ventilated plastic tubs (10 cm diameter × 6 cm high), stored at 24°C in the dark. Larvae for use as fourth and fifth instars were subsequently divided into groups of approximately 20 when in the third instar to provide sufficient room for growth. For experiments, larvae were removed within 24 h of moulting, so that they were of standard age (± 12 h). Only the first batch of larvae to reach the appropriate instar from each box were used, so that all larvae were of equal development rate. Infection with virus was carried out by placing larvae in a petri dish containing small drops of virus solution which they rapidly imbibe. The solution was coloured with blue food dye (5%) (Pierce Duff & Co., UK), so that the virus solution was visible through the cuticle of larvae once imbibed. Although the amount imbibed may vary between larvae, this method does allow for precise synchronisation of infection. A concentration of $10^8$ polyhedral inclusion bodies (PIBs) per ml was used for all instars, which gave almost 100% mortality. Little is known of the dose larvae are likely to ingest during natural epizootics, but it is likely to be highly variable given the patchy nature of virus distributions (e.g. Goulson et al. 1995). Control larvae were infected with distilled water and dye. Larval mortality was negligible in both control and infected larvae for the first 5 days post-infection (< 2%).

### Larval dispersal in the laboratory

Dispersal between food patches was measured under artificial conditions to obtain a crude indication as to whether dispersal rates differed between larvae infected with NPV and uninfected larvae, and also to examine differences between instars. Larvae were confined singly in 9-cm-diameter clear plastic petri dishes, lined with filter paper dampened with 1 ml of distilled water. Two pieces of artificial diet were placed in each dish, as far apart as possible. A line was drawn across the filter paper to separate each dish into two halves each containing a piece of diet. Larvae were introduced to the centre of the dish immediately after infection. Thirty replicates each of control and infected larvae were used for each of the first five larval instars, and experiments repeated twice (120 larvae/instar in total). Sixth instar larvae were not used since they rarely develop baculovirus infection even when infected with high doses (Evans 1983). Larvae were maintained under 12 h light:12 h dark at 22°C, with lighting provided from above, and examined every 24 h (during the middle of the light period). Observations ceased when the infected larvae became moribund (exhibiting white coloration typical of the late stages of infection). At each time-point the position of the larvae was scored, in terms of which half of the dish it was in (clearly this method cannot distinguish between larvae which have not moved and those which have moved but subsequently returned to the same position).

### Time-lapse photography of larval dispersal

Third instar larvae were chosen for more detailed analysis of movement since in petri dish experiments the most marked differences in dispersal rates of infected and uninfected larvae were found in the first three instars (see below), and because third instar larvae were sufficiently large to distinguish them clearly using the video equipment available.

Single larvae were confined within 25-$cm^2$ squares each 1 cm deep and with a glass lid. Each square contained four pieces of artificial diet, one at each corner. Diet was replaced every 2 days. Larvae were infected immediately before the experiment, and larvae were maintained under 12 h light:12 h dark at 22°C, with lighting provided from above. Larvae were filmed using a Canon EX1 camcorder, recording 0.5 s of film every 20 s, for 3 h during the light period (no difference was found in the night versus daytime activity of *M. brassicae* larvae; P. Jeffreys personal communication). Filming was continued daily until day 8 when most of the infected

larvae were moribund. Twelve larvae were set up and filmed simultaneously (six infected and six controls), and the experiment repeated twice. Movements were analysed by playing back the recording onto a monitor overlaid with an acetate sheet onto which larval positions were marked so that distances moved could be measured.

### Positions of infected larvae at death compared to healthy larvae of the same age

Cabbages (var. Spitfire) were reared in an environmental chamber until they had eight unfolded leaves surrounding the heart. Larvae were introduced at the rate of five per plant, 5 days after infection, and again maintained under 12 h light:12 h dark at 22°C, with lighting provided from above. Forty cabbages were used per replicate (20 controls and 20 with infected larvae), and experiments repeated for first, third and fifth instar larvae. The experiment was continued until infected larvae were moribund. The positions of both healthy and infected larvae was then recorded as the height of each larvae above soil level and their position on the leaf (underside, upperside or edge). Larvae in the heart of the cabbage were recorded separately since it was impossible to distinguish their position within the loose ball of folded leaves

### Larval behaviour in the field

The field experiment was carried out on a ploughed and harrowed arable field at the University of Oxford field station, Wytham, United Kingdom, during August 1994. Nine cabbages (var. Spitfire 240) were planted in a cross arrangement (Fig. 1) inside each of sixteen 1-$m^2$ plots. Plots were constructed following Goulson et al. (1995), using 1.2-m wooden stakes at each corner. A barrier of clear polythene 30 cm high was stapled around each plot, and buried 10 cm into the soil, to minimise ingress of predators and egress of larvae. Birds were excluded by netting. The cabbages were allowed to establish for 24 days before the introduction of larvae.

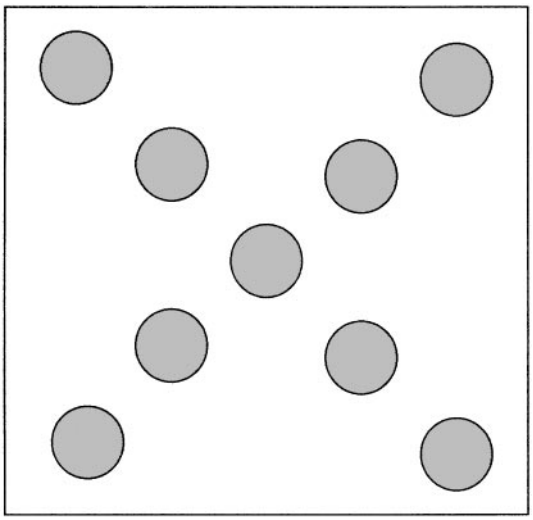

**Fig. 1** Layout of the nine cabbage plants within each experimental plot (aerial view). Plots were 1 m square. Fifty third-instar *Mamestra brassicae* larvae were introduced per plot on to the central cabbage and allowed to disperse naturally. For analysis of larval dispersal within the plots, cabbages were grouped into three zones according to their distance from the point of release of larvae. Zones consisted of the central cabbage alone, the four cabbages adjacent to the central cabbage, and the four outermost cabbages

Third instar larvae were infected with virus, and placed in the field plots immediately afterwards at a rate of 50 per experimental plot. Infected larvae were placed in nine plots; the remaining seven plots receiving control (uninfected) larvae. Larvae were transferred to the central cabbage in each plot (Fig. 1) using a paintbrush. A polythene roof was stapled across the top of five plots prior to introduction of larvae (two over control plots and three over plots containing infected larvae) to examine differences between plots due to exposure to rainfall.

To examine dispersal rates of uninfected versus infected larvae the cabbages within each plot were searched for larvae every 24 h, and the number per cabbage recorded. To prevent artificial cross-contamination of cabbages with virus this procedure was discontinued after 7 days when the first moribund larvae were observed. At 9 days post-infection all larvae which had been infected appeared to be moribund. Six plots were destructively sampled (three control plots and three containing infected larvae, using only uncovered plots). Each cabbage was severed at ground level and searched leaf by leaf to record the height above soil level and the position of each larvae. The remaining ten plots consisted of four controls and six containing virus, of which rain was excluded from half the plots in each treatment. They were then left a further 5 days to allow breakdown of cadavers and release of virus onto the foliage before assaying plots to determine the distribution of virus. Since little rain fell during this period, after 3 days uncovered plots were artificially watered to simulate rain. Each cabbage received 3 s of water from a watering can with a fine rose attachment held at a height of 50 cm above the cabbage.

Fourteen days post-infection (2 days after watering) the remaining plots (both controls and those containing virus) were destructively sampled to examine vertical and horizontal variation in the distribution of virus. Virus distribution was measured indirectly by bioassay, by exposing uninfected second instar larvae to field samples. Four leaves were removed per cabbage, the first, fourth, seventh and last leaves counting up the stem (ignoring the heart leaves), and the height of the centre of the leaf above soil level recorded. A 65-mm-diameter circle was cut from the centre of each leaf, and placed in a 95-mm-diameter plastic petri dish. Five second instar larvae were placed in each petri dish and allowed to graze on the leaf disc for 24 h before being transferred individually to clean diet and reared at 24°C in the dark until death or pupation (second instar larvae have an $LD_{50}$ of approx. 4,000 PIBs per larva, Goulson et al. 1995). A 5-$cm^3$ soil sample was taken from beneath each cabbage, and assayed for virus. One milliliter of distilled water was added to each soil sample, and ten second-instar larvae confined with the sample for 24 h before rearing individually on clean diet until death or pupation. For larvae used to assay both leaf and soil samples, larvae which died without exhibiting the typical symptoms of viral infection (a whitish coloration followed by lysis) were subjected to further examination. Cadavers were smeared and stained with Giemsa, and examined under a light microscope (× 1000) for the presence of polyhedral inclusion bodies. If this test was negative the larvae were excluded from further analysis.

### Statistical analysis

The proportion of larvae moving between diet patches in petri dishes within a 24-h period was analysed using GLIM (Generalized Linear Interactive Modelling) (McCullagh and Nelder 1989) with binomial errors, with each time-point/instar combination analysed separately, to test whether dispersal rates differed between control and infected larvae. The degree of overdispersion was within acceptable limits (Pearson's $\chi^2$ divided by the residual degrees of freedom was < 3), and a dispersion parameter was calculated and used to adjust the scale parameter. When scaling was between 1 and 1.5, $\chi^2$ values are presented, and when between 1.5 and 3, *F*-values are given

(McCullagh and Nelder 1989). Because of the large number of tests carried out (37) significance values were adjusted using a sequential Bonferroni procedure (Rice 1989). Time-lapse measurements of larval movement for the seven consecutive days of the experiment were analysed by repeated-measures analysis of variance to test for differences between infected and control larvae.

Larval height (above soil level) at death was compared to the height of healthy control larvae of the same age using analysis of variance, with each cabbage treated as a replicate (rather than using individual larvae). The proportion of larvae adopting different positions (upperside, edge, underside or heart) were compared to those adopted by healthy larvae using GLIM as above.

For the field experiment, the distribution of larvae within field plots was analysed separately for each of the six time-points at which cabbages were searched for larvae, using GLIM with binomial errors to compare the proportion of healthy versus infected larvae in each of the three zones of cabbages (central, intermediate and outer, Fig. 1). A relative estimate of virus distribution within the plots following larval lysis was obtained from assays of larval mortality after exposure to field samples. Proportionate mortality for each leaf discs and soil sample was assessed, with cabbage, zone (central, intermediate or outer), plot and treatment (control or infected) initially included as factors in the analysis, again using GLIM with binomial errors. Model simplification was achieved by stepwise removal of factors which did not contribute significantly to variation in proportionate mortality.

## Results

### Larval dispersal in petri dishes

Estimates of dispersal between food patches use the proportion of larvae to have moved patches during 24 h (Fig. 2). In general movement increased with time for each instar, and increased with instar. During the 6–9 days duration of each experiment larvae would typically moult once, after 3 or 4 days, so that each experiment spanned two instars. There was a slight decrease in movement rates evident after 3–4 days (except in fifth instar larvae) which may correspond to the period prior to moulting when larvae do not move.

Since larvae were not watched continually some movement would be missed: a larva moving randomly within the dish during a 24-h period is perhaps just as likely to end up in the half of the dish where it started. Hence estimates of the proportion of larvae moving are rarely above 0.5 even in the most mobile larvae (fifth instars). Despite this loss of information, significant differences in dispersal were found between infected and control larvae, although the effect varied with time and between instars (Table 1). There were significant differences between the proportion of larvae moving in infected and control groups in 6 out of 37 instar/time-point pairwise comparisons after adjustment of the significance level due to the large number of tests carried out (Table 1). In each significant comparison the dispersal rate of infected larvae was higher than that of controls. This pattern was most striking in the first three instars (Fig. 2). Differences between infected and control larvae according to the time post-infection do not exhibit a clear pattern, except that no differences were found at one day post infection. Subsequently infected larvae tended to show higher rates of movement throughout the course of infection until death.

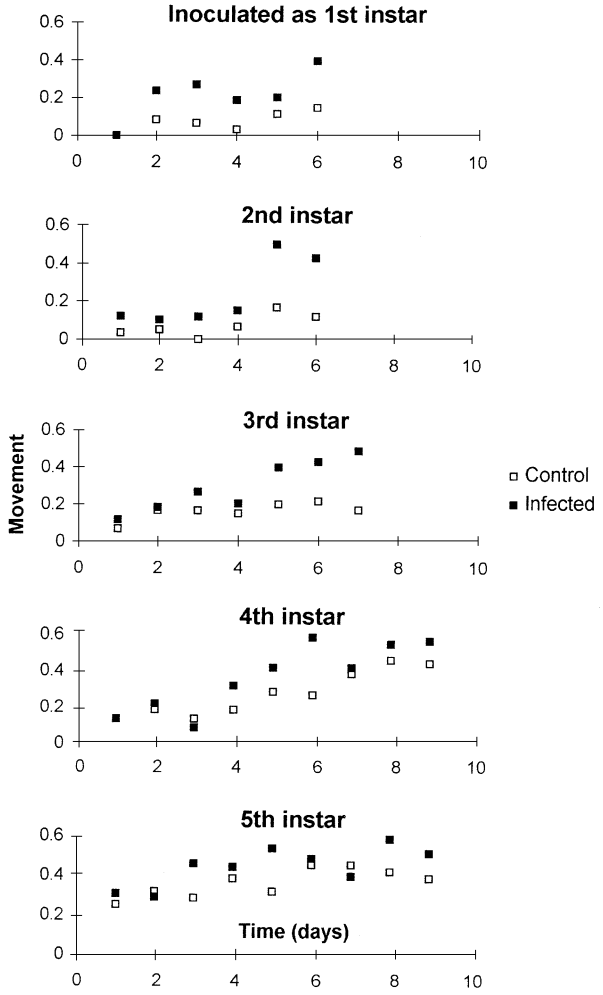


**Fig. 2** Estimates of the movement of control and infected larvae of *M. brassicae*. Following infection, 30 infected larvae and 30 control larvae were placed individually in petri dishes containing two pieces of artificial diet. Larvae were scored every 24 h as to which half of the dish they were in. The proportion of larvae which had moved since the previous time-point provides a measure of movement rates. Larvae were monitored until death of the infected group. Experiments were replicated twice (60 larvae/treatment in total) for each of the first five larval instars. $\chi^2$ values for differences in movement of control and infected larvae for each time-point/instar combination are given in Table 1

### Time-lapse photography of larval movement in the laboratory

The mean distance moved by infected larvae was consistently higher than that of controls for the first 6 days post-infection (Fig. 3), and a repeated measures analysis of variance revealed that this difference was significant ($F_{1,20} = 8.59$, $P < 0.01$). In this experiment the distances moved by healthy versus infected larvae

**Table 1** Differences in movement of control and infected larvae of *Mamestra brassicae* in laboratory experiments (values of $\chi^2$, $df = 1$). Immediately after infection 30 larvae each of control and infected were placed individually in petri dishes each containing two pieces of artificial diet. Larvae were scored as to whether they had moved between pieces of diet at 24 h intervals until death of the infected larvae. Experiments were replicated twice (60 larvae/treatment in total) for each instar. Significance levels were adjusted for multiple tests. The proportion of larvae which had moved at each time-point is shown in Fig. 2

| Days post infection | Instar 1 | Instar 2 | Instar 3 | Instar 4 | Instar 5 |
|---|---|---|---|---|---|
| 1 | 0 | 2.34 | 0.91 | 0 | 0.45 |
| 2 | 5.43 | 1.79 | 0.06 | 0.21 | 0.11 |
| 3 | 9.41* | 13.22* | 1.78 | 0.78 | 3.90 |
| 4 | 7.98 | 29.54** | 0.58 | 2.87 | 0.40 |
| 5 | 1.49 | 8.86 | 5.56 | 2.36 | 5.78 |
| 6 | 0.34 | 16.7* | 5.91 | 12.55* | 0.13 |
| 7 | – | – | 12.64* | 0.14 | 0.39 |
| 8 | – | – | – | 0.88 | 3.14 |
| 9 | – | – | – | 1.73 | 1.80 |

*$P < 0.05$, **$P < 0.01$

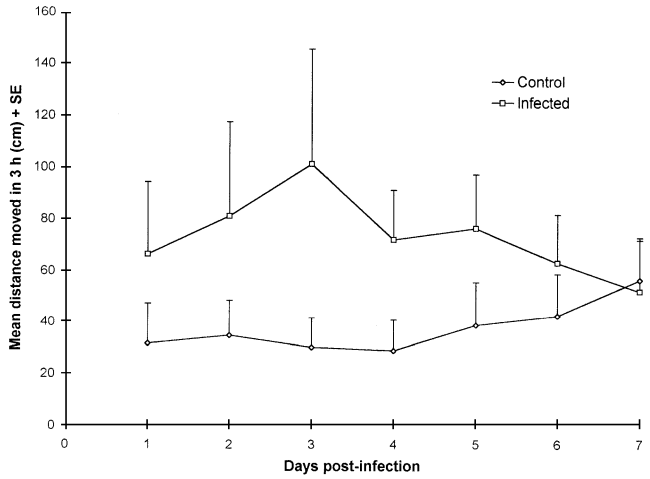


**Fig. 3** Mean distances moved by larvae in a three hour period (+ SE), recorded using time-lapse photography. In total 24 larvae were used (12 infected with virus and 12 controls) and filmed daily until infected larvae became moribund

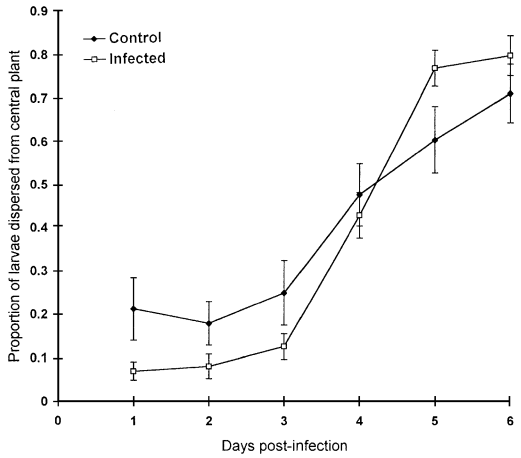


**Fig. 4** The proportion of larvae dispersed from the central cabbage (where they were released) to either the intermediate or outer cabbages in field plots. Based on 16 plots (9 with infected larvae and 7 with control larvae), each containing 50 third-instar larvae. Infection and release of larvae occurred on day zero. Infected larvae were moribund by day 7. $\chi^2$ values for the difference between control and infected larvae are given in Table 2

converged by day 7, a trend which was absent from the petri dish experiments.

## Larval dispersal in the field

Larval dispersal rates within the field plots were estimated from daily records of larval positions for the first 6 days post-infection. Differences in dispersal rates between infected and control larvae can most clearly be seen by considering the proportion of larvae which had dispersed from the central cabbage (Fig. 4). A significantly smaller proportion of infected larvae had dispersed during days 1–3, compared to controls (Table 2). However, since data for each day were not independent this repeated significance need only represent differential dispersal on the first day, and so must be treated with caution. Movement away from the central plant increased markedly in infected larvae during the later stages of infection, so that by day 5 significantly more infected larvae had dispersed than controls (Table 2). By the final time-point (day 6) more infected larvae had dispersed than controls, but the difference was no longer significant.

## Positions of infected larvae at death in the laboratory

When infected larvae became moribund (approximately eight days post infection) larvae infected with MbNPV tended to have adopted a higher position on the foodplant than uninfected larvae ($\chi^2_1 = 5.99$, $P < 0.05$) (Fig. 5). This effect was strongest in the third instar, although neither instar nor the instar – treatment interaction contributed significantly tothe model ($\chi^2_2 = 3.50$ and $\chi^2_5 = 4.40$ respectively).

**Table 2** Differences in the movement of control and infected larvae of *M. brassicae* under field conditions (values of $\chi^2$, $df = 1$). Since repeated measures were used on the same larvae, significance on days 2 and 3 does not imply differential movement of control and infected larvae on these days. Calculations were based on the proportion of larvae occupying the central, intermediate and outer zones within each plot, after being released within the central zone. There were 16 plots (9 with infected larvae and 7 with control larvae), each containing 50 larvae. Larvae were counted every 24 h from infection until infected larvae became moribund

| Days post infection | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Effect of infection | Decreased movement | Decreased movement | Decreased movement | – | Increased movement | – |
| $\chi^2_1$ | 21.4 | 11.3 | 12.0 | 1.65 | 7.6 | 3.32 |
| $P$ | *** | *** | *** | ns | ** | ns |

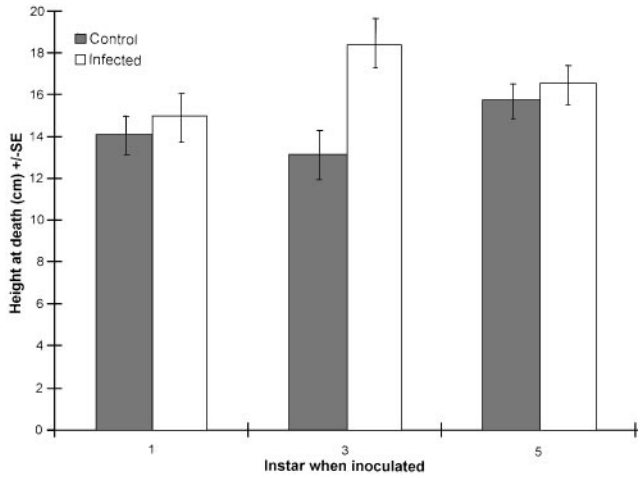


**Fig. 5** Height of positions adopted by moribund larvae compared to controls of the same age (± SE). Larvae were infected in the first, third or fifth instar, and placed on potted cabbages (five/plant) in the laboratory. Twenty cabbages (100 larvae) were used for each treatment

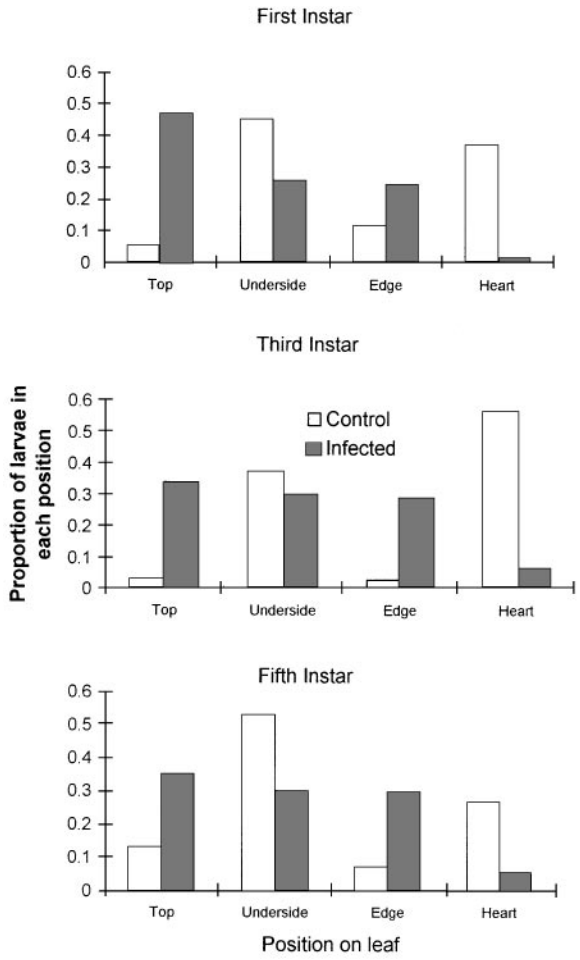


**Fig. 6** The frequency of larvae adopting different positions on cabbage plants under laboratory conditions, comparing moribund larvae with controls of the same age. Larvae were infected in the first, third or fifth instar, and placed on potted cabbages (five/plant) in the laboratory. Larvae were recorded as being on the top, edge or underside of the leaf, or in the heart of the cabbage. Twenty cabbages (100 larvae) were used for each treatment

Position on the plant differed more strikingly according to treatment and instar (Fig. 6). In all instars examined, healthy larvae tended to sit on the underside of leaves or within the heart of the cabbage, few being recorded on the edge or top side of leaves (17.3, 6.0 and 20.4% of larvae for instars one, three and five, respectively). Infected larvae were rarely found within the heart, but commonly occurred on the top side and edge of leaves (72.0, 63.3 and 64.6%, respectively). The proportion of larvae in each position differed significantly between treatments ($\chi^2_1 = 27.9$, $P < 0.001$), but once again neither instar nor the instar – treatment interaction contributed significantly to the model ($\chi^2_1 = 3.15$ and $\chi^2_5 = 7.49$ respectively).

## Positions of infected larvae at death in the field

The heights on the plant adopted by larvae in the field prior to becoming moribund did not differ from those of healthy control larvae of the same age (mean (cm) ± SE: 11.6 ± 2.5 and 11.3 ± 3.1 for control and infected larvae respectively, $F_{1,4} = 0.49$). However, the positions on the plant did differ between control and moribund larvae, and correspond well with differences found in the laboratory ($F_{1,4} = 9.13$, $P < 0.05$) (Fig. 7). Healthy larvae were found predominantly in the heart of the cabbages (61.8%), almost all of the remainder being on the underside of leaves (29.3%). Infected larvae were

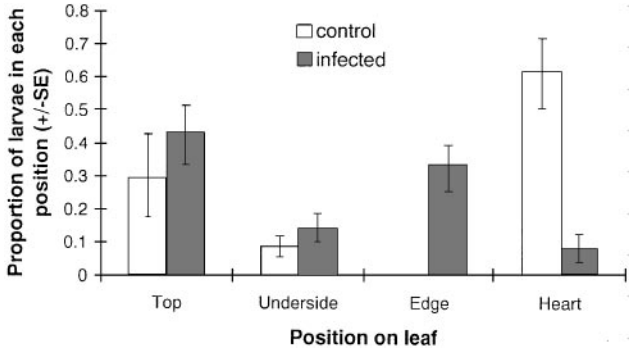


**Fig. 7** The frequency of larvae adopting different positions on the cabbage plants in field plots. Larvae were released immediately after infection as third instars, and allowed to position themselves naturally during the 7 days prior to becoming moribund. Larvae were recorded as being on the top, edge or underside of the leaf, or in the heart of the cabbage. Sample sizes were 50 for infected larvae and 91 for controls

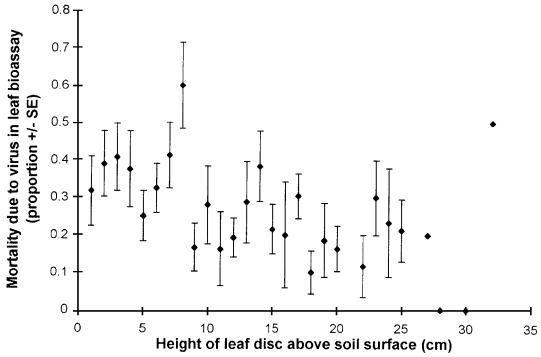


**Fig. 8** The vertical distribution of virus within field plots following an artificially induced epizootic in fifty third-instar *M. brassicae* larvae. Virus abundance was estimated from bioassay of leaf discs, according to height of the leaf discs above soil level. Three hundred and sixty discs (four per plant) were each assayed by allowing five second-instar larvae to feed on them for 24 h

rarely found in the heart of the cabbage (8.3%), mainly occupying positions on the edge, top or underside of outer leaves. Notably, 33.9% of infected larvae were recorded on leaf edges, a position in which no healthy larvae were found.

## Virus distribution following lysis of larvae in the field

The distribution of virus within field plots was assessed by bioassay, exposing uninfected larvae from laboratory culture to samples of field material. There were few deaths due to virus infection following exposure of larvae to field samples from control plots (overall 1.4% of larvae). I suggest that these deaths were most likely to be due to contamination of leaves after harvesting. Only plots which were stocked with infected larvae are considered further. The horizontal distribution of virus within the plots as measured by larval mortality was remarkably uniform given that the infected larvae were initially placed on the central cabbage. There was no significant difference in larval mortality according to whether they were exposed to leaf discs from cabbages in the central, intermediate or outer zone of cabbages (Fig. 1) ($F_{2,209} = 2.78$, $P > 0.05$). There were differences in mortality according to the height of leaves, indicating that there tended to be more virus on leaves lower down on the plant ($F_{1,210} = 6.16$, $P < 0.05$) (Fig. 8). Most strikingly, larval mortality was consistently higher in leaf samples taken from open plots (which received artificial watering) ($F_{1,210} = 12.54$, $P < 0.001$) (Fig. 9).

The same patterns were evident in soil samples taken from beneath each plant. There was no significant difference in mortality according to where the soil samples were taken within the plots ($F_{2,50} = 0.79$), but mortality was greater in plots exposed to rainfall ($F_{1,52} = 22.5$, $P < 0.001$) (Fig. 10).

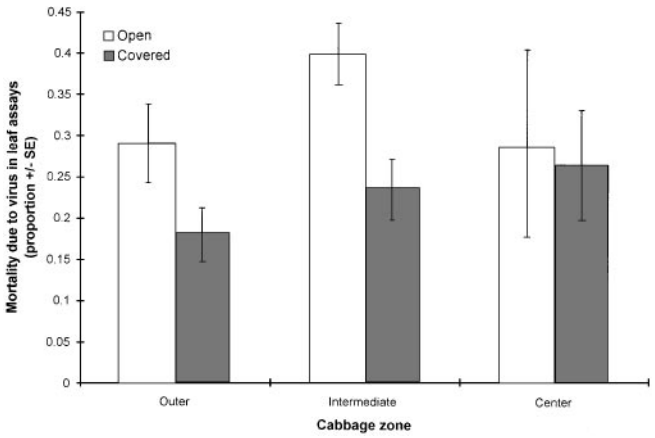


**Fig. 9** The horizontal distribution of virus within field plots, and the effect of rainfall, following an artificially induced epizootic in fifty third-instar *M. brassicae* larvae. Larval mortality was used as an indicator of virus abundance in leaf samples taken from field plots. In total 360 leaf discs were assayed by exposing five larvae to each disc for 24 h, and the larvae subsequently monitored for mortality due to viral infection. Mortality did not differ according to zone (position of the cabbage) but was higher in plots exposed to simulated rainfall (open) than in covered plots

## Discussion

This is the first study to quantify differences between the behaviour of healthy larvae and those infected with baculovirus, and describes broadly consistent results from both laboratory and field experiments. The baculovirus MbNPV produces marked behavioural changes in host larvae during the course of infection. These changes include enhanced dispersal in the later stages of infection, and a change in position on the foodplant at death compared to healthy larvae of the same age. Compared to controls, moribund larvae were higher on the foodplant and found on the edges and top of leaves, whereas healthy larvae were found on the underside of leaves and in the heart of the plant.

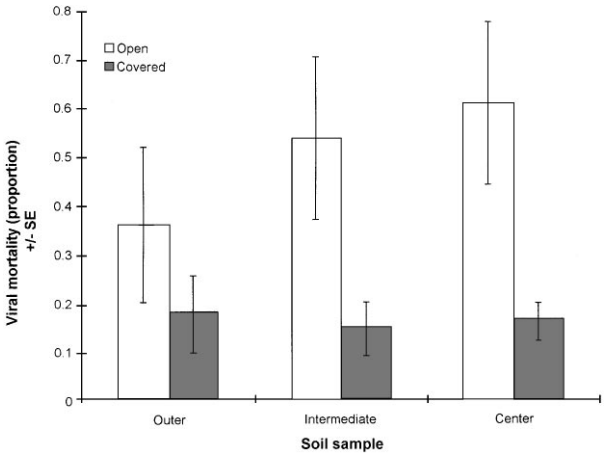


**Fig. 10** The horizontal distribution of virus within soil samples taken from field plots, following an artificially induced epizootic. Larval mortality after exposure to soil samples was used as an indicator of viral abundance. Ninety soil samples were assayed using 10 larvae per sample. Larvae which died due to causes other than viral infection were excluded from the analysis

Changes in host behaviour have been recorded from hosts infected with nematodes, acanthocephalans and fungi (Holmes and Bethel 1972; Moore 1984; Hechtel et al. 1993; Maitland 1994), but have not previously been quantified in an insect/baculovirus system. For example the fungi *E. muscae* induces infected yellow dung-fly hosts (*Scatophaga stercoraria*) to perch in a characteristic elevated position not observed in healthy hosts (Maitland 1994). The significance of these changes is not always clear; depending on the system studied, behavioural changes in the host have been interpreted as adaptive for the parasite, adaptive for the host, or of no adaptive significance (Moore and Gotelli 1990; Poulin 1994). Perhaps most frequently they are interpreted as adaptive manipulations by the parasite or pathogen, for example to increase horizontal transmission to subsequent hosts (Maitland 1994) or to avoid hyperparasitisation (Brodeur and McNeil 1989, 1992). Whatever the evolutionary origins of the phenomenon in this system, assessment of the distribution of virus following death suggests that behavioural changes may be of benefit to the pathogen. Infected larvae were released at a focus in the centre of experimental plots, but following larval death the horizontal distribution of virus on both foliage and in the soil was approximately uniform. Presumably the increased dispersal of infected larvae facilitates spread of the virus. Dispersion of cadavers increases transmission rates of NPV infection to early instar Douglas-fir tussock moth, *Orgyia pseudotsugata* (Dwyer 1991), and horizontal dispersal of infected larvae may play an important role in the spatial spread of waves of epizootic (Dwyer 1994).

Despite the significantly higher position adopted by moribund larvae compared to controls (in the laboratory), there was more virus on lower leaves than on upper ones. This may have been due to a combination of more rapid degradation of virus on the upper leaves by UV light and the presumed tendency of virus to drip and wash downwards following death of larvae. If larvae did not climb upwards prior to death the distribution would probably be more heavily biased towards lower leaves and the soil.

Differences in position on the plant were more striking than differences in height in both laboratory and field. The tendency of infected larvae to adopt positions on the upper side of leaves may encourage spread of virus over the leaf surface rather than dripping onto the soil below where prospects for transmission to other larvae are low. Since healthy larvae rarely adopt positions on the upper side of leaves they may only come into contact with upper leaf surfaces when they eat through the leaf from underneath. However, cadavers on the upper leaf surface are exposed to raindrops which may redistribute virus. Rainfall may play a major role in increasing virus transmission, since levels of larval infection were far higher in bioassays of both foliage and soil samples from open plots exposed to simulated rainfall. One might predict increased infectivity of soil in open plots due to the washing of virus downwards, but this ought to reduce the amount of virus on the foliage. I suggest that the increased infectivity of both foliage and soil is not a measure of the amount of virus, but rather of its distribution. In the absence of rainfall, virus distribution is likely to be highly aggregated (confined to the immediate vicinity of a cadaver). Many of the foliage samples taken from the unwatered plots may have missed these aggregations by chance. Rainfall is likely to spread the virus more evenly over the foliage, resulting in consistently higher mortality in this experimental system and presumably also in the wild.

It is worth noting that under field conditions dispersal rates were actually lower in infected larvae for the first three days following infection, although they subsequently became more dispersed than control larvae by day 5 (in contrast to laboratory experiments in which movement rates tended to be higher throughout the period of infection). If adaptive explanations are to be used for enhanced dispersal in later stages of infection they are also necessary to explain this reduced dispersal. However, as larvae are not infective at this stage it is difficult to see how this behavioural modification benefits either host or virus.

There are alternative explanations for changes in larval behaviour following infection; they may plausibly have evolved to increase host fitness, either by reducing transmission of infection to relatives or via increasing the likelihood of recovery. *M. brassicae* eggs are laid in large batches so the nearest neighbours to larvae are likely to be their siblings. Since larvae in late stages of infection with a baculovirus never recover, their expected future reproductive success is zero. Hence enhanced horizontal dispersal of infected larvae may represent a kin-selected Captain Oates style

self-sacrifice which serves to avoid infecting relatives, as occurs in both aphids and bumblebees (Smith Trail 1980; McAllister and Roitberg 1987; Müller and Schmid-Hempel 1993). Similarly, moving to the top of the leaves may encourage predation by birds and so remove the moribund larvae from the presence of relatives. Climbing behaviour is also likely to raise larval temperature by increasing exposure to solar radiation, and thus could be interpreted as behavioural fever (increasing body temperature by behavioural means in response to infection) (Boorstein and Ewald 1987; Horton and Moore 1993). Behavioural fever has been reported in diverse ectotherms including several insects species and as a result of infection by a range of microparasites, and may help the host to eliminate the parasite (e.g. Bronstein and Conner 1984; McClain et al. 1988; Carruthers et al. 1992; Watson et al. 1993). To our knowledge no studies have assessed effects of temperature on baculovirus-induced mortality. However, as in this system climbing occurs in the last stages of infection just prior to death, it is unlikely to influence larval survival.

An alternative possibility is that dispersal is a generalized response to stress which may not be adaptive for either virus or host. The virus initially infects mid-gut cells and may adversely affect food absorption so inducing food stress, in turn possibly prompting dispersal (although if this were true it does not exclude the possibility that either host or virus may ultimately benefit from dispersal). Distinguishing between such hypotheses presents a challenge for future research. If behavioural changes do represent adaptive manipulations by the pathogen they presumably depend upon mechanisms which are common to many Lepidoptera, for MbNPV has a broad host range, particularly within the Noctuidae (Doyle et al. 1990). It would be instructive to examine whether similar behavioural changes occur during infection of other host species.

Conclusively testing whether pathogen-induced behavioural changes are adaptive for either host or pathogen is difficult. Whatever the evolutionary explanation, behavioural changes in the host are likely to influence the population dynamics of the system. Research has proliferated on the application of epidemiological models to insect-virus population dynamics, and baculovirus-Lepidoptera systems have proved to be suitable for empirical testing of model assumptions (Dwyer 1991, 1994; Dwyer and Elkinton 1993; Goulson et al. 1995). Increased dispersal of infected late-instar larvae may contribute to stage-related differences in transmission; inclusion of a spatial component may substantially improve the predictive accuracy of such models when applied to baculovirus-Lepidoptera systems. Clearly further research to establish the generality and type of behavioural modifications induced by baculovirus infections may expand our understanding of the ecology and dynamics of this host-pathogen interaction.

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