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INTRA-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 within-host mechanisms of intra- and interspecific competition involving the gregarious braconid endoparasitoid, *Apanteles glomeratus*, and the granulosis virus (GV) of *Pieris brassicae*.

(2) Increasing doses of the GV resulted in increases in mortality and decreases in life-span of the host. Susceptibility to the virus decreased markedly with host age.

(3) High doses of the GV resulted in substantial reductions in production of pathogen progeny, consistent with intraspecific competition for a limited (but growing) resource.

(4) Survival of the parasitoid brood within GV-infected hosts depended on the relative timing of parasitoid emergence (at the spinning of the host pupal mat) and host death due to the virus.

(5) Interspecific competition between the natural enemies did not involve changes induced by the parasitoid in the pathogen's ability to infect the host. However, the expected life-span of hosts harbouring both competitors was significantly shorter than hosts infected with the virus alone.

(6) Interspecific competition within the host was, on average, highly symmetrical. The presence of virus reduced the average weight of the parasitoid brood by 29%, whereas the parasitoid brood reduced the potential reproductive output of the virus by 28%. However, when the size of the parasitoid brood was taken into account, the parasitoid individual was best off when in small broods, whereas the virus did best when competing with larger parasitoid broods.

INTRODUCTION

Interspecific competition, when one species contests the utilization of a limited resource with another, is generally considered to be a pivotal force in moulding the structure and dynamics of communities. A particularly fascinating class of interspecific interactions is interkingdom competition, where species from different kingdoms contend for a common resource (for review see Hochberg & Lawton, in press). Much of the work on interkingdom competition has considered interactions between hymenopteran or dipteran parasitoids and infectious disease (such as viruses, bacteria and fungi). Although very informative from a pathological perspective, only a small number of such studies have addressed ecological questions.

Much of the growing literature on host–parasitoid–pathogen interactions has involved lepidopterous hosts and nuclear polyhedrosis viruses (NPV). For instance, Irabagon &

Brooks (1974) showed that the outcome of competition between *Campoletis sonorensis* (Cameron) and a NPV in larvae of *Heliothis virescens* (F.) depended upon the timing of attacks of the natural enemies. If exposure to the virus followed that of the parasitoid by more than 48 h, then some of the parasitoids could survive to maturity, otherwise the virus essentially eliminated the parasitoid. The virus appeared to have no effect on the development period of the parasitoids (if they survived). Beegle & Oatman (1975) showed that the relative timing of attacks between the parasitoid, *Hyposoter exiguae* (Viereck), and a NPV in hosts of *Trichoplusia ni* (Hübner) determined the ultimate outcome of competition. If the NPV infected the lepidopterous host prior to parasitization, then the parasitoid larvae always died. But, if the virus entered the host after the parasitoid, then some or all of the parasitoids could survive. Those parasitoids which did survive spent significantly less time in hosts than did parasitoids within virus-free hosts. Surviving parasitoids were otherwise unaffected by the disease (e.g. in terms of fecundity, longevity, etc.). Hotchkin & Kaya (1983) found directly adverse effects of pathogens on a parasitoid, but in this case involving the interaction between *Glyptapanteles militaris* (Walsh) and the NPV of *Pseudaletia unipuncta* (Haw.). In contrast to the results of the previous study, Vail (1981) showed that the parasitoid *Voria ruralis* (Fallén) was not directly adversely affected by the NPV of *T. ni*; rather, survival of the parasitoid depended on the relative timing of the attacks between the competitors. Relative timing of attacks also appears to determine the competitive outcome of various aphid–parasitoid–fungus systems (e.g. Milner, Lutton & Bourne 1984; Powell *et al.* 1986).

Broadly similar qualitative interactions have been noted involving pathogens other than NPVs attacking lepidopterous hosts. For instance, Siegel, Maddox & Ruesink (1986) found that infection by the microsporidian, *Nosema pyrausta* (Paillot), of both the parasitoid, *Macrocentrus grandii* (Goidanich), and its host, *Ostrinia nubilalis* (Hübner), resulted in fewer parasitoids surviving to adulthood. Cossentine & Lewis (1988) found that the same microsporidian had no significant effect on the ability of the parasitoid, *Lydella thompsoni* (Herting), to eclose, but a related microsporidian, *Nosema* sp., did. This is apparently because the latter pathogen directly affected the parasitoid larvae, whereas the former did not. Competition may also manifest itself in 'inapparent' ways. For example, Weseloh *et al.*'s (1983) study of the interactions between the parasitoid *Apanteles melanoscelus* (Ratzeburg) and *Bacillus thuringiensis* (Berliner) in larvae of the gypsy moth, *Lymantria dispar* (L.), show that the retarding effect on host development of infection by the pathogen permits higher rates of parasitism by *A. melanoscelus* (due to the smaller size of infected hosts).

Several past studies have considered parasitoid–pathogen interactions involving Pierids. For example, the intensity of infection of *Pieris brassicae* (L.) by the microsporidian *Nosema mesnili* (Paillot) determines to what extent the parasitoid *Apanteles glomeratus* (L.) survives to adulthood (Blunck 1954; Tanada 1955; Larsson 1979). Similar outcomes have been observed for *Pieris rapae* (L.) attacked by *A. glomeratus* and a granulosis virus (Kelsey 1960; Levin, Laing & Jaques 1981).

This is the first part of a two-part study on mechanisms of competition between the gregarious braconid endoparasitoid, *Apanteles glomeratus*, and the granulosis virus of *Pieris brassicae* (hereafter abbreviated GV) contending for *P. brassicae* larvae. The second part explores the interactions outside of the host between these two species (Hochberg 1991). Among the questions addressed are: (i) How does the intensity of an attack by one species affect intraspecific competition among individuals? (ii) Does the presence of the parasitoid affect the ability of the GV to infect the host? (iii) How is each

competitor affected by the other when co-occurring within the host individual? Biological reviews can be found elsewhere for *P. brassicae* (Feltwell 1982), for *A. glomeratus* (Laing & Levin 1982), and for the GV (David 1978).

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 the GV was obtained from Dr N. Crook (AFRC Institute of Horticultural Research, Littlehampton, England), and stored at −20 °C. Required concentrations of the virus were prepared by successive dilutions from an original estimated concentration of $2{\cdot}50 \times 10^{12}$ occlusion bodies (OBs) ml^{-1}. The aqueous solution used in these dilutions consisted of 0·15% phenol red (to make the solution visible) and 1% (v/v) Teepol (ICI, London) (for suspension and spread of the GV over leaf surfaces).

Mortality attributable to the virus was assessed visually (Tanada 1953; Vago, Lepine & Croissant 1955); however, in cases where the cause of mortality was in question, the host cadaver was examined microscopically. All counts of parasitoid larvae were made by first dissecting them from the host, either under an isotonic Ringer's Solution or without added solutions.

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) unless there were significant differences between treatments, larvae dying from unknown causes were omitted from statistical analyses. Other general methods employed in this study can be found in Hochberg (1989).

Host response to different doses of the granulosis virus

Samples of all five larval instars of the host were tested for their response (survival or death) to increasing doses of the virus. A range of virus dilutions were prepared just prior to each trial using a separate study as a guideline to doses required to kill between 5% and 95% of a given sample of *P. brassicae* (Payne, Tatchell & Williams 1981) (for actual doses used, see Hochberg 1989). The results of Payne, Tatchell & Williams (1981) were also used as guidelines for deciding when each experiment was to be terminated. Terminating the experiment too late could mean that some of the larvae died of infections initiated after the 24-h infection period.

For the first instars, synchronously hatched (3–6-h time interval) larvae were placed into the pits of ELISA microtitre plates. The virus was introduced to awaiting larvae as a dried deposit (from a 2·5-nl micropipette) on a 1-mm^2 disc of Brussels sprouts (*Brassica oleraceae* L. var. *gemmifera* (Zenk)) leaf. The ELISA pits (containing larvae and virus) were then covered with *c.* 8 mm diameter glass balls (BDH Chem. Ltd, Poole, England) and placed in a humidified chamber. Only those larvae that consumed the whole disc after 24 h were used in the experiment.

The effects of virus dose on instars 2 and 3 were conducted in much the same way as for the first instars, except that the larvae were put into the microtitre plates just before they were due to ecdyse to the tested instar. Tests on the fourth and fifth instars were conducted

by placing the larvae into 3·3 cm diameter × 1·0 cm deep plastic Petri dishes. A microsyringe was used to distribute droplets of 0·5 μl onto leaf discs which were cut to sizes of 3·5 and 12·6 mm^2 for instars 2 and 3, respectively. Droplets of 1 μl were placed on discs of 30·1 and 86·5 mm^2 for instars 4 and 5.

Trials resulting in less than 5% or greater than 95% mortality were not used in the analyses as non-linearities in the relationship between dose and response could occur at these extremes (Hughes *et al.* 1984; van Beek, Wood & Hughes 1988). The data were analysed by a standard probit analysis (Finney 1978), and trials run on different dates were used together in the analyses since χ^2 comparisons revealed no heterogeneity between the treatments for any given instar.

Effects of high doses of the granulosis virus

Newly hatched first instars were fed one of four doses of the GV (spanning almost two orders of magnitude, Table 2), well above the predicted lethal dose to kill 99% of the sample (LD_{99}). In order to record more precisely the time of death due to the GV, the experiment was conducted at 15 °C rather than 20 °C. Intact larvae dying from the GV were dessicated using silica gel (BDH Chem. Ltd, Poole, England) and then weighed on a microbalance. Otherwise, the methods were the same as for the response experiment conducted on the first instars.

Parasitoid survival in infected hosts

Pre-trials indicated that if infection by the virus occurred prior to the beginning of the fifth instar of the host, then parasitoids rarely emerged from the host and pupated successfully. This is because these hosts died prior to comencing to spin their pupal mat (the necessary cue for emergence of *A. glomeratus* from *P. brassicae*; e.g. Hamilton 1935).

Trials were conducted using parasitized fifth instars (newly moulted), fed one of three different doses of the pathogen ($2{\cdot}50 \times 10^9$, $5{\cdot}00 \times 10^9$, or $1{\cdot}00 \times 10^{10}$ OBs), or a GV-free aliquot. Larvae were checked daily for death due to virus and/or parasitoid emergence, and if the check was positive for either, then the larvae were immediately dissected and the parasitoids within counted. Individual parasitoid larvae were counted as surviving if they successfully pupated.

Effect of parasitism on host susceptibility to infection by the virus

Parasitized and unparasitized samples of newly emerged first, second, third and fifth instars, and 48-h old fourth instars were fed virus as outlined above in the host-response experiments (doses given in Table 3). Hosts were checked daily for death due to the virus and/or emergence of the parasitoid.

Outcomes of competition between the granulosis virus and the parasitoid

To evaluate how the larval parasitoid and the virus compete within individual host larvae, four treatments were evaluated for each of instars 2 to 5: (i) control (no treatment), (ii) estimated LD_{95} of the GV (see Table 1), (iii) parasitoid, and (iv) estimated LD_{95} of the GV and parasitoid. Samples were freeze-killed (−20 °C) at 6, 7, 6 and 5 days post-inoculation for instars 2–5, respectively. Larval specimens that were in any way damaged so as to make full recovery (for weighing) of the specimen impractical, were discarded. Undamaged larvae were weighed in a frozen state to prevent loss of samples due to oozing of the GV from the host. Larvae in treatments (iii) and (iv) were subsequently dissected to confirm the presence of the parasitoid. Parasitoids from the fifth instar treatments (i.e. iii

TABLE 1. Summary of dosage–mortality responses of larvae of *Pieris brassicae* to infection by the granulosis virus.* Exponential notation abbreviated as E (e.g. $2{\cdot}54E4 = 2{\cdot}54 \times 10^4$)

	95% limits of LD_{50}			95% limits of LD_{95}			
Instar (n, d.f.)†	LD_{50}	Lower	Upper	LD_{95}	Lower	Upper	Slope (S.E.)
1 (198, 3)	1·71E2	1·36E2	2·10E2	9·39E2	6·32E2	1·86E3	2·23 (0·332)
2 (220, 3)	2·74E4	1·59E4	5·18E4	4·39E6	9·74E5	9·94E7	0·746 (0·137)
3 (374, 7)	1·53E6	1·01E6	2·35E6	2·33E8	6·55E7	2·51E9	0·754 (0·120)
4 (356, 6)	1·98E8	1·41E8	3·10E8	1·27E10	3·79E9	1·47E11	0·911 (0·161)
5 (206, 2)	1·39E9	1·82E8	2·43E9	2·01E10	1·14E10	1·65E11	1·42 (0·435)

* All regressions significant ($P < 0{\cdot}05$) and none of the slopes are heterogeneous ($P < 0{\cdot}05$). See Finney (1978) for interpretations of statistical tests in probit analyses.
† n = sample size, d.f. = degrees of freedom in probit analysis.

TABLE 2. Median lethal times (LT) and dry weights of first instar *Pieris brassicae* larvae subjected to high doses (in occlusion bodies) of the granulosis virus at 15 °C. Exponential notation abbreviated as E (e.g. $2{\cdot}54E4 = 2{\cdot}54 \times 10^4$)

Dose	Sample size (weighed)	LT median (range)*	Mean dry weight (mg) (S.E.)†
2·31E5	43 (32)	6 (6–11)	8·64E-2 (5·60E-3)[a]
6·93E5	42 (39)	6 (4–11)	9·01E-2 (4·80E-3)[a]
2·08E6	44 (42)	6 (4–10)	8·40E-2 (5·93E-3)[a]
6·25E6	38 (38)	5 (4–7)	6·34E-2 (2·58E-3)[b]

* Significant negative correlation between dose and life-span: $r_s = -0{\cdot}5657$, $P < 0{\cdot}001$.
† Means followed by different letters are significantly different, $P < 0{\cdot}05$.

and iv) were removed from the host, washed with distilled water, counted, pat-dried, and each brood weighed.

RESULTS

Host response to different doses of the granulosis virus

Table 1 presents the dosage–mortality responses of larvae of *P. brassicae* to infection by the granulosis virus. As hosts age, increasing doses of the GV were required to kill them (Table 1). Lethal doses (LD_{50}) differed by one to two orders of magnitude between any two consecutive instars; there was a ten-million-fold difference between the LD_{50} of first and fifth instars.

In agreement with the results of Payne, Tatchell & Williams (1981), within each instar, survival decreased significantly ($P < 0{\cdot}05$) with increasing dosage of the GV (Table 1). There was no apparent trend in the slopes of the regression lines of the probit plots.

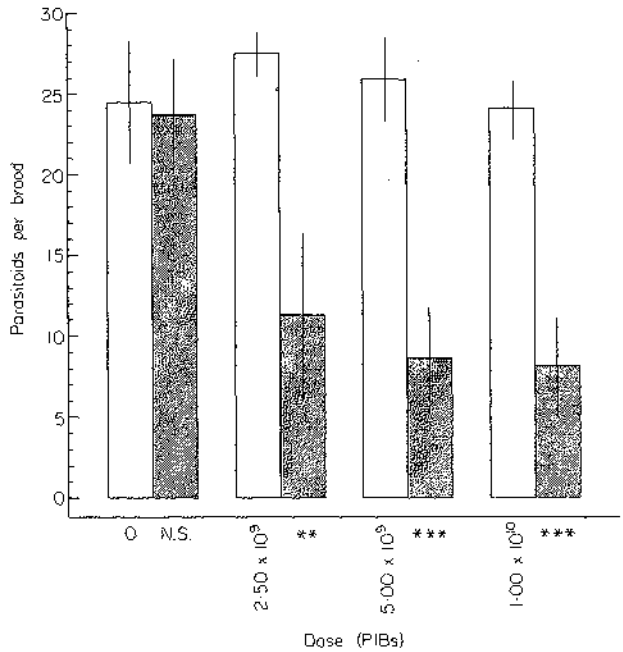

FIG. 1. Effect of virus on the survival of *Apanteles glomeratus* larvae in fifth instar *Pieris brassicae* hosts: control (no virus), $2{\cdot}50 \times 10^9$ OBs, $5{\cdot}00 \times 10^9$ OBs, and $1{\cdot}00 \times 10^{10}$ OBs, respectively; □ mean number of parasitoids in samples; ▨ mean number of parasitoids surviving in sample. Statistical comparisons (*t*-tests): N.S. = not significantly different at $P = 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

However, the slope of the regression of the first instar was significantly greater than those of instars 2, 3 and 4. This implies a more uniform response of the first instars as compared with older insects (Payne, Tatchell & Williams 1981).

Also in agreement with Payne, Tatchell & Williams (1981), increasing the virus dosage resulted in a decrease in the median time to death of the host. (Only treatments administered on the same day for any given instar were used in the analysis.) Significant negative correlations were detected for instars 1 ($r_s = -0{\cdot}2487$, $n = 88$, $P < 0{\cdot}05$), 2 ($r_s = -0{\cdot}2204$, $n = 102$, $P < 0{\cdot}05$), both groups of instar 3 ($r_s = -0{\cdot}2588$, $n = 75$, $P < 0{\cdot}05$; and $r_s = -0{\cdot}4453$, $n = 110$, $P < 0{\cdot}001$), and one of two groups of 4 ($r_s = -0{\cdot}3453$, $n = 36$, $P < 0{\cdot}05$; $r_s = 0{\cdot}0070$, $n = 98$, $P > 0{\cdot}05$). An insignificant correlation was found for instar 5 ($r_s = -0{\cdot}1346$, $n = 104$, $P > 0{\cdot}05$).

Effects of high doses of the granulosis virus

Table 2 shows the effects of virus dose on host longevity and on the dry weight of the host after succumbing to the virus. Consistent with the results of the host-response experiments, there was a significant negative correlation between the time to death and the dose administered ($r_s = -0{\cdot}5657$, $n = 167$, $P < 0{\cdot}001$). There is also a significant negative correlation between dose and the weight of the dying larvae ($r_s = -0{\cdot}3416$, $n = 151$, $P < 0{\cdot}001$). As suggested by these significant correlations, there were significantly positive correlations between the lethal time of infection and dry weight of the host for the three lowest doses ($r^2 = 0{\cdot}6540$, $n = 32$, $P < 0{\cdot}001$; $r^2 = 0{\cdot}7131$, $n = 39$, $P < 0{\cdot}001$;

TABLE 3. Effects of *Apanteles glomeratus* on the susceptibility of *Pieris brassicae* to its granulosis virus. Symbols: +, parasitoid present; −, parasitoid absent. Statistical comparisons: ? $0{\cdot}05 < P < 0{\cdot}1$; $*P < 0{\cdot}05$; $**P < 0{\cdot}001$. Exponential notation abbreviated as E (e.g. 2·54E4 = $2{\cdot}54 \times 10^4$)

	Sample size		Fraction dying of virus		Days to death median (range)	
Instar (dose)	+	−	+	−	+	−
1 (2·00E2)	27	40	0·741?	0·500?	6 (5–7)?	7 (5–7)?
1 (2·50E2)	35	37	0·743	0·595	6 (5–7)	6 (4–7)
2 (2·50E4)	45	44	0·244*	0·477*	7 (7–8)?	7 (7–8)?
2 (3·75E4)	33	41	0·818	0·683	7 (4–8)	8 (5–8)
3 (5·00E6)	45	47	0·933	0·851	8 (5–9)	8 (6–9)
4 (2·00E8)	29	12	0·828	0·833	8 (7–10)*	9 (8–11)*
5 (2·50E9)	8	49	0·625	0·673	7 (5–13)*	9 (7–13)*
5 (5·00E9)	14	46	0·714	0·739	7 (5–8)**	10 (6–14)**
5 (1·00E10)	17	39	0·824	0·947	7 (5–9)**	9 (5–13)**

$r^2 = 0{\cdot}3816$, $n = 42$, $P < 0{\cdot}001$, for low to high, respectively), but at the highest dose the correlation is not significant ($r^2 = 0{\cdot}0965$, $n = 38$, $P = 0{\cdot}058$).

Parasitoid survival in infected hosts

Even though the GV reduced the survival of the parasitoid as compared with controls (Fig. 1) the proportion of parasitoids within a brood surviving to pupation did not correlate significantly with virus dose ($r_s = -0{\cdot}0840$, $n = 39$; $P > 0{\cdot}05$; all larvae included). This is possibly because of the narrow range of the doses tested or a non-linearity in the response. Hosts harbouring both competitors showed no correlation between their life-spans and increases in virus dose ($r_s = 0{\cdot}1091$, $n = 28$; $P > 0{\cdot}05$).

The proportion of parasitoids surviving to pupation for the control did not correlate with number of the parasitoids within the brood (including survivors and non-survivors) ($r_s = -0{\cdot}030$, $n = 12$, $P > 0{\cdot}05$) and for the GV treatment at $2{\cdot}50 \times 10^9$ OBs ($r_s = 0{\cdot}0990$, $n = 8$; $P > 0{\cdot}05$). However, a significant positive correlation was detected at the dose of $5{\cdot}00 \times 10^9$ OBs ($r_s = 0{\cdot}7487$, $n = 14$, $P < 0{\cdot}01$), and a positive correlation at $1{\cdot}00 \times 10^{10}$ OBs just missed significance ($r_s = 0{\cdot}4130$, $n = 17$, $0{\cdot}05 < P < 0{\cdot}1$). These latter results suggest that parasitoids in larger broods have a better chance at survival to pupation than do those in smaller broods.

Furthermore, there were no significant correlations between time to death of the host (due to virus or parasitoid emergence) and the fraction of the parasitoid brood surviving to pupation for the control ($r_s = -0{\cdot}184$, $n = 12$, $P > 0{\cdot}05$) and at doses of $2{\cdot}50 \times 10^9$ ($r_s = -0{\cdot}1049$, $n = 8$; $P > 0{\cdot}05$) and $5{\cdot}00 \times 10^9$ ($r_s = 0{\cdot}0819$, $n = 14$; $P > 0{\cdot}05$); however, a significant positive correlation was detected at the highest dose of $1{\cdot}00 \times 10^{10}$ OBs ($r_s = 0{\cdot}7091$, $n = 17$, $P < 0{\cdot}01$). In this latter case, parasitoids were more likely to pupate from hosts which survived longer periods (see below).

Finally, a Kolmogorov–Smirnov goodness-of-fit test was employed to see if the survival of the parasitoid brood in GV-infected hosts was effectively 0% or 100%. Three classifications were employed: (i) all parasitoids survive, (ii) no parasitoids survive, or (iii) some, but not all parasitoids survive. Rejection of the null hypothesis 'H_0: some but not all parasitoids survive' would indicate that, when challenged with an infected host, either all

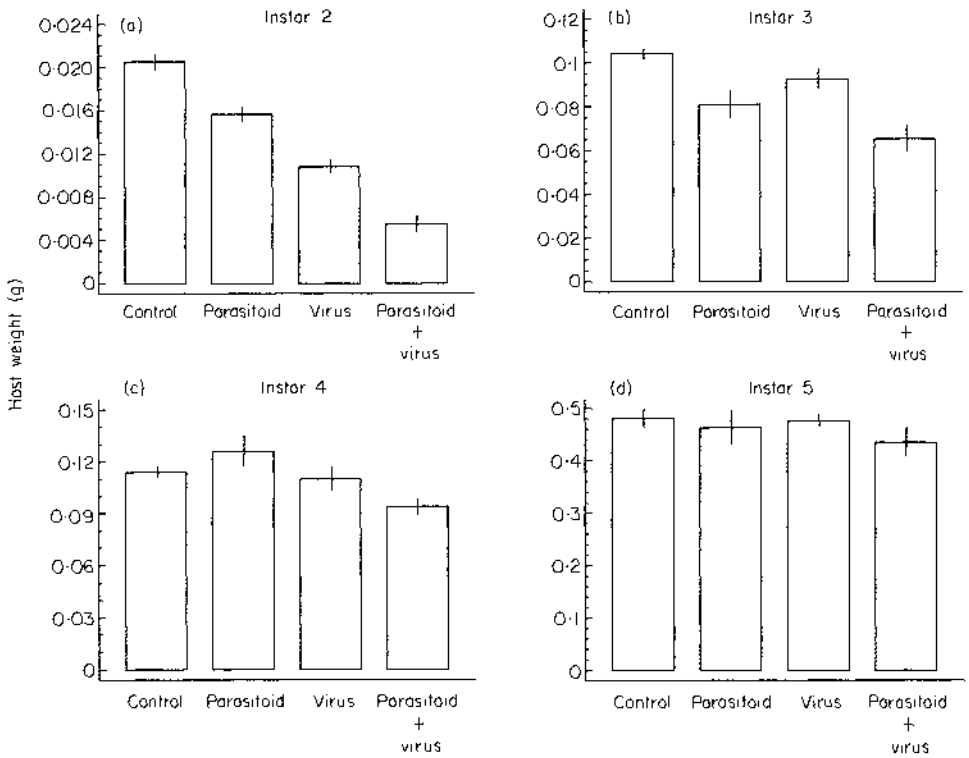

FIG. 2. Means and standard errors for weights (g) of *Pieris brassicae* larvae, subject to one of the following treatments: control, parasitoid, virus, and parasitoid + virus. Hosts are treated in either (a) instar 2; (b) instar 3; (c) instar 4; or (d) instar 5. See text for statistical comparisons.

of the parasitoids survive or none survive at all; however, this test does not specify the fraction of the parasitoid broods that fall into one or the other extreme. The test rejected the null hypothesis that parasitoid survival within the brood is intermediate to 0% and 100% ($d_{(8)}=4$, $P<0{\cdot}05$; $d_{(14)}=8$, $P<0{\cdot}001$; $d_{(17)}=10$, $P<0{\cdot}001$ for low to high doses (i.e. $2{\cdot}50\times10^{9}$, $5{\cdot}00\times10^{9}$, $1{\cdot}00\times10^{10}$), respectively, and $d_{(12)}=6$, $P<0{\cdot}01$ for the controls). In other words, essentially all parasitoids within a given brood either survived to pupation or died (failed to pupate); observations suggest that this depended on whether the host lived to begin spinning its pupal mat (a prerequisite for parasitoid emergence).

Effect of parasitism on host susceptibility to infection by the virus

Table 3 presents the fraction of larvae in a given sample dying from the GV and the median times to death for parasitized and unparasitized larvae. Only for one of the nine possible comparisons (i.e. dose $2{\cdot}50\times10^{4}$ given to instar 2) was there a significant decrease in the fraction of a larval sample succumbing to the infection when the parasitoid was present (Mann–Whitney test, $P<0{\cdot}05$). Note that at the other dose given to the second instars that a (non-significant) increase in susceptibility was observed. (The comparison for first instars at a dose of $2{\cdot}00\times10^{2}$ OBs just missed significance at $P=0{\cdot}0514$.) Although these results are inconclusive for the effect of the parasitoid on infection by the GV, once infected, older larvae which harboured the parasitoid lived for a significantly

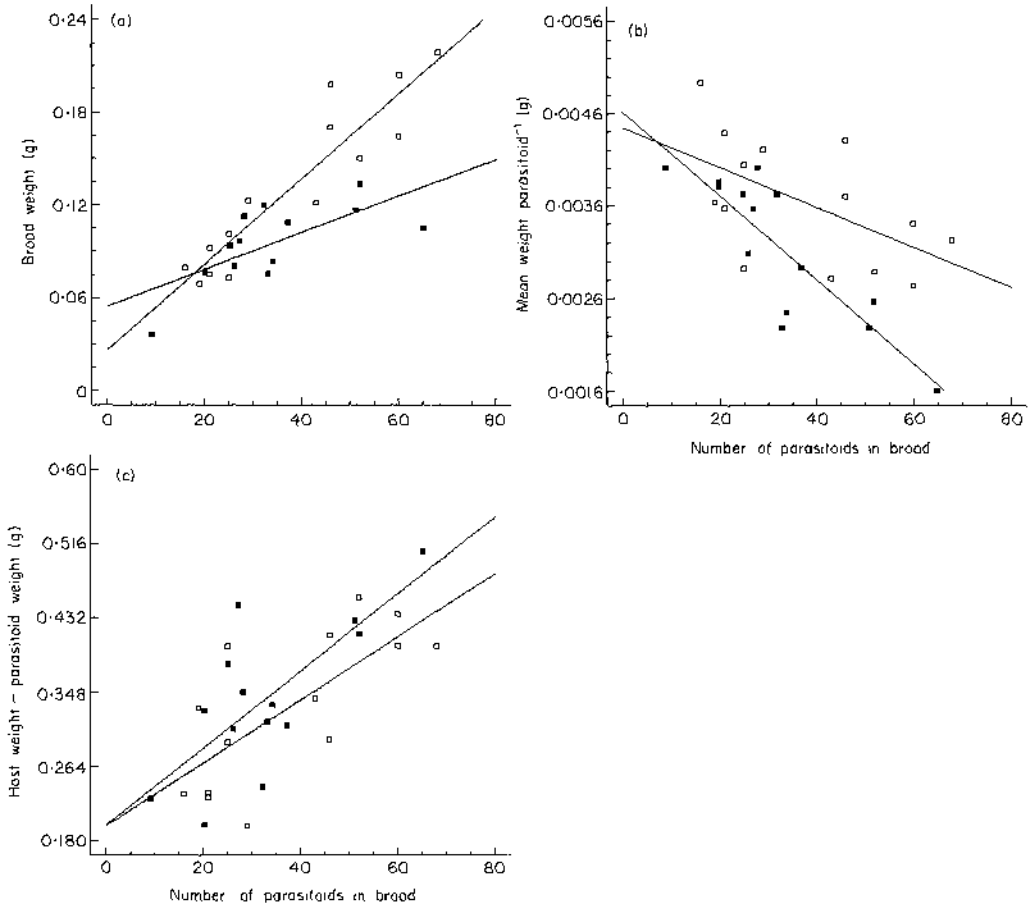

FIG. 3. Regressions of the size of the parasitoid brood on (a) brood weight ($Y=0{\cdot}00276X+0{\cdot}0266$ and $Y=0{\cdot}00118X+0{\cdot}0547$ for healthy and virus-infected hosts, respectively), □ $r^2=0{\cdot}8539$***, ■ $r^2=0{\cdot}4973$**; (b) mean weight per parasitoid larva ($Y=-0{\cdot}0000216X+0{\cdot}00444$ and $Y=0{\cdot}0000453X+0{\cdot}00461$), □ $r^2=0{\cdot}3153$*, ■ $r^2=0{\cdot}7215$***; and (c) remainder weight of host larva (wt with parasitoids − wt of parasitoid brood) ($Y=0{\cdot}00357X+0{\cdot}197$ and $Y=0{\cdot}00436X+0{\cdot}198$) □ $r^2=0{\cdot}5247$**, ■ $r^2=0{\cdot}5352$**. Healthy hosts (□) and granulosis virus-infected (■) hosts. *$P<0{\cdot}05$; **$P<0{\cdot}01$; ***$P<0{\cdot}001$. See text for other statistical comparisons.

shorter time than did unparasitized hosts (Mann–Whitney tests, $P<0{\cdot}05$, $P<0{\cdot}05$, $P<0{\cdot}001$, $P<0{\cdot}001$, for the fourth instar and for the three doses given to the fifth instar, respectively). The first instars given $2{\cdot}00\times10^2$ just missed showing a significant effect ($P=0{\cdot}069$) as did the second instars given $2{\cdot}50\times10^4$ OBs ($P=0{\cdot}058$).

Outcomes of competition between the granulosis virus and the parasitoid

Figure 2 shows the influence of the virus, the parasitoid, and both together on the weight of instars 2–5 of the host. The addition of the virus treatment resulted in significant decreases in larval weight compared to the controls for instars 2, 3 and 4 (two-way ANOVAS, $F_{(1,81)}=161{\cdot}96$, $P<0{\cdot}001$, Fig. 2a; $F_{(1,129)}=7{\cdot}70$, $P<0{\cdot}01$, Fig. 2b; $F_{(1,74)}=7{\cdot}34$, $P<0{\cdot}01$, Fig. 2c, respectively), whereas the parasitoid only did so for instars 2 and 3

($F_{(1,81)}=34{\cdot}11$, $P<0{\cdot}001$, Fig. 2a; $F_{(1,129)}=26{\cdot}67$, $P<0{\cdot}001$, Fig. 2b, respectively). Only in the fourth host instar was an interaction effect detected between the virus and the parasitoid ($F_{(1,74)}=4{\cdot}82$, $P<0{\cdot}05$, Fig. 2c). In the fifth instar neither of the natural enemies, nor a combination of them, had a significant effect on host weight (Fig. 2d).

Figure 3 shows the influence of parasitoid number on the weight of the parasitoid brood (Fig. 3a), the average weight per parasitoid larva (Fig. 3b) and the weight of the host less the parasitoid brood (Fig. 3c) in healthy and GV-infected hosts. Below, the statistics in the parentheses refer to healthy and GV-infected hosts, respectively ($n=14$ for all treatments). There was a significantly positive relationship between the number of parasitoids within a host and the total weight of the brood ($r^2=0{\cdot}8539$, $P<0{\cdot}001$; $r^2=0{\cdot}4973$, $P<0{\cdot}005$, Fig. 3a), negative relationship with the mean weight per parasitoid larva ($r^2=0{\cdot}3153$, $P<0{\cdot}05$; $r^2=0{\cdot}7215$, $P<0{\cdot}001$, Fig. 3b), and positive relationship with the weight of the host less the parasitoid brood ($r^2=0{\cdot}5247$, $P<0{\cdot}005$; $r^2=0{\cdot}5352$, $P<0{\cdot}005$, Fig. 3c). A similar positive relationship between the size of the *A. glomeratus* brood and weight of fifth instar *P. brassicae* individuals has been demonstrated by Fuhrer (1976) and Fuhrer & Keja (1976). (Note that the number of parasitoids did not differ between healthy and infected hosts ($F=0{\cdot}700$, $P>0{\cdot}05$).)

Furthermore, the presence of the virus significantly decreased both total brood weight (Fig. 3a) and average parasitoid weight (Fig. 3b) over cases in which the virus is absent, but these differences were only significant for the interaction terms (i.e. the slopes of the regressions differ but the intercepts do not) ($F_{(1,24)}=21{\cdot}2$, $P<0{\cdot}001$, Fig. 3a; $F_{(1,24)}=14{\cdot}6$, $P<0{\cdot}002$, Fig. 3b). In other words, the virus had a disproportionately larger negative effect on the size of larger parasitoid broods than smaller ones. Finally, hosts harbouring both virus and parasitoid had smaller remainder weights ($0{\cdot}341\pm0{\cdot}0234$ g) than did those infected by the GV alone ($0{\cdot}476\pm0{\cdot}0124$ g) ($F_{(1,26)}=28{\cdot}47$, $P<0{\cdot}001$). This was attributable to the presence of the parasitoid and not an effect of the presence or absence of the virus ($F_{(1,24)}=1{\cdot}75$, $P>0{\cdot}05$, Fig. 3c).

DISCUSSION

This study demonstrates that *A. glomeratus* and the granulosis virus of *P. brassicae* compete both intra- and interspecifically within larval hosts. The outcome of the competitive interaction depended on (i) the timing of infection by the GV, (ii) the number of infecting pathogens, and (iii) the number of parasitoids oviposited. Under no circumstances did the parasitoid emerge the clear victor; some GV was always produced by infected hosts, even in cases where the host died from the virus after the parasitoids had emerged.

Competition occurring prior to the emergence of the parasitoid larvae from the host was, on average, highly symmetrical. The parasitoid had a substantial impact upon the pathogen, reducing the potential amount of pathogen produced per fifth instar larva by 28% on average ($0{\cdot}341\pm0{\cdot}0234$ g with parasitoid vs. $0{\cdot}476\pm0{\cdot}0124$ g without). The pathogen also had a considerable effect on the parasitoid, even in cases where the parasitoid larvae survived to pupate from GV-infected hosts, reducing the weight of the parasitoid brood by an average of 29% after 5 days of infection by the virus. However, when the number of parasitoid larvae within the host were taken into account, this apparent symmetry between the competitors was lost: an individual parasitoid larva competed best when a member of a small brood, whereas the virus did best in hosts

harbouring larger parasitoid broods. It was brood size and not the presence or absence of the virus which determined this relationship. As the experiment was conducted only on hosts of a single age (i.e. newly moulted fifth instars), it cannot be concluded that this peculiar competitive relationship operates at other periods in the host's larval development.

Given that, when in competition with the virus, the per capita weight of the parasitoid larva was highest in small broods, but its chances of survival were highest when in large broods, it would appear that the parasitoid is best off when a member of an intermediate sized brood. It is interesting to note the typical clutch sizes observed for *A. glomeratus* (20–40 eggs per host, see leMasurier 1987) are near the minimum levels required for some or all of the brood to survive competition with the virus (data shown in Hochberg 1989).

The survival of the parasitoid within virus-infected hosts hinged on when the host succumbed to infection. If the host died prior to the construction of the pupal mat (the cue for the parasitoid larvae to emerge from and pupate outside of the host), then parasitoids rarely survived to pupation. In cases where the virus killed the host during or soon after the synchronous emergence of the parasitoid, most or all of the parasitoids survived. That the survival of the parasitoid brood is essentially an 'all-or-nothing' effect is in contrast to the findings of Levin, Laing & Jaques (1981) for competition between *A. glomeratus* and the GV within *Pieris rapae* larvae. They found that intermediate levels of emergence were possible as long as the infection followed the parasitoid by *c.* 4 days at 25 °C. The reason for the discrepancy between these two Pierid hosts is unknown, but may be due to an acceleration in the development time of the parasitoid within virus-infected as compared to healthy *P. rapae* (Levin, Laing & Jaques 1981).

Another aspect of the competitive interaction involved the influence of the presence in the host of one competitor on the successful parasitization or infection of the other. The results presented here suggest that the parasitoid had no significant effect on the ability of the pathogen to infect the host. There was, however, the potential for *A. glomeratus* to increase the host's susceptibility to the virus as the parasitoid is known to slow the host's development rate (Fuhrer 1976; Fuhrer & Keja 1976).

Although it is reasonable to assume that the production of virus by the host is proportional to the weight of the host tissue, it is not known precisely how the concentration, infectivity, or pathogenicity of the virus may have changed in the presence of competing virus particles or competing parasitoid larvae. One indirect way to gauge the impact of the parasitoid on the GV would have been to conduct bioassays of the liquefied contents of the host. For instance, if the parasitoid were to adversely affect the GV, say through preferential consumption of tissues normally attacked by the virus, then one would expect a diminished response of the host to bioassay treatments involving virus originating from parasitized hosts. In this vein, a recent study by Teakle & Byrne (1989) suggests that the production of a nuclear polyhedrosis virus in *Heliothis armigera* (Hübner) does not increase continually with host age at the time of infection, and that the infectivity of the progeny virus may be related to these sudden changes in production.

The consequences of the interactions explored here on field populations of the pathogen and the parasitoid are unknown. A recent theoretical exploration of the dynamics of host–parasitoid–pathogen interactions shows that within-host competition may critically determine the outcome of the competitive interactions between the natural enemies for the host (Hochberg, Hassell & May 1990). The possible outcomes of the interaction include (i) competitive exclusion of one natural enemy by the other, either depending upon or independent of initial densities, (ii) competitive coexistence at

constant densities, (iii) coexistence at periodically or chaotically fluctuating densities, or (iv) aperiodic invasions and extinctions of the pathogen. Inclusion of some of the intricacies of within-host interactions into mathematical models should shed further light on how the fine structure of competitive mechanisms may influence competition at the population level.

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