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EXTRA-HOST INTERACTIONS BETWEEN A BRACONID ENDOPARASITOID, *APANTELES GLOMERATUS*, AND A BACULOVIRUS FOR LARVAE OF *PIERIS BRASSICAE*

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SUMMARY

(1) This study explores the extra-host interactions between the braconid endoparasitoid, *Apanteles glomeratus*, and the granulosis virus (GV) of *Pieris brassicae*, focusing on factors influencing the transmission of the virus within and between host broods.

(2) Transmission of the virus by *A. glomeratus* adult females was shown to correlate positively with the time since death of the virus-killed donor larvae. Transmission was most likely to occur from donors which were dead for 24–48 h.

(3) In the absence of the parasitoid, the risk of infection to a given *P. brassicae* larva was dependent on (i) the host age at the time of the introduction of the virus, and (ii) the initial number of the larvae infected. The initial number of uninfected hosts, however, had no effect on the per capita risk of infection.

(4) The parasitoid accelerated the spread of the GV in the host brood, but the eventual number of hosts succumbing to the infection was unaffected.

(5) Whereas a field experiment demonstrated the parasitoid as an important disseminator of the GV, percentage parasitism and the number of virus-infected cadavers were significantly negatively correlated. This latter result suggests that the parasitoid is somehow repelled by virus-killed hosts.

INTRODUCTION

A central feature of the biology of many microparasites (e.g. viruses, fungi, bacteria, protozoans) is their dependence upon abiotic (e.g. rain, gravity) and biotic (e.g. predators, parasitoids) agents for their dispersal and transmission (Fuxa & Tanada 1987). Biotic vectors are of particular interest with respect to community ecology because in transmitting the microparasite they are, in effect, aiding a competitor. The potential impacts of such interactions on the dynamics of the system and on the evolution of avoidance mechanisms by vectors, nonetheless, remain largely unexplored.

The actual mechanisms by which predators and parasitoids may contribute to the dispersal and transmission of pathogens are numerous (see reviews by Entwistle 1982; Kaya 1982). They can be conveniently divided into two categories. First, the natural enemy may aid the microparasite via normal routes of infection. Here, the natural enemy is simply a mechanical vector, or translocation agent, of the pathogen and the pathway of

infection is identical to transmission in the absence of the natural enemy. There are a plethora of examples of mechanical transmission in the laboratory (Liago & Tamashiro 1966; Brooks, 1973; Bell, King & Hamalle 1974; Irabagon & Brooks 1974; Beegle & Oatman 1975; Raimo, Reardon & Podgwaite 1977; Larsson 1979; Levin, Laing & Jaques 1979) and field (Boucias *et al.*, 1987; Young, Lack & Yearian 1987; Young & Yearian 1987). Second, the natural enemy may aid in both the translocation and infection of the pathogen. A commonly noted example is of adult parasitoid females serving as a 'flying hypodermic needle', contaminating the ovipositor shaft on a donor host and injecting the pathogen into a healthy recipient. Few studies, however, have adequately substantiated claims that the infection actually originates from injected pathogen or pathogen otherwise entering an injection wound. The most likely of those have involved bacteria (Kurstak 1966; Bell, King & Hamalle 1974), microsporidia (Brooks 1973), or fungi (Kaya & Anderson 1974; El-Sufty & Fuhrer 1981). In practice, confirmation of infection would require concurrent ultrastructural studies.

The impact of parasitoids (and predators) in disseminating pathogen inoculum in the field remains unclear, in part because of the difficulties involved in assessing spatial and temporal changes in the pathogen population and the contribution of the natural enemy to the translocation of the pathogen. Hence, many studies on host–pathogen–parasitoid interactions in the field have relied on spatial and temporal correlations in the abundances of parasitized and infected hosts as a measure of parasitoid-aided dispersal and, more generally, competition between the natural enemies. For example, in studying *Lymantria dispar* (L.) and its natural enemies, Reardon & Podgwaite (1976) found significant positive correlations in the incidences of a NPV and two different parasitoid species, *Apanteles melanoscelus* (Ratzeburg) and *Parasetigena silvestris* (Robineau-Desvoidy). They interpreted this finding, along with other evidence of the ability of the parasitoids to translocate the pathogen in the laboratory (Wollam 1974, cited in Reardon & Podgwaite), as evidence that the parasitoids transmit the pathogen in the field. However, they did not actually quantify directly transmission rates. In a study of the interactions between parasitoids in the genus *Aphidiidae* and fungi in the genus *Entomophthorales*, Powell *et al.* (1986) point out that a variety of interactions between natural enemies (and factors independent of the interactions) may lay behind correlations from field data, implying that only carefully monitored, controlled experiments can reveal the role of natural enemies in aiding in the transmission of microparasites. An example of a series of such experiments is illustrated by the work of S. Y. Young and colleagues (Young & Yearian 1986, 1987; Young, Lack & Yearian 1987), who have investigated the roles of natural enemies of *Anticarsia gemmatalis* (Hübner) in the dispersal of a NPV using carefully controlled caged experiments in the field. They were able to quantify, to a limited extent, the potential of these natural enemies to disperse pathogen inoculum in the field. The effects of such dispersal on the population dynamics of host–parasitoid–pathogen interactions is currently being addressed (M. E. Hochberg, unpublished).

This is the second part of a two-part study of the intra- and interspecific interactions involving a braconid endoparasitoid, *Apanteles glomeratus* (L.), and the granulosis virus (GV) of *Pieris brassicae* (L.). The first part explored within-host aspects of the interactions (Hochberg 1991). The present investigation examines various aspects of the extra-host interactions of these two natural enemies. In particular, I aim to explore what influences the spread of the virus within the host brood, and the role that the parasitoid plays in the dissemination of the GV, both in the laboratory and in the field.

METHODS AND MATERIALS

The culture of *P. brassicae* originated from the 'virus-free' Cambridge stock (David 1962). The *A. glomeratus* stock came from field-caught material at Silwood Park, Ascot, England. A purified preparation of GV was obtained from Dr N. Crook (AFRC Institute of Horticultural Research, Littlehampton, England), and stored at −20 °C.

Unless otherwise indicated: (a) all experiments were conducted at 20 ± 1 °C, 16:8 L:D photoperiod, and *c.* 70% RH; (b) first instar *P. brassicae* larvae were parasitized by 2–6-day-old mated female parasitoids; (c) larvae were checked daily noting their instar, death due to unknown causes, disease symptoms, or parasitoid emergence; (d) barring significant differences between treatments, larvae dying from unknown causes were omitted from statistical analyses; (e) non-parametric two-sample statistical testing was employed (see pp. 138–141 in Zar 1984). Other general methods employed in this study can be found in Hochberg (1989).

Mechanisms of granulosis virus transmission by the parasitoid

Adult female parasitoids were introduced for 1 hour into the pits of ELISA plates containing virus-infected, second instar 'donor' larvae that were: (a) within 24 h of death (but still responsive to stimuli); (b) less than 24 h post-death (integument intact); (c) 24–48 h post-death (i.e. liquefied); (d) 48–72 h post-death (somewhat desiccated); or (e) healthy (i.e. control) larvae. A sixth treatment was as in (a), except the parasitoids were allowed 6 h to attack the donor. A seventh treatment was as for (d), except two donor larvae were employed. Insertion of the parasitoid ovipositor was not confirmed, leaving the possibility that some of the parasitoids did not parasitize or even come into contact with their donors. After each treatment the parasitoid was transferred to another ELISA pit containing either (i) a second instar 'recipient' larva with a 12·6-mm^2 leaf disc present, or (ii) a leaf disc without a recipient larva. After 1 h the parasitoid was removed. The recipient larvae from (i) were reared in the same ELISA plates in which they were exposed to the parasitoid. Unparasitized larvae were added singly to the pits of the ELISA plates of treatment (ii). All larvae were provided with fresh leaf discs 3 days post-treatment, and every 2 days thereafter. Larvae were checked daily for death due to the virus. The trial was terminated after 10 days.

Transmission of the granulosis virus within the host brood

Potted Brussels sprouts (*Brassica oleraceae* L. var *gemmifera* (Zenk)) plants were placed in the bases of Watkins–Doncaster cages and covered with two 20·5 cm × 9·5 cm plastic dishes (giving total dimensions of 20·5 cm diameter × 29·0 cm height) with muslin covers on top (Fig. 1).

Unless otherwise indicated, twelve replicates of each of twelve treatments were conducted. Each treatment consisted of placing a specified number of healthy first instar hosts from a common brood onto the second leaf from the apical meristem of a Brussels sprout plant. The brood was given 2–3 h to settle down on the leaf. (If after this time the larvae were not all massed together and feeding, then these larvae were removed and a new brood put in its place.) A predetermined number of larvae were then removed from the brood and infected with the estimated LD_{95} of the GV (see Table 1 in Hochberg 1991). These infected larvae were then replaced into the brood 24 hours later. During the course of the experiment, excised Brussels sprout or cabbage (*B. oleraceae* var *capitata*) leaves

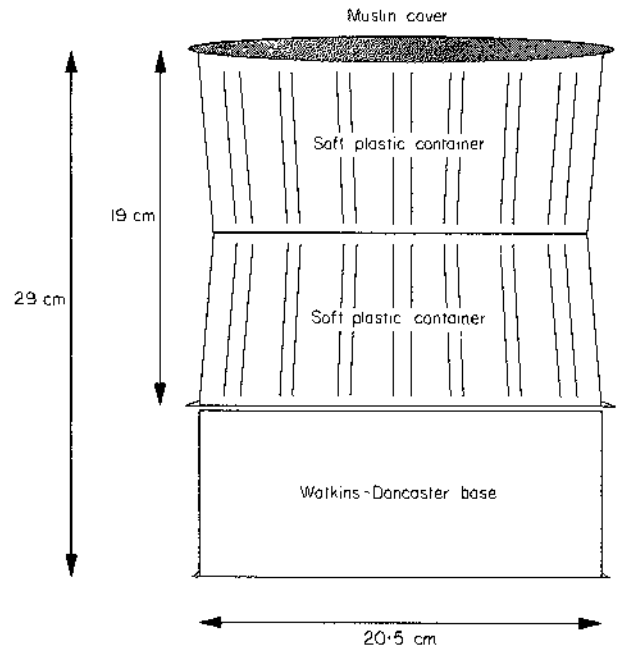

FIG. 1. Design of the cages used for the within-brood transmission experiments described in the text.

were supplied at the bottom of the cage on a daily basis. Old leaves were routinely removed from the bottom, even if they contained the remains of GV infected larvae. Day 0 of the experiment was taken to be the day on which the larvae emerged from the egg. In the twelve treatments conducted, three variables were manipulated (Fig. 2).

Number of infected larvae. Zero (control), 1, 2, 4 or 8 larvae were infected with *c.* $1{\cdot}00 \times 10^3$ occlusion bodies of the GV at emergence from the egg and then replaced into the brood on day 1. In some replicates, fewer or more than the number infected by the GV were observed to succumb to the virus in the days following infection (days 4–7). In all statistical tests, the number of initial infections was taken as the number of larvae dosed with the inoculum. In no case did larvae in the control treatment (one out of every thirteen plants) die from the disease, indicating no cross-contamination between replicates.

Total number of larvae. The initial total number of larvae (infecteds and susceptibles) was set at 10, 20, 40 or 80. Six replicates of the trials with 80 larvae were performed.

Larval age at initial infection. Larvae were infected with their estimated LD_{95} of the GV (Hochberg 1991) on days 0–1 (newly emerged first instars), days 2–3 (48-h-old first instars), days 4–5 (newly emerged second instars), or days 7–8 (newly emerged third instars). This involved removing larvae (arbitrarily) from the plant for infection, and then replacing them 24 h later on the same leaf from which they were removed.

A GLIM maximum likelihood analysis (McCullagh & Nelder 1983) was employed to assess the contributions of various factors to the proportion of the initially uninfected brood succumbing to the virus by the end of the larval period (i.e. the generational risk of infection).

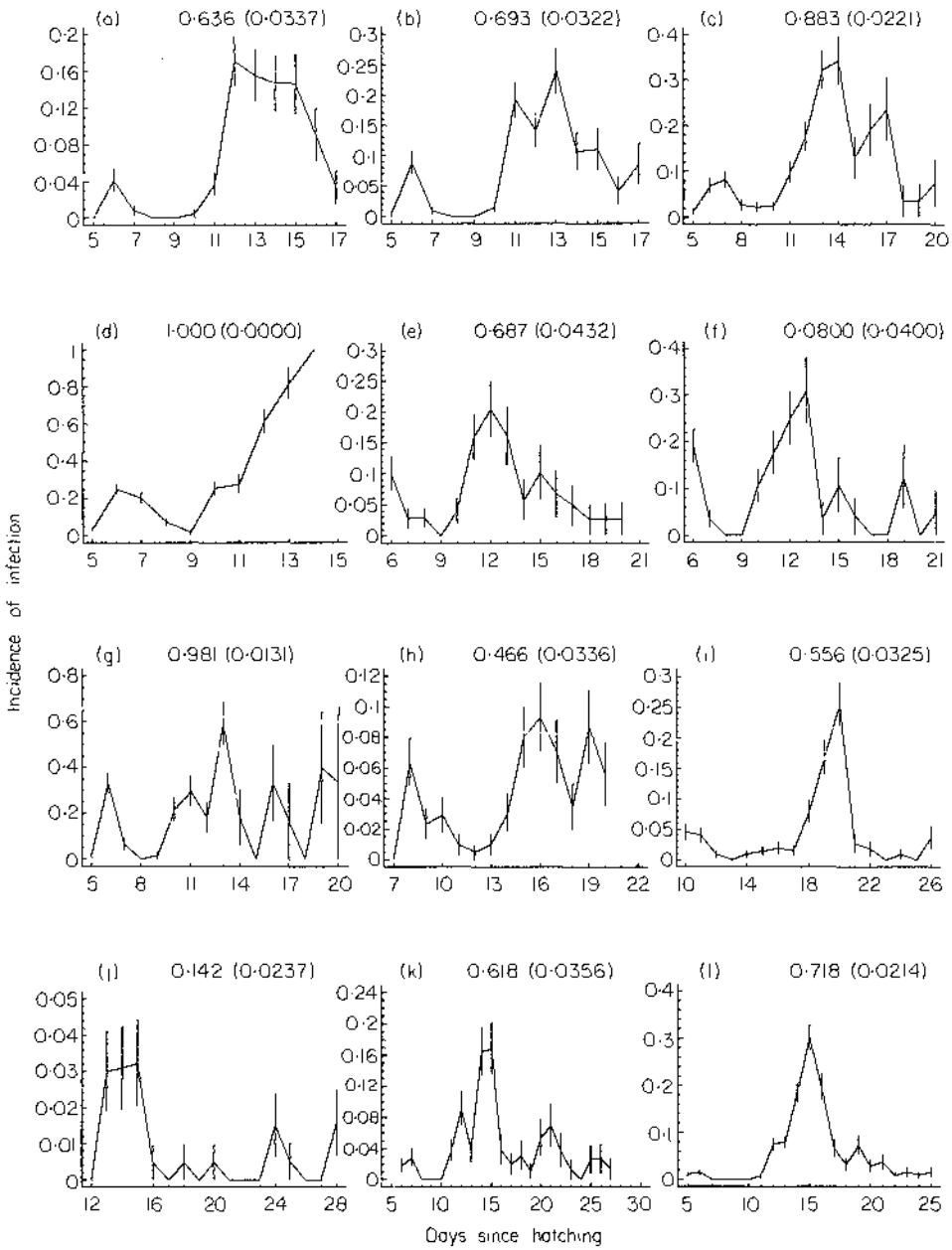

FIG. 2. The effects of various factors on the daily incidence of the virus. Unless otherwise stated, the total number of larvae (density) was twenty, the initial number of infected larvae was two, and larvae were initially infected the day after egg hatch (i.e. day 1): (a) infected = 1; (b) infected = 2; (c) infected = 4; (d) infected = 8; (e) density = 10, infected = 1; (f) density = 10, infected = 2; (g) density = 10, infected = 4; (h) day = 3; (i) day = 5; (j) day = 8; (k) density = 40; (l) density = 80. Mean incidence (± S.E.) of infection after the disease has run its course is given in upper right hand corner of each figure. Mean incidence levels (total or at a given observation) are estimated (assuming a binomial distribution of the data) as the sum of deaths due to virus divided by the total number of larvae (live + virus killed − unattributable deaths). Bars represent the standard errors of the means. Note the differing scale ranges on the x and y axes.

Influence of the parasitoid on virus transmission

Twelve replicates each of one control and one treatment were conducted. In the control, one fresh GV-killed larva (late first instar) was placed, arbitrarily, on the same side of the leaf approximately 1 cm from nineteen newly moulted feeding larvae. The treatment was the same as the control, except a single 2–4-day-old female parasitoid was placed in the cage for a period of 1 h. If the parasitoid did not exhibit searching and oviposition behaviours after 30 min, then she was replaced by a different parasitoid. This procedure was repeated until a suitable parasitoid was found. Otherwise, the experimental methods were the same as for the transmission experiments outlined in the previous section. Dissections of the twenty-nine larvae surviving to day 18 of the experiment showed that 24% (range of 0% to 50% between replicates) were parasitized.

Transmission of the granulosis virus in the field

The experiment was conducted at Imperial College Field Station, Silwood Park, Ascot, and consisted of interspersing potted Brussels sprouts plants in a *c.* 20 m × 20 m caged plot of planted Brussels sprouts (*c.* 400 plants, spaced 1 m apart). One replicate each of nine different treatments was executed, with each treatment consisting of four recipient plants (the 'patch'), placed an equal distance from one another and an equal distance from a central donor plant. (The final configuration is a square with the donor in the centre.) The centres of any two treatments were separated by *c.* 6 m.

For each treatment, an equal number of *P. brassicae* larvae were placed on each of the donor and recipient plants. In addition, a specified number of virus-killed cadavers were placed on each donor plant. The following variables were manipulated:

Number of susceptible hosts. Each set of four recipients and the donor contained one of the following numbers of healthy larvae: 5, 10 or 20 second instars (24-h-old) per plant.

Number of infected larvae on the donor. In addition to the susceptible larvae, the donor plant had one of the following numbers of fresh (less than 24 h after dying during moult to second larval instar) cadavers: 0 (controls), 1, 5 or 10 per plant.

Distance between the donor and the recipients. Three distances were tested: 30 cm, 1 m, and 2 m.

The trial started at 11.00 h on 15 August 1988 and finished at 11.00 h on 16 August 1988. Sterile techniques were strict. All susceptible larvae were in place before virus-infected cadavers were placed on the donors. Upon the termination of the experiment, recipients were removed together with their leaf. (The larvae from the donor plant were not collected.) The larvae from each recipient were reared together in a single container for 10 days in the laboratory as described elsewhere (Hochberg 1989). Observations were made daily, noting the age of the larvae, and death due to GV, parasitoid or due to a combination of the GV and the parasitoid. Larvae unaccounted for from the field were not included in statistical analyses.

A GLIM maximum likelihood analysis was used to deduce how the effects of various manipulated factors explained residual deviance in the data. Two variables were considered: (a) the proportion of the patch (i.e. four recipient plants) parasitized by *A. glomeratus*, and (b) the proportion of the patch infected by GV. In the case of (a), three independent variables were considered: (i) initial number of healthy larvae, (ii) initial number of GV killed (inoculum) larvae, and (iii) the distance between the donor plant and the recipient plants. For (b), the same three independent variables were considered with the addition of a fourth variable: (iv) the proportion of the patch parasitized by *A. glomeratus*.

RESULTS

Mechanisms of granulosis virus transmission by the parasitoid

Table 1 presents the numbers of larvae surviving and dying from GV. The age of the donor larva was the only variable explaining a significant amount of the variation in the probability of a recipient succumbing to the virus (GLIM analysis, $P < 0{\cdot}001$). The time of exposure to the recipient larva, presence or absence of the recipient, and the number of donor larvae had no significant effects.

Transmission of the granulosis virus within the host brood

Figure 2 shows the course of infection as indexed by the daily incidence of death due to the virus (number of virus deaths divided by the total number of hosts on a given day). The following general observations were made: (i) in most treatments there were either two or three distinct peaks of infection; (ii) there was a characteristic delay of *c.* 7–8 days between the first and second peaks of infection; and (iii) the variability in disease incidence (between replicates) for any given treatment increases with time (due, in part, to fewer larvae surviving per replicate.

A GLIM analysis revealed that the most important factors determining the proportion of healthy hosts from a brood succumbing to the GV (a measure of the per capita risk of infection) were (i) the initial number of cadavers and (ii) the time at which the inoculum was introduced into the brood (Table 2). The risk of infection increased with larger inoculum densities and decreased with the age at which the inoculum was introduced. However, the initial total number of larvae in the brood had no significant effect on the

TABLE 1. Numbers of *Pieris brassicae* larvae surviving and succumbing to the granulosis virus after exposure to *Apanteles gomeratus*

Group[g]	Donor treatment	Recipient?	Survived	Virus
1	Control	y	20	0
		n	18	0
2	Moribund[a]	y	21	0
		n	20	2
2	Moribund[ab]	y	15	0
		n	23	0
3	Cadaver[c]	y	18	3
		n	16	5
4	Cadaver[d]	y	9	8
		n	14	6
5	Cadaver[e]	y	21	2
		n	21	2
5	Cadaver[ef]	y	19	5
		n	21	3

[a] Responds to stimulus.
[b] 6-h exposure to recipient.
[c] < 24 h after death.
[d] 24–48 h after death.
[e] > 48 h after death.
[f] 2 donors.
[g] Group effect explains 80·8% of the deviance in GLIM analysis ($P < 0{\cdot}001$).

TABLE 2. Summary of significant effects in GLIM model* for the percentage infection by the granulosis virus within broods of *Pieris brassicae* in the laboratory

Factor	Regression estimate	Standard Error
Constant	−0·748	0·163
Number of infecteds†	0·827	0·0816
Host age at which virus is introduced‡[a]	−1·02	0·166
Host age at which virus is introduced‡[b]	−0·7343	0·152
Host age at which virus is introduced‡[c]	−3·94	0·351

* 39·5% of the deviance (variance) is explained by the logistic regression model based on 129 observations.

† Statistically significant correlation between cadaver number and proportion of brood succumbing to infection at $P<0·001$.

‡ Statistically significant difference between time of inoculation for all possible pairwise comparisons ($P<0·001$ for each comparison), except for comparison between day 3 (instar 1) and day 5 (instar 2): [a] regression coefficient for day 3; [b] coefficient for day 5; [c] coefficient for day 8 (instar 3).

proportion of the larvae succumbing to the infection. In no cases were interactions between various factors statistically significant.

Influence of the parasitoid on virus transmission

Figure 3 presents the course of the disease for both the control and the treatment with the parasitoid. The introduction of the parasitoid accelerated the initial spread of the GV (GLIM comparison for treatment effects from day 10 to day 12, $P<0·001$ (no

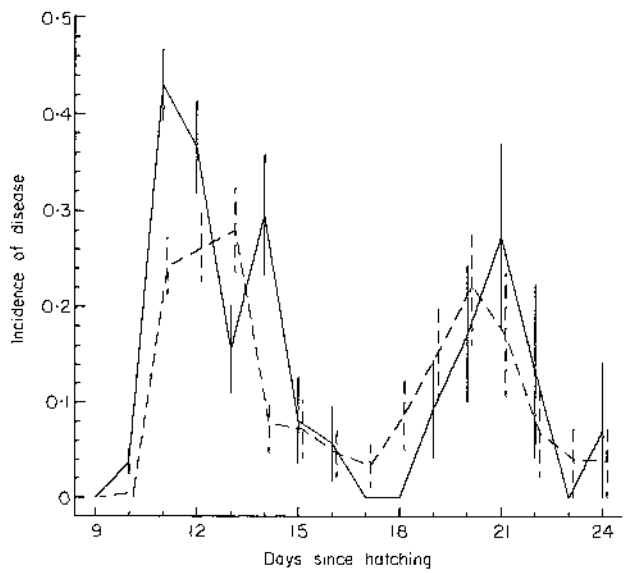

FIG. 3. The daily incidence of infection by the virus within broods of *P. brassicae* in the presence (——) and absence (– – –) of the parasitoid. Total incidence of infection: parasitoid absent, 0·837 (0·0375); parasitoid present, 0·839 (0·0396). Means and standard errors as for Fig. 2.

autocorrelation assumed)), but had no effect on the proportion of the brood eventually succumbing to the GV, and the median and mean days upon which the larvae within a given brood died (for median and mean, Mann–Whitney tests, $P > 0{\cdot}05$). In the control, there were two peaks of incidence of infection (days 13 and 20), whereas in the parasitoid treatment, there were three peaks in infection (days 11, 14 and 21). (Note that the higher total incidence of infection in these treatments as compared with the similar trial in the previous section (see Fig. 2a) is due to the direct placement of the cadaver in the vicinity of the feeding brood).

Transmission of the granulosis virus in the field

Percentage parasitism in the field was 55–98% between treatments (at the level of the patch) and 0–100% at the level of the recipient brood (Table 3). Levels of infection by the GV were generally low: 0–6% at the patch level, and 0–25% at the recipient plant level (Table 3). Levels of multiple parasitism (host attacked by both parasitoid and virus) were more elevated than simple infection by the GV, the former being 0–25% at the patch level, and 0–88% at the recipient brood level.

The GLIM analysis indicated that parasitism was significantly affected by (i) the number of infected larvae on the centre plant, (ii) the distance between the centre plant and recipient plants, and (iii) the number of susceptible larvae per plant (Table 3). In particular, percentage parasitism decreased with increases in cadaver number, and decreases in the distance between the plants (i.e. the clumping of the recipients) resulted in significant increases in parasitism. Increasing the number of susceptible hosts per patch resulted in significant increases in parasitism from densities of 20–40 per patch; but increasing number further (from 20 to 40 susceptible larvae per patch) resulted in significant decreases in percentage parasitism.

Furthermore, the GLIM analysis revealed that the per capita risk of infection by the virus increased significantly with increases in (i) the number of infected donor larvae and (ii) percentage parasitism (Table 3). The number of susceptibles per patch and the distance between the plants had no significant effects in explaining residual deviance. Note that if

TABLE 3. Incidences of parasitism, virus infection and mixed (both parasitoid and virus) mortalities in the field experiment

Number of recipients per patch*	Number of infected donors*†	Plant distance (cm)*	Fraction of larvae parasitized (range)†‡	Fraction of larvae infected (range)‡	Mixed mortalities (range)‡
40	0	30	0·977 (0·875–1)	0	0
40	0	30	0·759 (0·667–1)	0	0·037 (0–0·222)
40	1	30	0·944 (0·9–1)	0	0
40	5	30	0·555 (0·125–0·7)	0·056 (0–0·25)	0·25 (0–0·875)
40	10	30	0·757 (0·556–1)	0·027 (0–0·1)	0·135 (0–0·556)
20	10	30	0·625 (0·25–1)	0	0
80	10	30	0·594 (0·357–0·941)	0	0·014 (0–0·063)
40	10	100	0·703 (0·125–1)	0·027 (0–0·1)	0·027 (0–0·1)
40	10	200	0·629 (0–0·875)	0	0

* Significant effect on percentage parasitism ($P < 0{\cdot}05$) (89·9% of deviance explained by significant effects, GLIM analysis, $n = 9$).

† Significant effect on percentage infection by virus ($P < 0{\cdot}05$) (83·9% of deviance explained by significant effects, GLIM analysis, $n = 9$).

‡ Range of four recipient plants in treatment.

parasitism were not included in the model, then the two insignificant effects would enter the multiple regression as significant ones. This is because parasitism correlated with both number and distance (see first footnote to Table 3).

DISCUSSION

This study has explored some of the mechanisms responsible for the transmission of the granulosis virus within populations of *Pieris brassicae*. In particular, the parasitoid, *Apanteles glomeratus*, was shown to vector the GV both over short (e.g. within the brood) and long (e.g. between broods) distances. The short-distance effect was reflected by increased rates in the initial spread of the virus when the parasitoid is present. The long-distance effect involved the transfer of inoculum from virus-contaminated to virus-free broods of the host.

Transmission of the virus from host to host

In a separate study (Hochberg 1989), *A. glomeratus* was shown to be able to transmit the GV by mechanical means (e.g. contamination of the host's food source from the parasitoid's integument) but not by injection. In particular, manual injection of high doses of the GV ($> LD_{99}$) into second or third instar larvae resulted in virus deaths only in cases where the effluent from the puncture wound (resulting from the insertion of a 10-nl micropipette) was not washed from the integument (using a 1% solution of Teepol, ICI, London). The same result was found for injection by the parasitoid. Thus, simple contamination of the host's integument or surface of the leaf were sufficient to cause an infection.

In related studies, Levin and colleagues (1979, 1983) showed that *A. glomeratus* can transmit the GV to larvae of *Pieris rapae* L. either after having emerged from GV-infected hosts, or after having parasitized infected (but not dead) hosts. They suggested that transmission occurs via the ovipositor, but did not specify that infections arose via inter-hemocoelic injection. Although the results of the present study can only be used as a comparison, the much higher susceptibility of *P. rapae* than *P. brassicae* to the GV (Payne, Tatchell & Williams 1981) could explain the ease with which the parasitoid contributed to the transmission of the virus between host larvae as observed in the studies of Levin and colleagues. Only ultrastructural studies can establish with certainty the role of the parasitoid as an injecting vector of the GV.

In addition, parasitoid-aided transmission of the virus may be restricted to a narrow window of time. This is after the integument of the virus-killed host ruptures (i.e. *c.* 24–48 h after death), but before the cadaver becomes too dessicated (significantly fewer recipients succumbed to the disease in group 5 than in group 4, GLIM contrast, $P < 0{\cdot}05$, Table 1). Since the volume of GV-infected cadavers can be expected to increase (and thus maintain viscosity for longer) with the age of the growing host, it is likely that the parasitoid's role as a vector becomes more important in later instars (i.e. second and third) of the host.

Transmission of the virus within the host brood

The initial number of larvae in a brood infected by the virus significantly correlated with the eventual percentage of healthy larvae succumbing to the disease. When initial numbers were high enough (eight infecteds in a brood of twenty, Fig. 2d), then all healthy sibs died from the virus by day 14 (fourth larval instar), suggesting that there is an

increasing risk of infection as a function of initial virus contamination which asymptotes at around eight infected first instar larvae per brood. Since the dying larvae in the experiments were not weighed, any relationship between the initial number of infected larvae and the amount of virus produced by the end of the larval period could not be determined.

The larval age at which the virus is introduced into the host brood also contributed to the number of larvae eventually succumbing to infection. If the inoculum were introduced late enough (e.g. late third or fourth instar) then, at least under laboratory conditions, few or no subsequent infections resulted. Apparently, the larger amounts of virus produced by these larvae were not sufficient to compensate for increases in resistance to infection in older larvae (Hochberg 1991).

A separate maximum likelihood analysis (M. E. Hochberg, unpublished) to the GLIM analysis presented in Table 2 revealed that transmission of the virus within the *P. brassicae* brood follows the 'homogeneous' or 'random' mixing law commonly employed in host–pathogen models (e.g. Anderson & May 1981). From the host's viewpoint, this implies an equal risk of infection, over the whole of the larval period, between any two hosts (i.e. homogeneous vulnerability). From the point of view of the GV, this means that each GV-infected cadaver has an equal chance, on average, of being consumed by the susceptible larvae. Future studies should explore level of heterogeneity in transmission between broods in the field.

Interactions between parasitoid and virus in the field

The result that the parasitoid contributes significantly to the dispersal of the GV from brood to brood in the field supports the claim by Levin *et al.* (1983) that *A. glomeratus* may be important in transmitting GV in populations of *P. rapae*. Indeed, in areas where the two Pierids occur in sympatry, *Apanteles* potentially serves as a cross-species vector of the virus.

The field experiments also demonstrated that the adult parasitoid tended to avoid patches of the host which contain GV liberated from cadavers. Other studies have pointed out that *A. glomeratus* avoids obviously infected *P. rapae* (Kelsey 1960; Levin *et al.* 1983), implying that contamination of the parasitoid is unlikely to result directly from oviposition into moribund larvae, but rather from inadvertent trampling over virus-killed larvae. Although percentage parasitism was negatively correlated with the number of infecteds in the centre of the patch, some transmission of the GV did occur in four of the seven treated patches and in one of the two control patches (i.e. treatments with no infected donors, Table 3).

Potential pay-offs to the virus

In sum, this and a separate study (Hochberg 1991) suggest that increases in the pay-off to a virus (the quantity of virus produced by the end of the host generation) introduced into a susceptible brood of *P. brassicae* occur when:

The number of susceptible hosts in the brood is large. The results presented here demonstrate a directly proportional relationship between hosts available and hosts eventually succumbing to the infection.

Low to intermediate numbers of virus particles are introduced into the brood. In cases where the initial inoculum is very small, few or no larvae will become infected and the disease will not spread. On the other hand, if the initial inoculum is very large, then most or all of the larvae will succumb quickly and little magnification of the virus will occur.

Introduction of large quantities of virus could substantially magnify if they are introduced later in larval development when the larvae are less susceptible to infection.

The virus is introduced early in the host's larval development. Invasion of the GV early in the larval period not only increases the ability of a given propagule to cause an infection (due to the low resistance of the host when young; see Hochberg 1991), but also gives the GV a longer period in which to magnify its reproduction once released from the host. As the lethal period of infection lasts approximately 5–8 days at 20 °C (Payne, Tatchell & Williams 1981; Hochberg 1989), inoculum present in the first larval instar can potentially have as many as four magnifications (in the early second instar, mid-third instar, and mid-fourth instar and late fifth instar). In addition, a recent study on the 'productivity ratio' (virus produced: virus required to give LD_{50}; Evans, Lomer & Kelly 1981) of a nuclear polyhedrosis virus on *Heliothis armigera* (Hübner) demonstrated that the highest productivity ratio occurred in new-born insects (Teakle & Byrne 1989).

Given that the virus is ultimately the superior competitor within the host, and that the parasitoid may aid in the dispersal of the virus from host to host, how does *A. glomeratus* persist in the system? And why is the GV of *P. brassicae*, in fact, rarely observed in the field? Addressing these and other fascinating questions requires further experimentation and the development of quantitative theory (Hochberg, Hassell & May 1990).

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