Effects of entomopoxvirus infection in *Pseudaletia separata* (Lepidoptera: Noctuidae) on the development of a pupal parasitoid, *Brachymeria lasus* (Hymenoptera: Chalcididae)

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Abstract

The effects of entomopoxvirus infection in *Pseudaletia separata* on the development of a pupal parasitoid, *Brachymeria lasus*, were investigated under laboratory conditions. Surviving fifth-stadium *P. separata* larvae infected with the *P. separata* entomopoxvirus (PsEPV) exhibited extended developmental time in the prepupal phase after molting to the last stage. Although about half of the PsEPV-infected larvae pupated, greater than half of those pupae were incompletely pigmented in the first abdominal segment and failed to develop into adults. Infection of *P. separata* pupae with PsEPV was detrimental and adversely affected the development of *B. lasus*. Parasitoid emergence was inversely related to prepupal duration. Significantly fewer parasitoids emerged from PsEPV-infected pupae, and these parasitoids spent an additional day or two in pupae with a longer prepupal phase. This lower rate of survival was due to a large number of parasitoid imagoes having died in the PsEPV-infected pupae, which was a function of prepupal duration. There was little or no growth in parasitoids developing in PsEPV-infected pupae with a longer prepupal phase.

Key words: *Brachymeria lasus*, *Pseudaletia separata*, entomopoxvirus, competition, host-parasitoid-pathogen interaction

INTRODUCTION

The ability of microbial control agents to infect non-target beneficial insects is undoubtedly the most important and potentially controversial issue in relation to the use of entomopathogens as microbial control agents. However, the interaction at a multitrophic level involving different natural enemies has not often been considered as a topic of research (Hochberg and Lawton, 1990). The growing importance of entomopathogens in biological control methods warrants an investigation of the likely effects of disease on parasitoids, a widely successful class of biological control agents. Interactions between insect parasitoids and entomopathogenic viruses are known to occur in simultaneous parasitism (Harper, 1986). Much of the previous work on host-parasitoid-entomopathogenic virus interactions has focused on hymenopteran larval

parasitoids in lepidopteran insects. However, literature on the effects of viral infections at the pupal stage of hosts on parasitoids is limited.

Brachymeria lasus (Walker) is a solitary polyphagous pupal endoparasitoid. *B. lasus* is distributed in the Pacific region that includes Japan, China, the Philippines, Vietnam, India, and Hawaii (Habu 1960; Husain and Agrawal, 1982). A female wasp lays a single egg in the host pupa. The developmental period from the egg through the larval stages to eclosion of the wasp lasts about 15 d at 25°C with a 16:8 (L:D) h photoregime (Mao and Kunimi, 1994a). Forty-five species of lepidopteran insects including the rice armyworm, *Pseudaletia separata*, have been recorded as hosts for this parasitoid (Habu, 1964; Kunimi, unpublished).

The rice armyworm is naturally controlled by an entomopoxvirus (EPV). *P. separata* entomopoxvirus (PsEPV) is considered to be an important natural mortality agent of this host in

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China (Dongrui et al., 1986). Entomopoxviruses are the most recently discovered group of occluded-type insect viruses and they occur in insect populations worldwide (Goodwin et al., 1991).

Entomopoxviruses have been used in trials to control insect pests (Entwistle, 1998), but the interaction of EPVs with non-target organisms is poorly documented and requires greater attention. The infection of pupae by some EPVs has been reported (Sutter, 1972; Retnakaran and Bird, 1972). Since there is no information regarding the interaction between pupal parasitoids and host pupae infected with entomopathogenic viruses, we studied the effects of entomopoxvirus infection in *P. separata* on the development of *B. lasus* under laboratory conditions. Such a vital information will be very essential for any biological control program involving these natural enemies and as a model for studying the effects of entomopathogenic viruses on pupal parasitoids. Several parameters were measured related to the development and survival of *B. lasus* progeny in PsEPV-infected *P. separata* pupae in the laboratory to establish the details of this parasitoid-pathogen relationship.

MATERIALS AND METHODS

Insects. The laboratory colony of *P. separata* was continuously maintained in our laboratory as described previously (Kyei-Poku and Kunimi, 1996, 1997). *P. separata* larvae were reared on an artificial diet (Hattori and Atsusawa, 1980) with minor modifications at a constant temperature of 25°C with a 16:8 (L:D) h photoregime. Three-day-old pupae were used for rearing the parasitoid and for the various experiments to be described.

The *B. lasus* used in this experiment was continuously maintained on *Homona magnanima* pupae in our laboratory (Mao and Kunimi, 1994a, b), but propagation was switched to *P. separata* pupae for five continuous generations before using them in the experiments. Wasps were collected within 24 h after eclosion, sexed, and then paired by placing them in a glass tube (30 mm diameter; 180 mm long) and fed a 10% sugar solution in cotton pads. The females were transferred into new glass tubes after a single

mating was observed. The life span of *B. lasus* adults fed on a 10% sugar solution is about 4 months, and their preoviposition period is ca. 7 days (Mao and Kunimi, 1994b). Thus, 14-day-old females were used in all the experiments, and only a single oviposition was permitted per host pupa.

Virus purification. The *P. separata* entomopoxvirus (PsEPV) used in these experiments was obtained from Dr. Hukuhara of our university and was propagated in host larvae in our laboratory. The viral occlusion bodies (OBs) were purified, and the concentration of OBs in the stock suspension was determined as indicated previously (Kyei-Poku and Kunimi, 1996, 1997). The stock suspension was stored at 4°C until use.

Virus inoculation. Fourth stadium larvae beginning to molt, as determined by head capsule slippage, were individually collected into 30-ml plastic cups without diet. Newly molted fifth instars within 24 h after molt were fed 125 mm³ (5 × 5 × 5 mm³) plugs of artificial diet (Hattori and Atsusawa, 1980) inoculated with 10 µl aliquots of purified suspension of PsEPV containing 10^{6.5} OBs/ml. This virus concentration was calculated to be the LD₅₀ based on larval mortality. The LD₅₀ dose of OBs was previously determined by a probit analysis. The control larvae were fed a diet plug treated with 10 µl sterilized distilled water. Larvae that completely consumed the treated diet within the administration period of 24 h were individually transferred into new 90-ml plastic cups, supplied untreated diet, and reared at 25°C with a 16:8 (L:D) h photoregime. The final stadium (sixth stadium) larvae that ceased to feed and became inactive were individually transferred into 90-ml plastic cups containing wet tissue papers for pupation. The larval developmental time as well as the duration of the prepupal period (the time elapsed between transfer of the quiescent larva to plastic cups and pupation) was recorded. In a preliminary experiment, 40% of pupae developed from PsEPV-infected larvae with symptomatic viral infection, as has been described by Melamed-Madjar and Raccach (1979).

Survival of *B. lasus* in PsEPV-infected pupae. Three-day-old pupae with varying

prepupal periods from the PsEPV-infected and uninfected larvae were dipped in 3% sodium hypochlorate solution, rinsed three times in deionized water, wiped dry, and subsequently exposed to *B. lasus* females. Washing ensured that the pupae were free of any possible virus traces from larval feces and regurgitation. Pupae used in all the experiments were treated similarly. High humidity has been observed to reduce the rate of parasitoid emergence (Welesoh, 1979). To eliminate this problem, the containers holding parasitized pupae were lined with filter paper to maintain a low relative humidity. Parasitized pupae were kept at 25°C with a 16:8 (L:D) h photoregime and were observed for parasitoid emergence. Twenty days after parasitization, pupae with no parasitoid emergence were dissected under a microscope to check for any remnants of the parasitoid. The tissues of these pupae were examined for the presence of viral occlusion bodies in a wet mount by phase-contrast microscopy.

Parasitoid offspring that emerged from PsEPV-infected and uninfected pupae were individually sexed. The sex ratio was calculated and expressed as the number of males/total number of emerging wasps. There were five replicates with more than 22 larvae (22–84; mean 55) for each treatment.

Growth rate of parasitoid larvae in PsEPV-infected pupae. To elucidate the effects of PsEPV infection of host pupae on the development of *B. lasus*, parasitized and PsEPV-infected pupae were dissected daily in physiological saline from day 1 after parasitization until 12 d following parasitization. Egg lengths and larval body lengths were measured using ocular micrometers in a dissecting microscope. The volume of each parasitoid egg or larva was calculated according to Brown et al. (1990). There were three replicates with 20 larvae for each treatment.

Statistical analysis. All data were analyzed by a one-way analysis of variance (ANOVA) and means were compared using Fisher's least significant difference (LSD). Frequency data were subjected to the arcsine square root-transformation before analysis.

Fig. 1. Cumulative pupation of *Pseudaletia separata* infected with *P. separata* entomopoxvirus (PsEPV). Newly molted fifth stadium larvae of *P. separata* were infected *per os* with PsEPV.

RESULTS

Developmental time of PsEPV-infected hosts

The effect of the PsEPV infection of *P. separata* on pupation rate is shown in Fig. 1. All uninfected and PsEPV-infected larvae molted to the sixth stadium. More than 90% of uninfected larvae pupated between 10 and 14 days after the fourth ecdysis (11.8 ± 0.9 ; mean $\pm$ SD), whereas 48% of the virus-infected larvae pupated between 11 and 16 days after the fourth ecdysis (13.2 ± 1.6 ; mean $\pm$ SD).

Several pupae that originated from PsEPV-infected larvae were found to be incompletely pigmented in the first abdominal segment (Fig. 2). No adults emerged from these pupae.

Emergence of parasitoids

The effect of PsEPV infection on the survival of *B. lasus* was monitored using pupae that developed from PsEPV-infected *P. separata* larvae.

More than 87% of parasitoids developing in the uninfected *P. separata* pupae emerged from their hosts (Table 1). The mean percentage for the emergence of parasitoids developing in PsEPV-infected pupae was significantly lower than that for parasitoids developing in uninfected pupae ($p < 0.05$), and the rate decreased with an increase in the prepupal period. The percent pupal mortality due to PsEPV increased with an increase in the prepupal period. The sex ratio of parasitoid offspring developing from PsEPV-infected pupae was significantly biased towards males compared with

Fig. 2. Uninfected (a) and entomopoxvirus-infected (b) pupae of *Pseudaletia separata*.

Table 1. Effect of entomopoxvirus infection of *Pseudaletia separata* pupae on percentage emerging from host and sex ratio in *Brachymeria lasus*

Prepupal period (days)	PsEPV infection ^a	Total no. observed	% pupae with parasitoid emergence ^b	% pupae without parasitoid emergence ^b	% pupae dead from PsEPV infection ^b	Sex ratio ^a (% male)
1	—	250	88.4±1.6 b	11.6±1.6 a	0	48.8±1.4 a
	+	336	63.7±3.4 a	26.7±3.9 b	9.6±0.6	59.1±2.8 b
2	—	250	87.2±0.8 b	12.8±0.8 a	0	47.5±1.4 a
	+	401	50.4±1.3 a	40.3±1.5 b	9.3±0.7	53.0±2.3 a
3	—	255	91.2±3.2 b	9.1±0.9 a	0	46.2±1.2 a
	+	398	48.4±1.6 a	41.5±1.5 b	10.1±0.5	54.7±1.3 b
4	—	147	93.3±2.2 b	6.7±2.2 a	0	44.7±4.1 a
	+	305	31.3±1.5 a	47.4±5.0 b	21.2±3.7	51.7±6.3 a
5	—	—	—	—	—	—
	+	217	31.0±2.2	43.2±3.6	25.8±2.9	52.0±6.5
6	—	—	—	—	—	—
	+	215	14.3±2.7	56.7±2.8	29.0±2.3	45.5±2.8

^a —, uninfected pupae; +, infected pupae.

^b mean ± SE (5 replicates).

Newly molted 5th-stadium *P. separata* larvae were orally infected by feeding pieces of artificial diet with PsEPV treated with 10 µl of PsEPV suspension (10^{6.5} OBs/ml). Three days after pupation, pupae were parasitized by *B. lasus* female. Means followed by the same letter showed no significant difference between uninfected and infected pupae by Fisher's LSD, *p* > 0.05.

that of parasitoid offspring developing from uninfected pupae (Table 1). Although the emergence of parasitoids developing in PsEPV-infected pupae with 5 and 6 prepupal periods

was delayed by about 1 to 2 days compared to those emerging from the uninfected pupae (Fig. 3, Table 2), there was no significant difference in the number of days required for emergence be-

Fig. 3. Cumulative percent emergence of *Brachymeria lasus* from uninfected and entomopoxvirus-infected pupae of *Pseudaletia separata*. PR1, PR2, PR3, PR4, PR5, and PR6 denote pupae with prepupal periods of 1, 2, 3, 4, 5, and 6 days, respectively.

tween the parasitoids which developed from PsEPV-infected pupae and those which developed from uninfected pupae ($p > 0.05$) (Table 2). Dead parasitoid imagoes were found in PsEPV-infected pupae without parasitoid emergence.

Development of parasitoids in pupae

Figure 4 shows the rate of parasitoid growth in pupae that developed from PsEPV-infected and uninfected larvae. The mean body size (volume) of parasitoids developing in PsEPV-infected pupae with a shorter prepupal period (1–3 days) did not differ significantly compared with that of parasitoids developing in the uninfected pupae ($p > 0.05$). However, the body sizes of parasitoids developing in PsEPV-infected pupae with a longer prepupal period (4–6 days) were significantly smaller than those of parasitoids developing in the uninfected pupae ($p < 0.05$). The observed parasitoid larvae were all alive.

Table 2. Days required for emergence of *Brachymeria lasus* from *Pseudaletia separata* pupae infected with *P. separata* entomopoxvirus (PsEPV)

Prepupal period (days)	PsEPV infection ^a	Total no. of pupae observed	Days from parasitization to emergence ^b
1	—	221	16.6 ± 0.09 a
	+	214	16.8 ± 0.09 a
2	—	218	16.6 ± 0.08 a
	+	202	16.8 ± 0.08 a
3	—	232	16.8 ± 0.07 a
	+	192	16.8 ± 0.09 a
4	—	138	16.8 ± 0.11 a
	+	95	16.8 ± 0.10 a
5	—	—	—
	+	66	17.8 ± 0.11
6	—	—	—
	+	31	17.8 ± 0.18

^a —, uninfected pupae; +, infected pupae.

^b Mean ± SE.

Means followed by the same letter showed no significant difference between uninfected and infected pupae by Fisher's LSD, $p > 0.05$.

Fig. 4. The growth rate of *Brachymeria lasus* developing in entomopoxvirus-infected pupae of *Pseudaletia separata* with various prepupal periods. PR1, PR2, PR3, PR4, PR5, and PR6 denote pupae with prepupal periods of 1, 2, 3, 4, 5, and 6 days, respectively. The vertical lines at each point indicate the standard error.

DISCUSSION

The inoculated dose of $10^{6.5}$ OBs/ml and subsequent infection extended the developmental time of the larval stage of *P. separata*.

Although about half of the PsEPV-infected larvae pupated, greater than half of those pupae were morphologically impaired and failed to develop into adults. Transstadial transmission of entomopathogens from larval to pupal stages has been reported in lepidopteran insects when infection takes place in the later larval stages (Sager, 1960; Stairs, 1965; Melamed-Madjar and Raccach, 1979; Young and Yearian, 1982, 1989). Our results corroborate those of previous studies, suggesting that when lepidopteran species are infected with some entomopathogenic viruses, the older the larvae is at the time of infection, the more likely it is to survive until pupation. Furthermore, it has been observed that sublethal infection of many insects with some entomopathogenic viruses leads to an extended developmental time in the larval stages (Sait et al., 1994; Goulson and Cory, 1995; Rothman and Myers, 1996; Volkman, 1997).

Morphological and textural differences between PsEPV-infected and uninfected pupae were presumed to have some, but minor, influence on pupal selection, as female wasps parasitize both PsEPV-infected and uninfected *P. separata* pupae (Kyei-Poku and Kunimi, unpublished). However, infection of *P. separata* pupae with PsEPV was detrimental and adversely affected the development of *B. lasus*. The adverse effects observed in this study were a retarded development of the parasitoid larvae and the failure of parasitoid imagoes to successfully emerge as adult wasps.

As has been reported by authors studying other combinations of pathogens and parasitoids (Hamm et al., 1985; Hotchkiss and Kaya, 1983; Eller et al., 1988; Nakai and Kunimi, 1997; Nakai et al., 1997), the developmental time of parasitoids developing in virus-infected hosts was retarded. Hotchkiss and Kaya (1983) have reported that the larval developmental times of *Hyposoter exiguae*, *Campoletis sonorensis*, and *Chelonus insularis* are longer in *Spodoptera exigua* and *Pseudaletia unipuncta* larvae infected with the Hawaiian strain of granulovirus (GV) or the hypertrophy strain of nucleopolyhedrovirus (NPV). They have suggested that the increase in larval developmental time can be attributed to insufficient nutrition in a host being exploited by a virus.

Nakai and Kunimi (1997) have reported that *Ascogaster reticulatus* larvae developing in GV-infected *Adoxophyes* sp. larvae accumulate numerous viral capsules in their gut lumen, which could prevent parasitoid larvae from absorbing sufficient nutrition. In this study, because PsEPV-infected pupae produced numerous viral occlusion bodies, *B. lasus* larvae developing in those pupae appeared to suffer from a nutritional imbalance.

Our data show that the mean percentage for the emergence of parasitoids developing in PsEPV-infected pupae is significantly lower than that of parasitoids developing in uninfected pupae, and that the rate decreases with an increase in the prepupal period. Previous reports have indicated that most parasitoids are unable to develop successfully in virus-infected hosts as a result of the premature death of their hosts (the host dies from the viral infection before the parasitoids complete their development), the production of substances that are toxic to the parasitoid larvae within their hosts, and the physiologically or nutritionally unsuitable nature of hosts infected with viruses for parasitoid development (Brooks, 1993). Even though these previous studies have used larval parasitoids, one of the above reasons may be applicable to the results obtained here.

The male-biased sex ratio of the progeny of *B. lasus* developing from PsEPV-infected pupae may have been due to differences in host quality between the PsEPV-infected and uninfected pupae. Laigo and Pasche (1968) have indicated that *Pteromalus puparum* exhibits an altered sex ratio when parasitized *Pieris rapae* larvae are infected with GV. Since hymenopteran parasitoids which have arrhenotokous reproduction can control the sex of each offspring by fertilizing or not fertilizing eggs that are laid, they often make offspring sex decisions according to the quality of the host resource (Waage, 1986; King, 1993; Luck et al., 1993).

This work is the first report of multitrophic interactions in relation to the possible effects of an entomopathogenic virus on the survival of a pupal parasitoid.

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