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Prey selection and baculovirus dissemination by carabid predators of Lepidoptera

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Abstract. 1. The interaction between coleopteran predators and baculovirus-infected larvae was studied in the laboratory and the field in order to assess the potential role of predators in the dissemination of a nucleopolyhedrovirus (NPV).

2. Preference tests using three carabid species, *Harpalus rufipes* De Geer, *Pterostichus melanarius* Illiger and *Agonum dorsale* Pont. showed no evidence of discrimination between healthy and diseased larvae of the cabbage moth *Mamestra brassicae* L. (Lepidoptera: Noctuidae) as prey items.

3. Virus infectivity was maintained after passage through the predator's gut. NPV mortality ranged from 97% to 20% when test larvae were exposed to faeces collected immediately after and 15 days post-infected meal respectively.

4. The potential for transfer of inoculum in the environment was estimated in the laboratory by soil bioassay. Carabids continuously passed infective virus to the soil for at least 15 days after feeding on infected larvae.

5. Field experiments showed that carabids which had previously fed on diseased larvae transferred sufficient virus to the soil to cause low levels of mortality in larval populations of the cabbage moth at different instars.

Key words. Baculovirus, prey selection, carabids, virus dispersal, nucleopolyhedrovirus, *Mamestra brassicae*.

Introduction

The success of entomopathogens as natural enemies is determined by their dispersal ability, transmission within the population and survival in the absence of hosts. Studies of baculovirus epizootics have clearly demonstrated the capacity for the disease to spread from the original foci of infection (Entwistle *et al.*, 1983; Dwyer, 1992; Fuxa *et al.*, 1993; Fuxa & Richter, 1994). Few studies, however, have attempted to estimate quantitatively the biotic mechanisms by which the virus can persist and spread in the enviroment. The virus may be solely dispersed by the host or the process may be enhanced by other organisms, especially predators and scavengers, which feed on diseased larvae (Biever *et al.*, 1982; Entwistle, 1982; Entwistle *et al.*, 1977, 1983; Boucias *et al.*, 1993; Fuxa & Richter, 1994). The contribution of predators to either altering the rate of epizootic development or initiating new foci of infection is unclear.

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The importance of predaceous arthropods in the dissemination of insect viruses depends on three factors: the acceptance of infected insects as food, the effect of passage through the predator gut on virus infectivity, and the interactive behaviour of predator and prey in relation to virus acquisition. Food intake by hemipteran predators varies, according to the species, from no discrimination between healthy and diseased larvae (Abbas & Boucias, 1984) to a strong preference for infected prey (Young & Yearian, 1987, 1989; Young & Kring, 1991). In both cases release of viable virus in the predator faeces occurred for at least 4 days after feeding on infected larvae (see also Beekman, 1980). Host larvae reared with hemipterans that had fed on virus-diseased larvae can also acquire moderate to high levels of infection (Young & Yearian, 1987, 1992). Less information is available on the interaction between viruses and coleopteran predators. Capinera & Barbosa (1975) demonstrated that the faeces of field-collected *Calosoma sycophanta* (Col.: Carabidae) contained enough NPV to induce mortality in *Lymantria dispar* (Lep.: Lymantriidae) larvae and that high mobility of both larvae and adults of the predator enhanced the potential for virus dispersal. Infective virus in carabid faeces was also reported by Young & Hamm (1985), who suggested that NPV infection does not alter the nutritional quality of the prey.

Ground beetles are usually generalist predators, feeding on a broad spectrum of prey (Thiele, 1977). Although the consumption of infected larvae and subsequent release of viable virus by carabids are likely to occur in nature, the ecological implications of these phenomena have not been studied under field conditions. This study investigated the role of predatory ground beetles (Col.: Carabidae) as passive agents of virus dispersal and addressed the following questions: (i) do carabids discriminate between healthy and diseased larvae as prey items? (ii) does the virus remain viable after passage through the carabid gut? (iii) for how long can carabids excrete viable virus in their faeces? (iv) can virus released by carabids be acquired by host larvae in laboratory and field situations and is this likelihood related to larval behaviour/instar?

Materials and Methods

Virus extraction and infection techniques. The multiply enveloped nucleopolyhedrovirus (MNPV, Baculoviridae) used in this study was isolated in Germany in 1973. Virus was propagated in cabbage moth, *Mamestra brassicae*, larvae from a culture established in 1977 and reared on semi-synthetic diet (Hunter *et al.*, 1984). Virus was purified by centrifugation on discontinuous sucrose gradients and counted using Wigley's (1980) dry film method.

Larvae were infected as follows: neonate larvae were allowed to drink from droplets of a 400 Polyhedral Inclusion Bodies (PIB)/μl virus suspension placed on a Petri dish lined with hydrophobic film. Second and third instar larvae were infected individually with a dose of 5×10^3 and 3.2×10^4 PIBs respectively using the diet plug system (Doyle *et al.*, 1990). Following virus ingestion, larvae were transferred to individual polypots containing diet and kept at 24°C. After 6 days, larvae infected as first and second instar were visibly moribund having developed to second and third instar respectively. Larvae infected at third instar were used at 7 days post-infection, at fourth instar.

Beetle collection and description. Carabids collected in pitfall traps at the University of Oxford Farm, Wytham, Oxfordshire, in the summers of 1993 and 1994 were kept in acrylic boxes with moist compost at 12°C and fed on *M.brassicae* larvae until further use. Three flightless, soil-dwelling species were chosen due to their abundance in the field and to differences in their predatory behavior. *Harpalus rufipes* and *Pterostichus melanarius* feed on many groups of invertebrates: whereas the latter is essentially carnivorous, plant material such as strawberry and umbelliferous seeds can make up half of *H.rufipes'* diet (Speight, 1976; Thiele, 1977). Their wide occurrence, together with their relatively large size (*H.rufipes* = 11–16 mm, *P.melanarius* = 13–17 mm), make them likely candidates as larval predators and thereby carriers of insect pathogens. The third carabid, *Agonum dorsale*, is smaller (5.8–7.5 mm), primarily carnivorous, and is reported to climb plants in the search for food such as aphids (Vickerman & Sunderland, 1975; Thiele, 1977).

Preference tests. Four weeks prior to starting the experiments, beetles were transferred to a 21 ± 2°C room under a 14:10 light:dark cycle, with red light to simulate night conditions, and fed on cooked minced beef to avoid any bias in the prey preference. After a 40 h starvation period, beetles were placed in a 140 mm × 15 mm Petri dish lined with a black paper circle to mimic the dark colour of the soil. One beetle plus eight third-instar *M.brassicae* larvae (four healthy and four infected of similar size) were placed in each arena and observed continuously for 30 min. The multiple choice of larvae enabled the detection of any consistent trend in prey selection given an abundant food supply. Larvae at a late stage of infection were used in order to emphasize the changes in texture, colour and behaviour due to virus infection, namely, pale and flaccid integument and reduced mobility.

Experiments were carried out under fluorescent light ('day') so that the sequence and condition of consumed larvae, the duration of each meal and the activity of each beetle could be recorded. The number of replicates was twenty-five for *H.rufipes* and twenty for *P.melanarius*. Feeding times, considered here to be the time spent by the predator between attacking and completing consumption of larvae, were compared for healthy and NPV-diseased larvae. Since the species tested are usually nocturnal, experiments under red light ('night' conditions) were also performed. In this case numbers of healthy and infected larvae remaining after the 30 min interval were recorded. Thirteen *H.rufipes*, thirteen *P.melanarius* and eight *A.dorsale* were used in 'night condition' tests. Due to their markedly smaller size and lower consumption rates, *A.dorsale* was observed for a longer period (6 h) and second instar larvae were used as prey. All beetles were used only once, after which they were killed and sexed.

Viability of virus realeased by carabid beetles. Virus carriage by predators was investigated in the laboratory in two ways: the effect of passage through the carabid gut was measured by bioassaying faeces collected at different days after an 'infected' meal, whilst virus transfer to the environment, via faeces and contamination of the beetle's cuticle, was estimated by soil bioassay.

(i) *Faeces bioassay. H.rufipes* beetles were allowed to feed on a moribund infected fourth-instar *M.brassicae* larva and placed in individual 4 cm diameter acrylic polypots containing moist compost ('soil'). At 1, 3, 5, 7, 9, 15 and 30 days after the infected meal beetles were transferred to clean polypots, suspended from the lid by a cotton thread to prevent contamination from their cuticle, and faeces were collected for a period of 48 h. Between the initial meal and faeces collection, carabids were transferred every 48 h to polypots with fresh soil plus food, i.e. a dead uninfected larva, to reduce re-ingestion of inoculum. Each timepoint comprised ten replicates; controls consisted of an equal number of beetles treated similarly after eating healthy larvae. Faeces collection was carried out on independent samples, that is, beetles were used only once throughout the experiment. Faeces were triturated in 100 μl of sterile water and each suspension was assayed for viable virus by feeding 1 μl to each of thirty second-instar *M.brassicae* larvae using the diet plug system. Larvae were kept individually on diet at 24°C and checked daily until death or pupation.

(ii) *Transfer of infective virus to soil. H.rufipes* adults fed on infected prey, as described above, were placed individually in 4 cm diameter polypots half-filled with moist compost. After 24 h the beetles were transferred to new polypots containing fresh soil plus food (dead uninfected larvae), and the procedure was repeated every 24 h for 30 days. Presence of virus in the soil was detected by placing five second-instar *M.brassicae* larvae in each polypot for 36 h at 24°C, after which the larvae were transferred to individual polypots containing diet and checked daily for

virus mortality. Ten timepoints were used; 0, 1, 2, 3, 4, 5, 6, 7, 15 and 30 days after feeding on virus-infected larvae. Thirty-five 'virus-fed' beetles were used, and fifteen carabids identically treated after feeding on healthy larvae acted as controls.

Field experiment. A field trial conducted in August 1994 at the University of Oxford Farm focused on the interactive behaviour of *M.brassicae* larvae and carabid predators and addressed two questions: (i) can carabids which have fed on infected larvae release enough virus to cause mortality in larval populations of the cabbage moth? and (ii) is the likelihood of predation and virus acquisition related to larval instar/behaviour? Earlier field observations suggested that later instars of *M.brassicae* were more mobile, and subsequently more likely to acquire virus from the soil. Alternatively younger larvae, being more susceptible, require smaller quantities of pathogen to develop lethal infections.

Experimental units consisted of 1 m^2 plots surrounded by polythene barriers extending 50 cm above and 20 cm into the soil to reduce insect escape. Plots contained five large cabbage plants (variety Spitfire 240) and were covered with a polythene roof to minimize the effect of rain. The experimental area was depleted of predators by continuous pitfall trapping prior to the trials. Treatments consisted of second or fourth instar *M.brassicae* larvae introduced into plots (1) without beetles, (2) containing beetles that had fed on healthy larvae, and (3) with beetles fed NPV-infected larvae. Each treatment was replicated four times. 'Clean' and 'infected' carabids were marked with enamel of different colours, and pitfall traps were scattered around the experiment field to monitor beetle escape. Fifteen *H.rufipes* beetles were introduced per plot. 12 h later, twenty larvae were placed, with minimal disturbance, in the centre of each plant, totalling 100 larvae per plot. Larvae and carabids were left in the plots for 5 days. This was the maximum length of time that larvae could be left before baculovirus-related death might occur in the field, which would alter inoculum density. After this period larvae were collected by destructive sampling, transferred to individual polypots with diet, and observed daily in the laboratory for virus mortality.

Following the experiment, five 30 ml samples of soil were collected from each plot and biossayed on second-instar *M.brassicae* larvae in the laboratory. Ten larvae were placed into each polypot with soil for 24 h at 24°C, then transferred to individual polypots containing diet and checked daily for NPV mortality.

Statistical analysis. Analysis was performed using GLIM (Royal Statistical Society, 1985). Initially all factors and their interactions are fitted in a model and their significance is evaluated by comparing the changes in the overall deviance due to their progressive removal from the model using *F*-tables (data with normal sampling errors) or χ^2 tables (Poisson and binomial errors). Arcsine transformation of values was carried out when the data showed a large degree of overdispersion (scale parameter larger than 3).

Results

Preference tests

The numbers of larvae consumed by *H.rufipes* and *P.melanarius* were analysed in a factorial ANOVA with Poisson errors using each treatment [sex (male/female); light condition (day/night); condition of larvae (healthy/infected) and species (*H.rufipes*/*P.melanarius*)] plus their interactions as factors. The proportion of healthy and infected larvae consumed (Fig. 1) did not differ significantly for either species tested ($\chi^2 = 0.04$; df = 1; $P > 0.80$, scale parameter 1.48). There was also no difference in response between male and female carabids ($\chi^2 = 0.74$; df = 1; $P > 0.30$, scale parameter 1.48). Surprisingly, given that both species are reported to be nocturnal, the consumption rate was slightly higher in daylight conditions ($\chi^2 = 4.29$; df = 1; $P < 0.05$, scale parameter 1.47). *P.melanarius* was clearly more voracious than *H.rufipes*, preying upon a significantly higher number of larvae ($\chi^2 = 46.04$; df = 1; $P < 0.0001$, scale parameter 1.47).

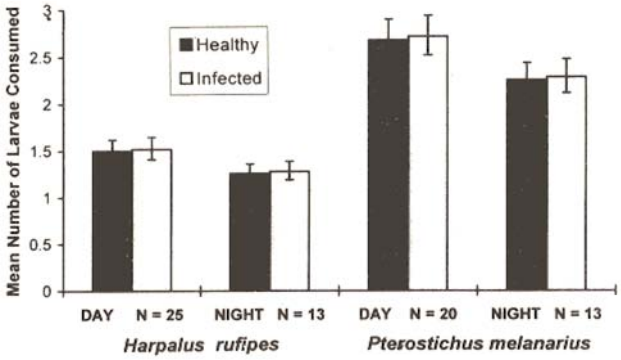

Fig. 1. Mean number of healthy and NPV-infected *M.brassicae* larvae consumed by carabids in a 30 min observation period. Error bars represent SE of the difference between the means of healthy and infected larvae consumed. (Healthy versus infected larvae consumed: $\chi^2 = 0.40$; df = 1; $P > 0.80$; daylight versus night: $\chi^2 = 4.29$; df = 1; $P < 0.05$; *P.melanarius* versus *H.rufipes*: $\chi^2 = 46.04$; df = 1; $P < 0.0001$.)

A complementary analysis was conducted to test whether the first choice by predators was biased towards healthy or diseased larvae, using a *G*-test (Sokal & Rohlf, 1995) to assess departure from non-discriminatory behaviour. There was no significant difference in the state of the larva chosen first for either species (*H.rufipes*: $G = 2.72$, df = 1; $P \approx 0.10$; *P.melanarius*: $G = 1.33$, df = 1; $P > 0.20$).

Feeding times for both beetle species tested in daylight were compared in order to detect whether they were affected by changes in larval response, texture and mobility due to infection. The duration of the first meal by each carabid was compared using ANOVA with normal errors and species, sex and larval condition as factors. Meals lasted slightly longer for infected than for healthy larvae for both species ($F_{(1,39)} = 4.19$; $P \approx 0.05$). This difference may be due to the more flaccid texture of infected larvae, since their bodies tend to liquefy while being consumed. The defensive response, which was stronger in healthy larvae, did not appear to represent much impediment against attack, presumably because both carabids are able to seize large and highly mobile prey. Besides killing more larvae, *P.melanarius* also consumed the prey in a shorter time than *H.rufipes* ($F_{(1,39)} = 12.09$; $P < 0.001$). The mean feeding times for *H.rufipes* were 8.7 ± 1.4 (min ± SE) for healthy larvae ($N = 9$) and 11.5 ± 1.1 for infected prey ($N = 13$). For *P.melanarius* the meals lasted on average 3.2 ± 1.2 min for healthy larvae ($N = 12$) and 6.0 ± 1.6 min for infected larvae ($N = 7$).

The number of prey consumed by *A.dorsale*, compared in an ANOVA test with Poisson errors, showed that there was no

evidence for discrimination between healthy or infected larvae ($\chi^2 = 0.8$; df = 1; $P > 0.30$, scale parameter 1.84). The mean number of larvae consumed was 2.04 healthy (SE = 1.58; +SE = 2.63: SE = standard error of the difference) and 2.56 infected (–SE = 1.98; +SE = 3.30)

Release of virus by carabids

Percentages of mortality in the faeces bioassay were initially arcsine transformed and analysed with normal errors using timepoint of faeces collection and type of original meal (healthy/infected) as factors. Following that, a series of models was fitted to the data to predict the release of inoculum. The release of virus was found to be non-linear with respect to time. The final model included treatments (virus/control) as factors and time as linear, quadratic and cubic factors. Inclusion of polynomial factors in the model proved significant, with fitted values in agreement with the observed data (Fig. 2). Presence of viable virus in carabid faeces was demonstrated by the mortality observed in *M.brassicae* larvae, which was highest for faeces collected immediately after feeding on infected prey (97%) and then declined steadily during the first week. A reversal in the decline of virus release was observed at the 15th day post-meal, which may be the result of sample contamination. By the 30th day after the infected meal virus was no longer detected in the faeces. Excretion of virus in controls was negligible throughout the experiment.

Virus transfer by carabids was assessed by measuring mortality in larvae exposed to soil where beetles had been present at different intervals after an infected meal. Mean mortalities were arcsine transformed and analysed separately for each timepoint, given the non-independence of samples, with normal errors and treatments (clean/infected carabids) as factors. Transfer of virus to soil, both the in faeces and (possibly) as a result of contamination of the beetle's cuticle, followed a similar pattern to the faeces biossay, showing that larvae readily acquire inoculum from the soil. Presence of virus in soil caused almost 100% mortality immediately after an infected meal and continued at a reduced level for at least 15 days (Fig. 3).

Field trials

Predation in the field was estimated by comparing the proportion of larvae recovered in an ANOVA (presence of beetles and larval instars as factors) with normal errors. Values were initially arcsine transformed to correct a slight overdispersion. Although fewer larvae were retrieved from plots with predators, the difference was not statistically significant ($F_{(1,13)} = 3.12$; $P \approx 0.10$) (Table 1). Recovery in all plots was higher for larvae at fourth than at second instar ($F_{(1,13)} = 4.56$; $P \approx 0.05$), which might be due to the fact that earlier instars are more susceptible to death by handling or pathogens and that their size and darker colour make older larvae more noticeable. Only one out of the 240 marked beetles released was captured in the pitfall traps outside the plots, suggesting that the barriers contained carabids for the duration of the trial.

Virus deaths were analysed in ANOVA (binomial errors) with beetle condition (healthy/virus-fed) and instar of test larvae (second/fourth) as factors. The mortality observed, although low, indicated that carabids which had fed on virus infected prey can pass this inoculum to foliar feeding larvae. Even though some viral deaths occurred in the controls, mortality in the plots with

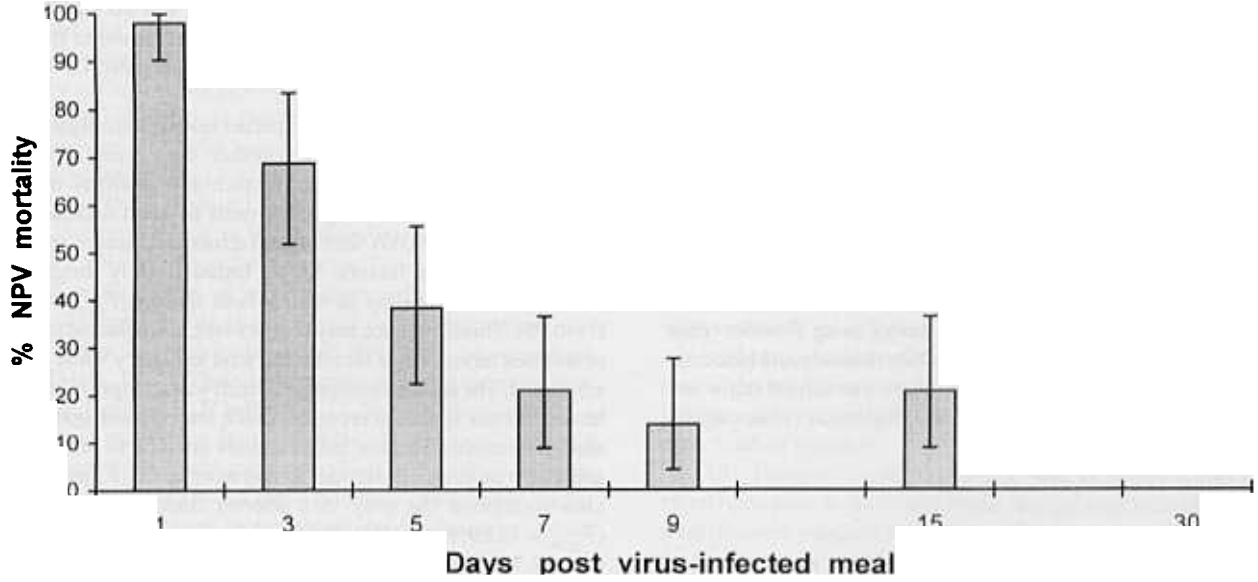

Fig. 2. Virus mortality in second instar *M.brassicae* larvae after ingestion of *H.rufipes* faeces collected at different timepoints after feeding on infected prey. Number of beetles tested: 10 (day 1); 7 (day 3); 6 (day 5); 6 (day 7); 9 (day 9); 6 (day 15) and 6 (day 30). Number of larvae tested per beetle sample: 30. Error bars represent SE of the difference between mean mortality in virus and control treatments. Fitted values follow the equation: $y = -1.71 - 0.31\text{day} + 0.021\ \text{day}^2 - 0.0004\ \text{day}^3$ for virus-fed beetles (day: $F_{(1,91)} = 22.04$; $P < 0.0001$); day^2: $F_{(1,89)} = 7.79$; $P < 0.01$; day^3: $F_{(1,87)} = 8.26$; $P < 0.01$). Excretion of virus in the controls was negligible.

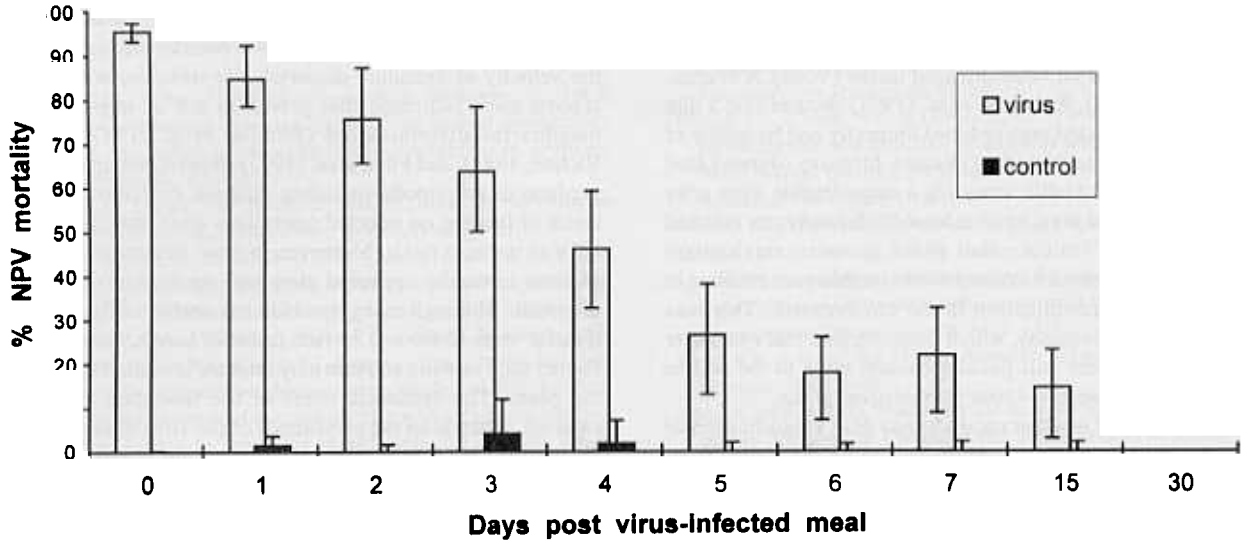

Fig. 3. NPV mortality in second-instar *M.brassicae* larvae exposed to soil where *H.rufipes* adults were introduced at different intervals after an infected meal. Error bars represent the SE of the difference between mean mortality in virus and control treatments. (Day 0: $F_{(1,45)} = 846.2$, $P < 0.0001$; day 1: $F_{(1,44)} = 173$, $P < 0.0001$; day 2: $F_{(1,32)} = 89.5$, $P < 0.0001$; day 3: $F_{(1,49)} = 39.0$, $P < 0.0001$; day 4: $F_{(1,46)} = 25.6$, $P < 0.0001$; day 5: $F_{(1,44)} = 8.5$, $P < 0.01$; day 6: $F_{(1,48)} = 6.3$, $P < 0.02$; day 7: $F_{(1,41)} = 5.1$, $P < 0.05$; day 15: $F_{(1,33)} = 2.4$, $P > 0.10$; day 30: $F_{(91,33)} = 0$). $N = 35$ virus-fed beetles and $N = 15$ controls; Number of larvae tested per beetle sample: 5.

'infected' carabids was significantly higher ($\chi^2 = 9.22$; df = 1; $P < 0.01$, scale parameter 1.40), irrespective of larval instar ($\chi^2 = 2.90$; df =1; $P < 0.05$, scale parameter 1.40) (Table 1).

Table 1. Field trials. The effect of carabids on (1) the recovery of *M.brassicae* larvae and (2) the development of NPV infection in test larvae in the field. Values correspond to the mean of four replicates. Means followed by the same letter are not statistically different. Fourth versus second instar larvae recovered ($F_{(1;13)} = 4.56$; $P \approx 0.05$); virus mortality resulting from virus-fed versus 'clean' carabids ($\chi^2 = 9.22$; df = 1; $P < 0.01$) virus mortality in second versus fourth instar ($\chi^2 = 2.90$; df = 1; $P > 0.05$).

	Trial 1: Carabids *v* Larval recovery		Trial 2: Carabids *v* NPV mortality	
Instar of test larvae	Presence of carabids	Recovery (% ± SE)	Condition of carabids	% NPV mortality (–SE; ±SE)
Second	Absent	68.8[a] ± 6.3	'Clean'	0.6[c] (0.3; 1.0)
	Present	55.3[a] ± 6.8	Virus-fed	2.8[d] (1.6; 4.8)
Fourth	Absent	84.4[b] ± 5.0	'Clean'	1.4[c] (0.8; 2.4)
	Present	71.4[b] ± 7.0	Virus-fed	6.5[d] (3.9; 10.7)

Deaths from the bioassay of soil samples collected from the plots were analysed by ANOVA (binomial errors) and showed that carabids effectively transfer inoculum to the surface layers of the soil. Although the overall mortality in plots with 'infected' predators was low [mean of eight plots = 6.1% (–SE = 2.2%; +SE = 15.8%)], it was significantly higher than in controls [mean of eight plots = 0.3% (–SE = 0.1%; +SE = 0.8%)] ($\chi^2 = 21.05$; df = 1; $P < 0.0001$, scale parameter 1.16).

Discussion

The results demonstrate that *Agonum dorsale, Harpalus rufipes* and *Pterostichus melanarius* do not discriminate between healthy and NPV-infected larvae of the cabbage moth, *M.brassicae*. As reported for other carabids (Thiele, 1977), the species tested in this study appeared to find the prey by wandering aimlessly, with larval mobility having limited importance as a releasing stimulus for prey recognition. Although a chemical sense may be important for prey localization in nocturnal carabids (Thiele, 1977), and can be involved in determining prey preference (Bilde & Toft, 1994), differences in texture, appearance and taste caused by NPV infection in lepidopteran larvae do not influence their selection by the three species tested. For hemipteran species, preference for infected prey may be due to a lack of a defensive response in moribund larvae (Young & Kring, 1991). Since coleopteran predators have a stronger set of mouthparts, specialized to grip and cut living tissues of prey, larval defensive response may represent less impediment for attack. Both *H.rufipes* and *P.melanarius* can feed on all larval stages of *M.brassicae* (Cory, 1984), even though late instars can be bigger than the predators themselves. Results of preference tests are relevant to the risk assessment of genetically modified microorganisms: if larval mobility does not influence prey suitability, ground beetles may also accept hosts infected with lengineered viruses that cause paralysis, such as the NPV expressing an insect-selective scorpion toxin (Cory *et al.*, 1994).

As expected, results from the faeces' bioassay confirmed that virus remains viable after passage through the carabids' gut. The capacity for NPVs to survive passage through predator guts is associated with the neutral to acid gut pH of invertebrate predators (Entwistle & Evans, 1985) which does not break down the inclusion bodies. Although lepidopteran NPVs cannot infect predaceous insects, whether viral infection alters the

nutritional quality of the prey is not clear. Although no effects were observed in species of Coleoptera, Hemiptera, Dermaptera and Neuroptera fed on virus-diseased larvae (Young & Hamm, 1985; Abbas, 1988), Ruberson *et al.* (1991) showed that a diet based on NPV-infected prey reduced longevity and fecundity of *N.roseipennis* (Het.: Nabidae). Faeces bioassay showed that *H.rufipes* excreted viable virus for a considerable time after feeding on diseased prey, up to at least 15 days after an infected meal. This may indicate that there is some mechanism whereby baculoviruses are retained within carabid guts, resulting in their prolonged dissemination in the environment. This was confirmed by soil bioassay, which demonstrated that even after 15 days beetles were still passing enough virus to the soil to cause mortality in second-instar *M.brassicae* larvae.

An indication of whether carabids may play a significant role as passive disease vectors is demonstrated by the results from the field experiment. Two factors will influence the importance of carabids as vectors; firstly, the frequency of contact between predator and prey (and therefore the likelihood of ingesting virus) and, secondly, whether inoculum released by carabids is likely to be acquired by healthy susceptible larvae. In the system studied, encounter between the carabids and larvae is likely to have been low because *H.rufipes* do not usually climb cabbage plants (Brown, 1986), and *M.brassicae* larvae tend to remain on the food source while it is available. This is reflected in the results: the carabids did not significantly reduce the larval population in the cabbage plots, although this may have been influenced by the short time that carabids and larvae were in contact (5 days). However, this rate of contact is likely to increase in the case of viruses genetically modified to express paralysis-inducing toxins which cause the infected larvae to fall off the plants (Cory *et al.*, 1994). The release of carabids which had been fed on virus-infected larvae enabled the likelihood of virus acquisition to be assessed. Mortality in the field, although not high, showed that carabids released infective virus in a form that could be acquired by susceptible lepidopteran hosts. However, the low level of mortality in the system studied indicates that predaceous beetles, such as carabids, would need to be more mobile (climb or fly onto plants) or the host larvae would need to exhibit behaviour that would bring them in more frequent contact with predator populations, to have a major impact on virus dissemination.

In most epizootics, disease expands from a multiplicity of endemic centres resulting in complex distribution patterns (Entwistle *et al.*, 1983). These patterns are thought to exhibit a 'travelling wave behaviour' (Murray, 1989), i.e. the infected proportion of the population develops into a moving wave across the landscape. The speed of baculovirus dispersal is determined by the processes of virus transmission, disease-induced mortality, host movement and the production and persistence of virus occlusion bodies (Dwyer, 1992). Among the mechanisms which may influence virus dispersal, predators have been recognized as disseminating inoculum for considerable distances. Birds commonly feed on infected larvae (Entwistle *et al.*, 1993) and are reported to disperse virus for up to 6 km from the initial site of infection (Entwistle, 1982; Entwistle *et al.*, 1977). The likelihood of consumption may be enhanced by the higher visibility of diseased prey which in many species become paler and migrate to the top of the plants as a result NPV infection.

Arthropod predators may act on an intermediate scale, and their higher mobility compared to host larvae may increase the velocity of inoculum dispersal. For instance, a number of reports have indicated that predators are of importance in baculovirus dissemination (Boucias *et al.*, 1987; Fuxa & Richter, 1994), and Fuxa *et al.* (1993) showed that up to 60% of predaceous arthropods, including carabids, contained virus as a result of feeding on infected caterpillars after application of an NPV in soybean fields. Moreover, in these studies, the presence of virus in nearby untreated plots was attributed to predators' dispersal. Although many carabids are unable to fly, they can transfer virus to the soil surface or lower leaves, where abiotic factors such as wind and rain may relocate inoculum upwards in the plant. The epizootic value of the inoculum spread by carabids depends on the persistence of the virus in the soil until acquisition by a new host, as soil can be regarded as the eventual repository for most insect viruses. The impact of carabid-mediated dispersal of baculoviruses may therefore be more relevant to populations of soil-dwelling Lepidoptera (e.g. cutworms) where host and vector (beetle) are in close proximity.

As stressed by Fuxa & Ritcher (1994), a full understanding of the spread of entomopathogens by predators is relevant not only to the successful application of biopesticides but also to predicting the environmental fate of natural and genetically modified microorganisms. Results presented here suggest that ground beetles have the potential to contribute to virus carriage and subsequent spatial redistribution of inoculum in the field. The magnitude of this role may depend on how the virus affects larval behaviour: future studies should focus on how the nature of the insect–virus interaction may influence the role of predators in disease dissemination. Whether the development and establishment of baculovirus epizootics in the field occurs with the same velocity and intensity in the absence of predators is yet to be determined.

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