

Effects of a protease-expressing recombinant baculovirus insecticide on the parasitoid *Cotesia marginiventris* (Cresson)

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Abstract

A recombinant baculovirus insecticide, AcMLF9.ScathL, has been constructed that expresses a cathepsin L-like protease. This protease degrades the basement membrane of infected-lepidopteran larvae. AcMLF9.ScathL kills larvae significantly faster than the wild type baculovirus. Before field trials can be conducted with this virus, risk assessment studies are required to examine the potential impact of the virus on nontarget organisms. We examined the impact of AcMLF9.ScathL on the survival, development, and behavior of the parasitoid *Cotesia marginiventris* (Cresson) that parasitizes infected second instar larvae. Larvae of *Heliothis virescens* were exposed to the parasitoid and infected with the wild type or recombinant virus at >LD99 at 72, 96, or 120 h after parasitism. Control larvae were mock infected. Choice tests showed that infection with AcMLF9.ScathL did not affect the host preference of the parasitoid. At 72 h post-parasitism, the survival of parasitoids emerging from hosts infected with AcMLF9.ScathL was lower than for parasitoids emerging from hosts infected with wild type virus or uninfected controls. There were no significant differences between AcMLF9.ScathL and wild type virus treatments for the larval emergence time, adult emergence time, sex ratio, size, and fecundity of *C. marginiventris*. These results indicate that use of AcMLF9.ScathL in insect pest management poses a slightly greater risk to the parasitoid at early stages of parasitoid development than use of the wild type virus at >LD99 dose.

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1. Introduction

Wild type baculoviruses have been used for management of lepidopteran pests in cotton, vegetable crops, forest, and ornamental plants (Inceoglu et al., 2001; Moscardi, 1999). The commercial use of baculovirus insecticides has been limited however in part because of the relatively slow speed of kill compared to chemical insecticides (Inceoglu et al., 2001). Recombinant baculoviruses that express insect-specific toxins, hormones or enzymes have been constructed for improved insecticidal

efficacy (Bonning and Hammock, 1996; Bonning et al., 2002; Inceoglu et al., 2001). The infected insect dies from the effects of the expressed protein, and not from virus infection.

Before a recombinant baculovirus can be tested under field conditions, risk assessment studies are required to evaluate the potential risks posed by the recombinant baculovirus to nontarget organisms (Black et al., 1997). Beneficial insects, such as pollinators, predators, and parasitoids, may come into contact with the recombinant baculovirus under field conditions (Richards et al., 1998). Risk assessment studies on potential nontarget effects of a recombinant baculovirus expressing the scorpion venom-derived toxins *Androctonus australis* (AaIT)

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or *Leiurus quinquestriatus hebraeus* (LqhIT2) showed that the recombinant baculoviruses posed no greater risk to honey bees or predatory arthropods than the wild type virus (Heinz et al., 1995; Li et al., 1999).

Parasitoids and viruses compete for host resources. Baculoviruses do not replicate in parasitoids so any impact of virus infection of the host insect on parasitoids is indirect (Bonning et al., 2002; Gröner, 1990). McCutchen et al. (1996) found no adverse effects of recombinant baculoviruses expressing AaIT or juvenile hormone esterase (JHE) on the parasitoid *Microplitis croceipes* (Cresson). The parasitoid *M. croceipes* transmitted the recombinant baculoviruses to uninfected larvae in this laboratory study (McCutchen et al., 1996), although virus was not detected in parasitoids in field experiments (Smith et al., 2000).

A recombinant baculovirus AcMLF9.ScathL that expresses a basement membrane-degrading protease has been constructed. The ST50 and feeding damage of AcMLF9.ScathL-infected larvae were 49 and 20% lower, respectively, than those of wild type virus-infected larvae (Harrison and Bonning, 2001). Boughton et al. (2003) reported that there are no greater risks to predatory arthropods associated with use of AcMLF9.ScathL than with use of wild type virus. However, given that a parasitoid would be bathed in the protease, AcMLF9.ScathL may represent a greater risk to parasitoids than to predators. In addition, AcMLF9.ScathL-infected larvae melanize, which may alter parasitoid host choice, or affect emergence of parasitoid larvae from the infected host. The purpose of this study was to evaluate the effect of AcMLF9.ScathL on the behavior, survival, development, and fecundity of a model parasitoid, *C. marginiventris* (Cresson). We also evaluated the potential of the parasitoid *C. marginiventris* to transmit the recombinant baculovirus to other hosts.

2. Materials and methods

2.1. Viruses

Construction of the recombinant baculovirus, AcMLF9.ScathL, has been described previously (Harrison and Bonning, 2001). The parental virus, AcMNPV clone C6 (Possee, 1986), was used as the wild type virus control.

2.2. Virus amplification and purification

The recombinant and wild type viruses were amplified and purified as described previously (O'Reilly et al., 1992).

2.3. Insects

The parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) was maintained on larvae

of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Tobacco budworms, *H. virescens*, were reared from eggs purchased from AgriPest (Zebulon, North Carolina) at 27°C, 70% RH on *H. virescens* diet obtained from Southland Products (Lake Village, AR). All insects were maintained with a photoperiod of 14 h light, 10 h dark.

2.4. Choice tests

Two types of choice tests were conducted to determine whether infection with AcMLF9.ScathL affected parasitoid host preference. The first choice test was conducted to examine whether feeding *H. virescens* larvae on different food coloring dyes affected the preference of parasitoids for the “colored” hosts. The dyes were used in the second choice test to indicate which *H. virescens* larvae received which treatment (virus or control). We examined whether the virus treatment affected parasitoid host preference in the second choice test. Both choice tests were performed in the laboratory. Lighting was provided by fluorescent ceiling lights and the temperature was 23–26°C. The experimental arena consisted of 35 × 10 mm tissue culture dishes filled one third full with *H. virescens* diet. The dishes were then divided into three equal sized 120° sections. Prior to use in the first choice test *H. virescens* larvae were reared on different colored diets for 48 h. Red or blue food color dyes (2%; McCormick, Hunt Valley, MD) were added to the *H. virescens* diet during diet preparation. A single *H. virescens* larva reared on red diet was placed at the center of the first section, a larva reared on blue diet was placed at the center of the second section, and a larva reared on plain diet was placed at the center of the third section. A female parasitoid was then introduced into the center of each arena. The female was exposed to uninfected larvae for oviposition prior to being transferred to the choice test arena. Experimental arenas containing host larvae and one female parasitoid were observed until the first attack. The choice of larva for the first attack was recorded. The first color-preference choice test was repeated three times, with 20 female parasitoids per replicate.

The second choice test was conducted to evaluate the effect of virus infection on parasitoid host preference. The droplet feeding method of Hughes and Wood (1981) was used for infection of *H. virescens* larvae. Prior to being used in the choice tests *H. virescens* larvae were reared on different colored diets for 24 h and then starved for another 24 h before virus infection. The colored diet, which was visible in the larval gut, allowed for identification of larvae that received each virus treatment: *H. virescens* larvae reared on red diet were infected with wild type virus, larvae reared on blue diet were infected with AcMLF9.ScathL, and larvae on plain diet were mock infected. Infected larvae were

reared on colored diet for 24 h prior to being used in the choice tests. In each choice test arena, one *H. virescens* larva reared on red diet was placed at the center of the first section, a larva reared on blue diet placed at the center of the second section, and a larva reared on plain diet was placed at the center of the third section. A single female parasitoid was then introduced into the center of each arena. Experimental arenas containing host larvae and a single parasitoid were set up at two-minute intervals. Data were recorded for the first attack only. Once the first attack was observed, another experimental arena was set up with a different female parasitoid and other treated larvae. Three replicate experiments were conducted for a total of 20 female parasitoids per replicate. The dose used for each virus was $100 \times \text{LC50}$. The concentration used for the wild type virus, AcMNPV C6, was 4.34×10^6 occlusion bodies (OB)/ml and for the recombinant virus, AcMLF9.ScathL was 3.44×10^6 OB/ml. The LC50 dose of each virus was determined from the mean value of two replicate lethal concentration bioassays conducted in prior experiments (data not shown).

2.5. Survival and development tests

Experiments to examine the impact of AcMLF9.ScathL on survival and development of the parasitoid were based on methods described by McCutchen et al. (1996). Mated female *C. marginiventris* with oviposition experience were placed individually into petri dishes (35 mm \times 10 mm) with one second instar for each female. *H. virescens* larvae were infected with AcMNPV C6 or AcMLF9.ScathL at 72, 96, or 120 h after parasitism (5–10 larvae per treatment per time point) by using the diet cube inoculation technique as described by Boughton et al. (2003). The larvae were infected with >LD99 (5×10^4 OB) dose of NPV (McCutchen et al., 1996). Any larvae that died before parasitoid emergence were dissected to determine whether a larval parasitoid was present. Survival and development experiments were replicated four times. Host insects were not infected prior to 72 h post-parasitism because preliminary studies with viral treatments at 0, 24, and 48 h post-parasitism resulted in few or no parasitoids completing development to the cocoon stage in wild type virus- or AcMLF9.ScathL-infected hosts, respectively. Uninfected parasitized larvae served as controls. Emergence of parasitoid larvae from the host and emergence of parasitoid adults from cocoons were monitored at 12 h intervals. Survival of larval and adult parasitoids from all treatments was monitored. The head widths of surviving adult parasitoids from each treatment were recorded as an indication of parasitoid size by using an ocular micrometer. The sex ratio of adults from the survival tests was also determined.

2.6. Fecundity tests

In fecundity tests, parasitoid adults surviving from *H. virescens* larvae infected with virus (>LD99) at 96 h post-parasitism or from the control treatment were allowed to mate and each female was provided with five healthy second instar larvae of *H. virescens* for oviposition each day until death. *H. virescens* larvae were then housed individually and the number of parasitoid progeny recorded. Successful oviposition was quantified as emergence of the larval parasitoid from the host (McCutchen et al., 1996). Fecundity experiments were replicated twice.

2.7. Virus transmission

The host larvae from fecundity tests were monitored for evidence of virus infection by examination for virus symptoms (pale coloration or melanization). In addition, the progeny *C. marginiventris* adults from each treatment of the survival and development tests were stored at -20°C and analyzed for the presence of virus by using polymerase chain reaction (PCR). The DNA extraction method used was as described previously with slight modification (Noda et al., 2002). Individual parasitoids were homogenized in 10 μl STE buffer (100 mM NaCl, 1 mM ethylenediamine-tetraacetic acid, pH 8.0, and 10 mM Tris-HCl, pH 8.0) and digested with 0.05% proteinase K. Samples were boiled for 2 min and used as template for PCR. Detection of wild type virus was performed by using two primers for AcMNPV C6. The two primers were 603F1 5' GTA GTT CTT CGG AGT GTG TTG 3' and PH 5' GTG GGA CGG TAT GAA TAA TC 3' based on ORF603 and polyhedrin gene sequences, respectively. PCR conditions consisted of an initial denaturation step for 2 min at 95°C , followed by 45 cycles of denaturation at 95°C for 20 s, annealing at 54°C for 1 min and extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR for the detection of recombinant virus was performed by using two primers for AcMLF9.ScathL. The two primers were OScL335 5' CCA CCT ACA TTC CTC CAG CAC 3' and OScL822R 5' ATT GTA TAC ACC TTC GCT GTA C 3'. PCR conditions were the same as for AcMNPV C6, except for annealing at 55°C for 1 min. Viral DNA samples purified from insect cell culture medium from approximately 0.135 and 5 plaque-forming units of virus were used as positive controls for AcMNPV C6 and AcMLF9.ScathL, respectively.

2.8. Statistical analyses

The χ^2 test (SAS-Institute, 2002) was used to identify differences in parasitoid host choice. ANOVA and Tukey adjustment's means separation test (SAS-Institute, 2002) were used to analyze differences in parasitoid

survival, emergence times, and proportions of parasitized hosts. The survival, sex ratio, and fecundity data were transformed by arcsin square root before analysis. Means plus standard deviations were reported after they were back-transformed to their original form.

3. Results

3.1. Choice tests

The parasitoids observed in the color choice tests and virus treatment choice tests did not show any preference for a particular color or treatment (Fig. 1). The larvae did not exhibit any obvious symptoms from virus infection in these tests (24 h post-infection). The parasitoid behaviors observed were walking around the dish, approaching the hosts, drumming the hosts with antennae, and attacking the host by inserting the ovipositor into the host larva. Most of the parasitoids parasitized as soon as they came into contact with the hosts, and most attacked more than one host. Data for the three replicates of each choice test were pooled with 61 parasitoid females observed for the color treatment choice test and 60 for the virus treatment choice test. The choice tests did not show any differences in parasitoid preference

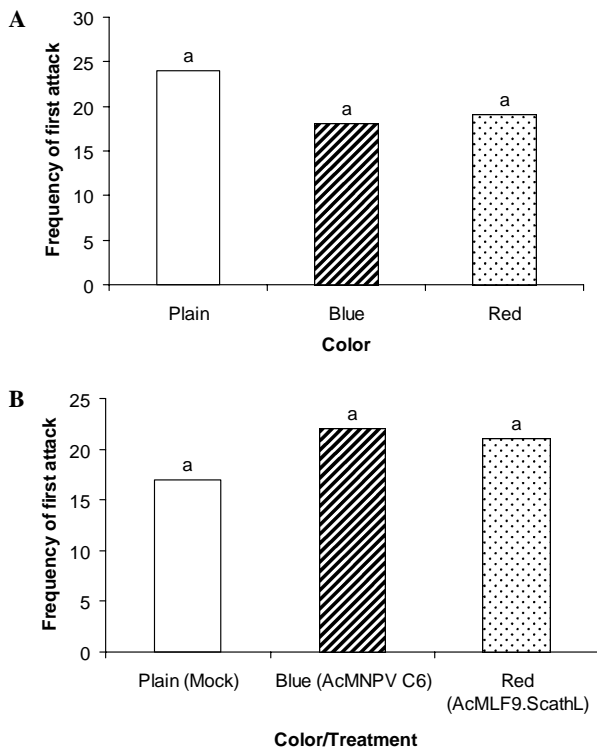


Fig. 1. Frequency of first attack in color (A) and color/treatment (B) choice tests for *C. marginiventris*. Data are based on 60 (A) and 61 (B) *C. marginiventris* attacks, pooled from three replicates. Columns with the same letter are not significantly different at the 5% significance level as determined by the χ^2 test.

between replicates or for pooled data for the hosts reared on blue, red or plain diet ($X^2=1.01$, $df=2$, $P=0.6016$; Fig. 1A). No preference was detected for uninfected *H. virescens* larvae or for larvae infected with the wild type virus or recombinant virus ($X^2=0.7$, $df=2$, $P=0.7047$; Fig. 1B).

3.2. Survival and development tests

Larvae of *H. virescens* were parasitized and then infected with virus (lethal dose >LD99) at 72-, 96-, 120-h post-parasitism. The emergence of parasitoids from the hosts was monitored. There was a significant difference in mean survival percentage of parasitoid larvae ($F=74.84$; $df=2,24$; $P=0.0001$) and adults ($F=93.88$; $df=2,24$; $P=0.0001$) among the treatments following virus infection at 72 h post-parasitism (Fig. 2). At 72 h post-parasitism, the number of survivors was significantly lower for parasitoids emerging from

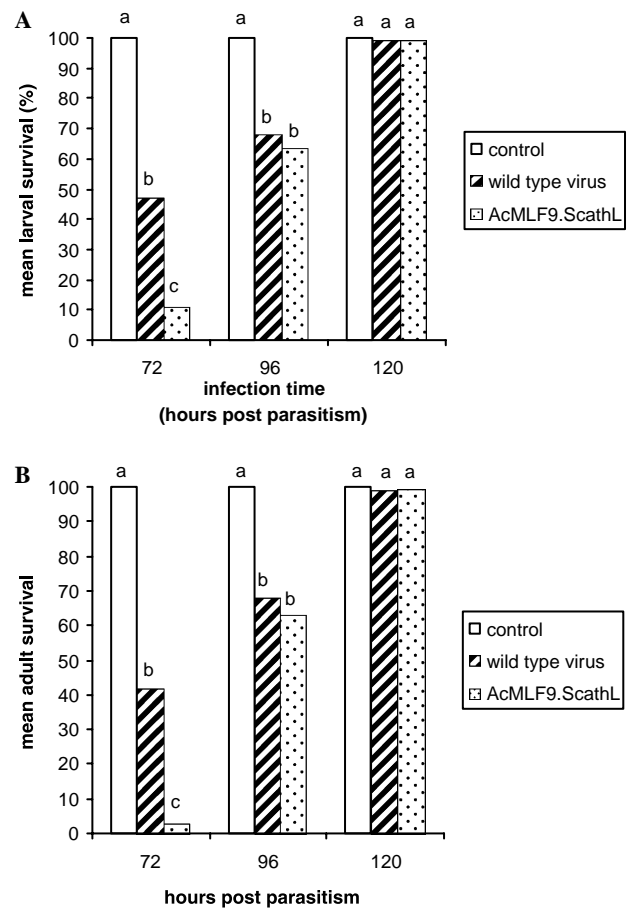


Fig. 2. Mean percentage survival of the larval (A) and adult (B) stage of *C. marginiventris* exposed to healthy hosts (control) or hosts infected at a high dose (>LD99) of wild type virus or AcMLF9.ScathL, at 72, 96, or 120 h post-parasitism. Columns with the same letter are not significantly different at the 5% significance level as determined by one-way ANOVA and the Tukey means separation test. Data were collected from four replicates with a total of 26–32 parasitoids per treatment.

Table 1
Development and sex ratio of *C. marginiventris* exposed as larvae to recombinant and wild type nucleopolyhedroviruses

Treatment	Mean time to larval emergence \pm SD in hours (<i>n</i>)	Mean time to adult emergence \pm SD in hours (<i>n</i>)	Mean pupal period \pm SD in hours (<i>n</i>)	Sex ratio (% males) (<i>n</i>)
Control (uninfected)	199 \pm 3a (81)	308 \pm 4a (81)	109 \pm 2a (81)	35a (81)
Recombinant AcMLF9. ScathL	195 \pm 3a (52)	304 \pm 4a (50)	108 \pm 2a (50)	43a (50)
Wild type AcMNPV C6	200 \pm 3a (63)	310 \pm 4a (60)	110 \pm 2a (60)	36a (60)

Numbers with the same letter within a column are not significantly different at the 5% significance level as determined by among treatment comparisons by using one-way ANOVA with Tukey adjustment's means separation. *n*, number of parasitoids. Experiments were conducted at 27 °C, 14 h light: 10 h dark.

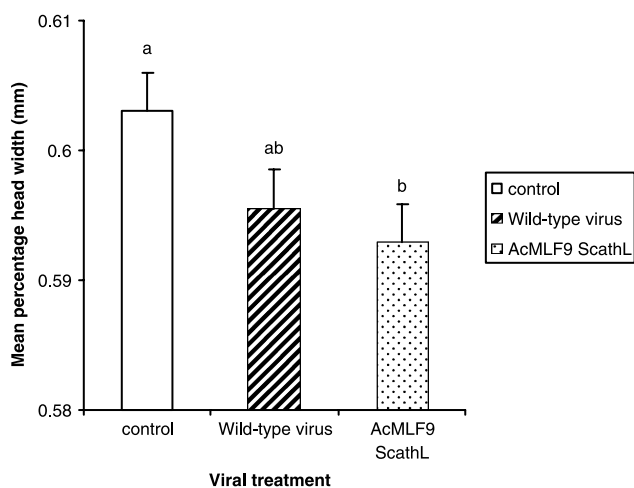


Fig. 3. Mean head width (mm) of adults of *C. marginiventris* surviving from hosts infected with wild type or recombinant virus. Adults from recombinant virus-infected hosts were significantly smaller than for the control group ($p = 0.05$). Columns with the same letter are not significantly different at the 5% significance level as determined by among treatment comparisons using one-way ANOVA with Tukey's means separation test. Data were pooled from four replicates with a total of 43–76 parasitoids per treatment.

recombinant virus-infected hosts than from wild type virus infected hosts and from uninfected controls. At 96 h post-parasitism, the mean percentage of larval and adult survival showed no significant difference between the wild type and the recombinant virus treatment, but was significantly lower than for control treatments. The mean percentage parasitoid survival showed no significant difference between the virus treatments and the control for virus infection at 120 h post-parasitism.

Our experiments showed no significant differences for mean time of larval ($F = 1.35$; $df = 2,190$; $P = 0.2626$) and

adult emergence ($F = 1.15$; $df = 2,185$; $P = 0.3186$), or pupal period ($F = 0.45$; $df = 2,185$; $P = 0.6398$) among the three treatments for infection at >LD99 (Table 1). There was no significant difference in the sex ratio of parasitoids that emerged from the different virus treatments ($F = 1.48$; $df = 2,28$; $P = 0.2447$). Parasitoids that emerged from the recombinant virus-infected hosts were not significantly smaller as determined by head width than those from the wild type virus infected-hosts (Fig. 3). Parasitoids emerging from the recombinant virus-treated hosts were significantly smaller than those emerging from uninfected larvae.

3.3. Fecundity

No significant difference was detected in fecundity between the parasitoids that emerged from the wild type virus-infected hosts and recombinant virus-infected hosts (Table 2; $F = 5.0$; $df = 2,29$; $P = 0.0180$).

3.4. Virus transmitted by parasitoids

Five percent and three percent, respectively, of hosts exposed to parasitoids that emerged from wild type virus- and recombinant virus-infected hosts died from virus infection (Table 2). The 431 bp-PCR product resulting from amplification of an AcMNPV C6 sequence was detected in 36% of the parasitoids that developed in AcMNPV C6-infected hosts. The 488 bp-PCR product was detected in 33% of the parasitoids that emerged from recombinant virus-infected hosts (Fig. 4). 25, 28, and 29 parasitoids were analyzed for uninfected, wild type virus- and AcMLF9.ScathL-infected treatments, respectively. During the course of these experiments, we observed that parasitized larvae

Table 2
Oviposition and virus transmission by parasitoids that emerged from virus-infected or uninfected hosts

Treatment	Number of parasitoid females	% of exposed hosts from which progeny parasitoids emerged (<i>n</i>)	% of exposed hosts killed by virus
Control (uninfected)	13	83a (324)	0
Recombinant AcMLF9. ScathL	11	66b (347)	3
Wild type AcMNPV C6	11	68b (293)	5

Numbers with the same letter are not significantly different at the 5% significance level as determined by among treatment comparisons using one-way ANOVA with Tukey adjustment's means separation.

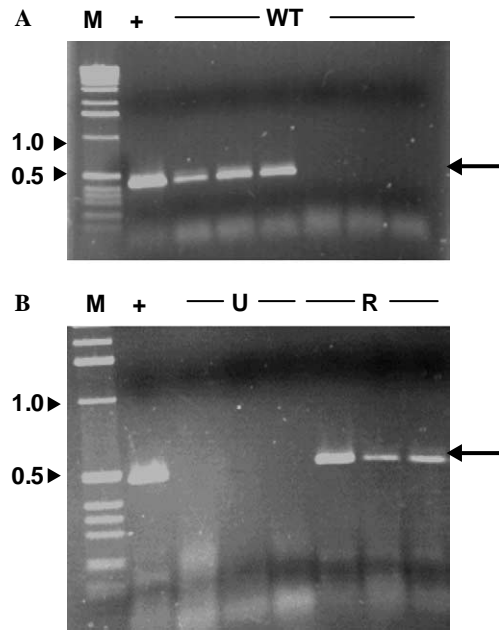


Fig. 4. Analysis of progeny adults of *C. marginiventris* for the presence of (A) AcMNPV C6 and (B) AcMLF9.ScathL by PCR. (A) M, 1 kb ladder; +, viral DNA extracted from 0.135 pfu of AcMNPV C6 (positive control). WT, six individual parasitoids from larvae infected with AcMNPV C6. The 431 bp PCR product (arrow) was detected in 36% of parasitoids that developed in AcMNPV C6-infected host larvae. (B) M, 1 kb ladder; +, viral DNA extracted from 5 pfu of AcMLF9.ScathL (positive control); U, three individual parasitoids from uninfected host larvae; R, three individual parasitoids from larvae infected with AcMLF9.ScathL. The 488 bp PCR product (arrow) was detected in 33% of parasitoids that developed in AcMLF9.ScathL-infected host larvae.

infected with AcMLF9.ScathL did not melanize (Fig. 5).

4. Discussion

4.1. Choice tests

Parasitoids use cues to assess the quality of potential hosts (Godfray, 1994). They attack the hosts by inserting their ovipositor to obtain additional information about host suitability, after externally examining the host by stroking or drumming with their antennae (Godfray, 1994). Some studies suggest that virus infection may affect parasitoid ovipositional behavior. Kyei-Poku and Kunimi (1997) suggested that the parasitoid *Cotesia kariyai* (Watanabe) might use sensory structures on the ovipositor to detect cues inside the host before making a decision to oviposit. In some cases, female parasitoids prefer to oviposit in uninfected hosts rather than virus-infected hosts (Versoi and Yendol, 1982; Caballero et al., 1991; Kyei-Poku and Kunimi, 1997; Hegazi and Abo Abd-Allah, 2004). However, in other cases no parasitoid preference was observed (Beegle and Oatman, 1975; Levin et al., 1983).

Harrison and Bonning (2001) noted that infection with AcMLF9.ScathL resulted in melanization of late instar larvae which might affect parasitoid ovipositional behavior. However, Boughton et al. (2003) observed that first and second instar larvae infected with AcMLF9.ScathL did not melanize completely prior to death, although they showed some dark mottling, indicating a low level of melanization. Our data show no effect of AcMLF9.ScathL infection on parasitoid preference or ovipositional behavior. The choice tests were done at 24 h post-infection, because 50% of AcMLF9.ScathL infected larvae were killed at 48 h post-infection (data not shown). The parasitoids may not detect the low level of melanization or physiological alterations caused by AcMLF9.ScathL infection. The implication is that *Cotesia marginiventris* will readily oviposit in hosts infected with AcMLF9.ScathL.

4.2. Survival and development tests

Host larvae infected by baculoviruses may die from virus infection before the parasitoids complete their development. Thus, the parasitoids may not survive (Brooks, 1993). Recombinant baculoviruses have been constructed to hasten the speed of kill relative to the wild type virus. McCutchen et al. (1996) showed that survival of parasitoids was not affected by recombinant baculoviruses expressing a scorpion-derived insect specific toxin (AcAaIT) or JHE (AcJHE.KK), even though the recombinant baculoviruses killed larvae 30–40% faster than the wild type virus. Their results also showed that parasitoid larvae emerged earlier from AcAaIT-infected hosts than from wild type virus infected hosts. These investigators suggested that the parasitoids might develop faster in response to declining nutrients in the host insect.

The survival time of AcMLF9.ScathL-infected larvae was reduced by 51% when compared to that of wild type virus-infected larvae (Harrison and Bonning, 2001). In addition, AcMLF9.ScathL killed *H. virescens* approximately 30% faster than recombinant baculovirus expressing the scorpion toxin, LqhIT2 (Harrison and Bonning, 2001). The rapid death of hosts infected with recombinant baculoviruses may impact parasitoid survival. The results of our study showed that the larval and adult survival of the parasitoids was deleteriously impacted by the recombinant virus relative to the wild type virus when the hosts were infected 72 h post-parasitism. Parasitoids did not survive if hosts were infected less than 72 h post-parasitism. In contrast to the results of McCutchen et al. with AcAaIT, there was no significant difference between the recombinant- and wild type virus treatment in time to larval or adult emergence. The low survival rate of *C. marginiventris* when hosts were infected 72 h post-parasitism may be caused by the inability of the parasitoids to complete their develop-



Fig. 5. Effect of parasitism on cuticular melanization of AcMLF9.ScathL-infected larvae. Nonparasitized (A–C) and parasitized (D–F) larvae of *H. virescens*: uninfected (A,D), infected with wild type AcMNPV C6 (B,E), or AcMLF9.ScathL (C,F). Note the absence of cuticular melanization in F relative to C. Larvae were infected at fourth instar and photographed four days post-infection. Bars: 3.5 mm.

ment within the AcMLF9.ScathL-infected hosts before the hosts died.

Kyei-Poku and Kunimi (1998) observed retarded growth of the parasitoid *C. kariyai* in entomopoxvirus-infected larvae. They also found that the parasitoid died inside the virus-infected host before host death. Dissection of the dead *H. virescens* infected with AcMLF9.ScathL at 72 h post-parasitism indicated that the parasitoid larvae were still alive and had developed to second or third instar. These observations suggest that the protease does not harm the parasitoid directly, because the parasitoid larvae were still able to

develop to the later instars. Our results indicate that AcMLF9.ScathL infection at 72 h post-parasitism results in indirect adverse effects on parasitoid survival.

On oviposition, some hymenopteran endoparasitoids (including *Cotesia* spp.) inject polydnviruses into the host insect (Shelby and Webb, 1999). Polydnviruses inhibit the immune response of the host thereby allowing the parasitoid to develop within the host without detection and inactivation (Shelby and Webb, 1999). Host inactivation of invading organisms results from encapsulation with hemocytes and melanization (Lavine and Strand, 2002). Given that the protease

ScathL induces melanization of AcMLF9.ScathL-infected larvae, it will be interesting to determine whether the presence of the polydnvirus alters the physiological effects induced by ScathL. If the presence of the polydnvirus reduces the impact of ScathL on the host insect, this may explain why no significant difference was detected in emergence times between wild type and recombinant virus-infected hosts. Indeed, parasitized larvae infected with AcMLF9.ScathL exhibited no melanization in contrast to unparasitized larvae.

The sex ratio and the size of parasitoids emerging from infected hosts may be affected by a recombinant baculovirus. Kunnalaca and Mueller (1979) reported that *C. marginiventris* female development takes slightly longer at 25 °C, although the development times for both sexes were the same at 30 °C. Thus, the sex ratio of parasitoids may change if the virus kills the hosts before most females complete their development. However, we found no difference in the sex ratio of parasitoids between treatments. McCutchen et al. (1996) also did not find significant differences in the sex ratio or fecundity of parasitoids emerging from recombinant virus or wild type virus treatments at 27 °C. McCutchen et al. (1996) found that parasitoids that emerged from AcAaIT- and AcJHE.KK-infected hosts were significantly smaller than those from the uninfected hosts, and that parasitoids developing in AcAaIT-infected hosts were significantly smaller than those from wild type virus infected hosts. In contrast, the parasitoids that emerged from AcMLF9.ScathL-infected larvae were similar in size to those from the wild type virus-infected larvae. As the protease ScathL disrupts the basement membrane, it may increase the availability of nutrients for the developing parasitoid in the hemolymph. Hence, reduced resources available to the parasitoid as a result of virus infection may be countered in part by the activity of the protease. Indeed, larvae of the green lacewing, *Chrysoperla carnea*, appeared to benefit from the increased availability of nutrients from the proteolytic action of ScathL in AcMLF9.ScathL-infected prey (Boughton et al., 2003).

4.3. Fecundity tests

The size of the adult female parasitoid may influence fitness and reproductive success by affecting searching efficiency, longevity, or egg production (Godfray, 1994). McCutchen et al. (1996) found that parasitoids that emerged significantly earlier from the recombinant virus-infected larvae were smaller than for the uninfected control group. However, there was no difference in fecundity or percentage parasitized hosts among parasitoids emerging from recombinant virus infected hosts, from wild type virus infected hosts, and from uninfected hosts (McCutchen et al., 1996). Our results showed that there was no significant difference in fecundity of para-

sitoids emerging from wild type virus- and AcMLF9.ScathL-infected hosts. The protease ScathL expressed by AcMLF9.ScathL does not affect the reproductive success of female parasitoids that develop in infected hosts. Fewer progeny were produced by parasitoids that emerged from virus infected larvae compared to the uninfected control larvae however. This may result from competition between the parasitoid and virus for resources, such that parasitoids from virus-infected larvae have fewer resources for production of progeny.

4.4. Virus transmission

Female parasitoids can carry baculoviruses on the ovipositor (Beegle and Oatman, 1975). Eller et al. (1988) reported that the parasitoid *M. croceipes* did not transmit HzSNPV from infected larvae to uninfected larvae via oviposition. They suggested that the inability of the parasitoids to transmit the virus was caused by the brief duration of oviposition and the short ovipositor. However, by using PCR McCutchen et al. (1996) recorded virus transmission by *M. croceipes* from wild type NPV, AcAaIT, and AcJHE.KK treatments with 4.0, 10.6, and 8.3% infection of the uninfected larvae. They also reported that approximately 40% of parasitoids that developed in AcAaIT-infected hosts carried the virus (McCutchen et al., 1996). However, in a field experiment none of the parasitoids tested positive for viral DNA (Smith et al., 2000).

Our study showed that *C. marginiventris* transmitted virus to uninfected larvae. Virus was detected by PCR analysis in approximately 33 % of parasitoids that developed in AcMLF9.ScathL infected hosts and in 36% of parasitoids that emerged from wild type virus infected hosts. Although a relatively crude method was used for extraction of DNA in these experiments, this approach was sufficiently sensitive for detection of <1 plaque-forming units (pfu) of AcMNPV C6 and 5 pfu of AcMLF9.ScathL. Five percent and three percent of *H. virescens* larvae exposed to female *C. marginiventris* that developed in wild type virus and AcMLF9.ScathL treatments, respectively, died from virus infection. In other cases, the virus dose transmitted by the parasitoids is assumed to have been sufficiently low that the parasitoid was able to outcompete the virus. The overall rate of virus transmission was not determined. These results confirm that parasitoids may carry and transmit recombinant baculoviruses to other host larvae.

Taken as a whole, our results indicate that use of AcMLF9.ScathL in insect pest management may pose a slightly greater risk to parasitoids at early stages of development than use of the wild type virus if host larvae are heavily infected. However, the degree of exposure of parasitoids under field conditions may not be as high as in this worst-case scenario study, such that the actual risk is likely to be negligible.

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