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Interaction of calyx fluid and venom from *Microplitis croceipes* (Braconidae) on developmental disruption of the natural host, *Heliocoverpa zea*, and two atypical hosts, *Galleria mellonella* and *Spodoptera exigua*

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Abstract

Polydnaviruses of many braconid and ichneumonid endoparasitoids play an important role in the successful parasitism of their hosts. The host's development is altered and its immune response is also suppressed. In this study, we compared the effects of calyx fluid and venom on the development of the natural host, *Helicoverpa zea*, and two atypical hosts that the parasitoid does not normally attack in nature, *Galleria mellonella* and *Spodoptera exigua*. The levels of calyx fluid and\or venom injected was 0.05, 0.1 and 0.2 female equivalents (FE)/larva. In *H. zea*, calyx fluid significantly reduced larval growth on day 5 post injection. Venom alone did not affect larval growth but it synergized the action of calyx fluid by reducing growth earlier and for a longer period after injection. Other effects of calyx fluid on the host, either alone or in combination with venom, were an increase in developmental period, and a reduction in percent emergence and weight of adult moths. The percentage of *H. zea* larvae that pupated was not affected by calyx fluid or venom. In *Galleria mellonella*, venom alone reduced larval growth comparable to calyx fluid and both tissues induced the effects on day 1 post injection. Other effects caused by calyx fluid or venom alone or the combination were a reduction in percent pupation and emergence, and the average adult weight. In *S. exigua*, high mortality occurred when 4th instar larvae were injected. Although the injection of larger fifth instars reduced overall mortality, the sham-injected larvae only gained weight during the first 24 hours after injection (from day 0 to day 1). However, adults were produced at all doses of calyx fluid or venom. The effects of the virus on development in this species were a prolongation of the larval stage and reduction of adult weight by calyx fluid in combination with venom. In conclusion, injections of calyx fluid and venom of *Microplitis croceipes* can differentially affect the growth and development of its natural host *H. zea*, and atypical host, *G. mellonella*, but only a minimal effect was observed in *S. exigua*. Published by Elsevier Science Ltd.

Keywords: Parasitoid; Braconidae; Calyx fluid; Venom; Noctuidae

1. Introduction

The survival of endoparasitoids in insects depends on multiple factors including biochemical and hormonal interactions and on suppression of the host's immune system. Suppression of immunity is modulated by complex mechanisms to help the parasitoids develop successfully (Lavine and Beckage, 1995). Certain braconid and ichneumonid endoparasitoids have developed a unique association with polydnaviruses which replicate in the calyx epithelium of the lateral oviducts (Stoltz and Vinson, 1979; Fleming, 1992). Calyx fluid, containing polydnavirus, and venom are injected into the host along with the parasitoid egg, and are important factors in the suppression of the host immune system. These factors are known to alter the physiology of the host (Vinson and Iwantsch, 1980; Coudron, 1991; Fathpour and Dahlman, 1995; Summers and Dib-Hajj, 1995).

Atypical hosts have been used in place of the natural host in rearing beneficial ectoparasitoids in order to reduce rearing costs (Maltby et al., 1973; Greany, 1991;

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Bratti, 1993; Rojas et al., 1995). This raises the question of what effects the virus of an endoparasioid has on an atypical host, in cases where the parasitoid successfully developed and emerged. Recently, expression of a polydnavirus injected into a natural host and an atypical host was reported within the tissues of the former but not the latter host (Hayakawa et al., 1994).

Six species of Lepidoptera were evaluated as candidate hosts for *in vivo* rearing of *Microplitis croceipes* (Blumberg and Ferkovich, 1995). All six atypical host species were acceptable for oviposition after treatment with frass and hemolymph from *Heliocoverpa zea* (Ferkovich and Blumberg, 1994). The parasitoid developed successfully only in *Galleria mellonella* and *Spodoptera frugiperda* (Blumberg and Ferkovich, 1995); however, the rates of parasitoid adult emergence were lower and the sizes of the adults were smaller than those reared on *H. zea* (Gupta et al., 1996a). The addition of supplemental nutrients to the *G. mellonella* diet did not improve development and emergence of *M. croceipes* (Gupta et al., 1996b). The lack of egg encapsulation and the successful emergence of *M. croceipes* from *G. mellonella* suggests at least partial suppression of this host's immune system. In contrast, in the atypical host *S. exigua, M. croceipes* eggs all were encapsulated, indicating ineffective suppression of the host's immune system. These findings raise the question of whether the polydnavirus associated with the parasitoid is more effective in suppressing the immune system of *G. mellonella* than of *S. exigua* which thus allowed low numbers of the adult parasitoids to emerge in *G. mellonella*.

The objective of this study was to evaluate the effects of calyx fluid and venom from *M. croceipes* on the growth and development of the atypical hosts *G. mellonella* and *S. exigua*, as compared with their effects on the natural host, *H. zea*.

2. Materials and methods

2.1. Host and parasite colony maintenance

H. zea was reared according to Lewis and Burton (1970) at the USDA-ARS, Insect Biology and Population Research Laboratory, Tifton, GA. We received eggs by mail and allowed them to hatch on *Heliothis* Premix diet® (Stonefly Industries, inc., Bryan, TX). *G. mellonella* was reared according to Bean and Silhacek (1989). *Microplitis croceipes* was reared as described earlier (Ferkovich and Dillard, 1986).

2.2. Preparation of calyx fluid and venom

Calyx fluid and venom were collected from four day old M. croceipes females. Generally, 20 females were anesthetized on ice and then surface sterilized by immersion in 70% ethanol for one minute. The ovipositor was gently pulled with forceps and the reproductive tissues were placed into a drop (25 μl) of Pringle's saline (pH 7.2) on a glass slide held on ice. The calyces (ovaries and oviduct) were dissected from the tissues and transferred to a 100 μl drop of Pringle's and gently punctured with forceps to release the contents into the saline and then transferred into an Eppendorf microfuge tube on ice. Similarly, the venom glands and reservoir were dissected into a 100 μl drop of Pringle's, then transferred into a disposable 1.5 ml sterile microfuge tube and opened by gently shearing with a plastic micro mortar and pestle (Kontes Biotechnology, Vineland, NJ). The tubes were stored on ice for no more than 3–5 min and then centrifuged at 800 **g** for 15 min. The crude supernatants of calyx fluid and venom were saved, appropriately diluted, and mixed in microfuge tubes for expression in wasp equivalents.

2.3. Injections

Immediately after collection of calyx fluid and venom, three doses of calyx fluid venom or calyx plus venom of 0.05, 0.1, or 0.2 female equivalents (FE) were injected in volumes ranging from 2 to 6 μl into the proleg of the host larva's sixth abdominal segment. Injections were performed with a microinjector (Instrumentation Specialties Company, Inc. Lincoln, NB) fitted with a 0.25 ml insulin syringe with a 35 gauge, stainless steel needle. Prior to injection larvae were relaxed with CO_2; bleeding was minimal. Fresh preparations of calyx fluid and venom were used for each of the replicates for each experiment. In each set of experiments, 10 to 15 larvae were injected on day 0 and three replicated experiments were carried out over a period of several weeks and the data were pooled. The treatments also included a control (Pringle's saline injection). Injected larvae were placed in 28 cc plastic cups on standard diets and were weighed each day until they pupated. Adults were weighed on the day of emergence.

The injections were performed in two sets of experiments. In the first set of experiments, calyx fluid and venom were injected separately into 4th instars of *H. zea*, and 5th instars of *G. mellonella* and *S. exigua* weighing 0.032 ± 0.002, 0.026 ± 0.001 and 0.073 ± 0.006 g SD/larva, respectively. Fifth instars of *S. exigua* were used because of the high mortality rate that resulted with 4th instars after injections. In our colony of *S. exigua*, larvae that stopped feeding prior to wandering had an average weight of 0.216 ± 0.008 g.

In a second set of experiments, calyx fluid and venom were injected in combination. To avoid a high rate of mortality from the injections, larvae of each species were a day older and weights for *H. zea*, *G. mellonella*, and

S. exigua were 0.068 ± 0.003, 0.055 ± 0.005, and 0.098 ± 0.006 g SD/larva.

2.4. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and treatment means were compared against control means by Dunnet's procedure using SAS (SAS Institute, Cary, NC).

3. Results

3.1. Effects of calyx fluid and venom in H. zea

3.1.1. Larval growth

Growth of *H. zea* larvae was affected by calyx fluid on day five after injection (Fig. 1A). As the larvae approached pupation, which began at 9–11 days, this effect of virus was diminished. Venom alone did not affect growth; however, it enhanced the effect of calyx fluid by inducing an earlier reduction in growth on day one after injection and by extending the inhibitory effects through to day nine.

3.1.2. Developmental responses

Mortality and pupation were not affected by calyx fluid, venom or the combination of the two materials. The developmental period (from injection to pupation) and the emergence of adult moths was significantly reduced by calyx fluid alone and with venom. The average weight of adult moths was reduced by the combination of calyx fluid and venom.

3.2. Effects of calyx fluid and venom in G. mellonella

3.2.1. Larval growth

Growth of *G. mellonella* was significantly prevented as early as day one through day five relative to the control larvae by both calyx fluid and venom (Fig. 2A and B). By day seven, the sham-injected control larvae had either died or pupated. The combination of calyx fluid plus venom reduced growth on day one through to day nine (Fig. 2C). The control larvae in this experimental set did not begin pupating until after day eight so that a significant reduction in growth was observed for an additional four days longer than the larvae shown in Fig. 2A and B). The reduction of growth relative to that sham injected control larvae in Fig. 2C), therefore, could not be attributed to enhancement by the venom.

3.2.2. Developmental responses

Pupation and emergence were reduced by calyx fluid alone and by calyx fluid with venom at all three doses, and the size of adults was reduced at all three treatments

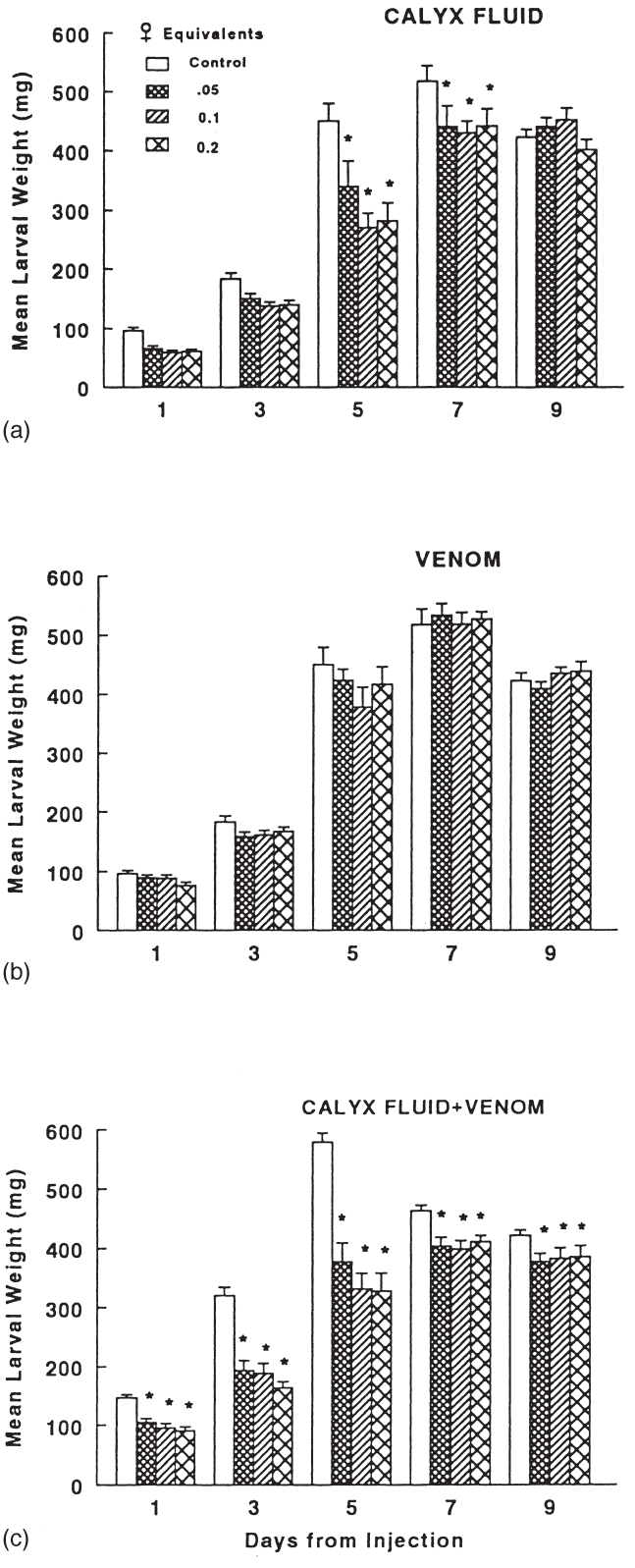

Fig. 1. Mean daily weights (mg ± SE) of *Heliocoverpa zea* larvae injected with female equivalents of *Microplitis croceipes* calyx fluid and venom separately and in combination. Control larvae (sham) were injected with Pringle's saline. Injections were done on day 0. *Significantly different from the control at $P < 0.05$.

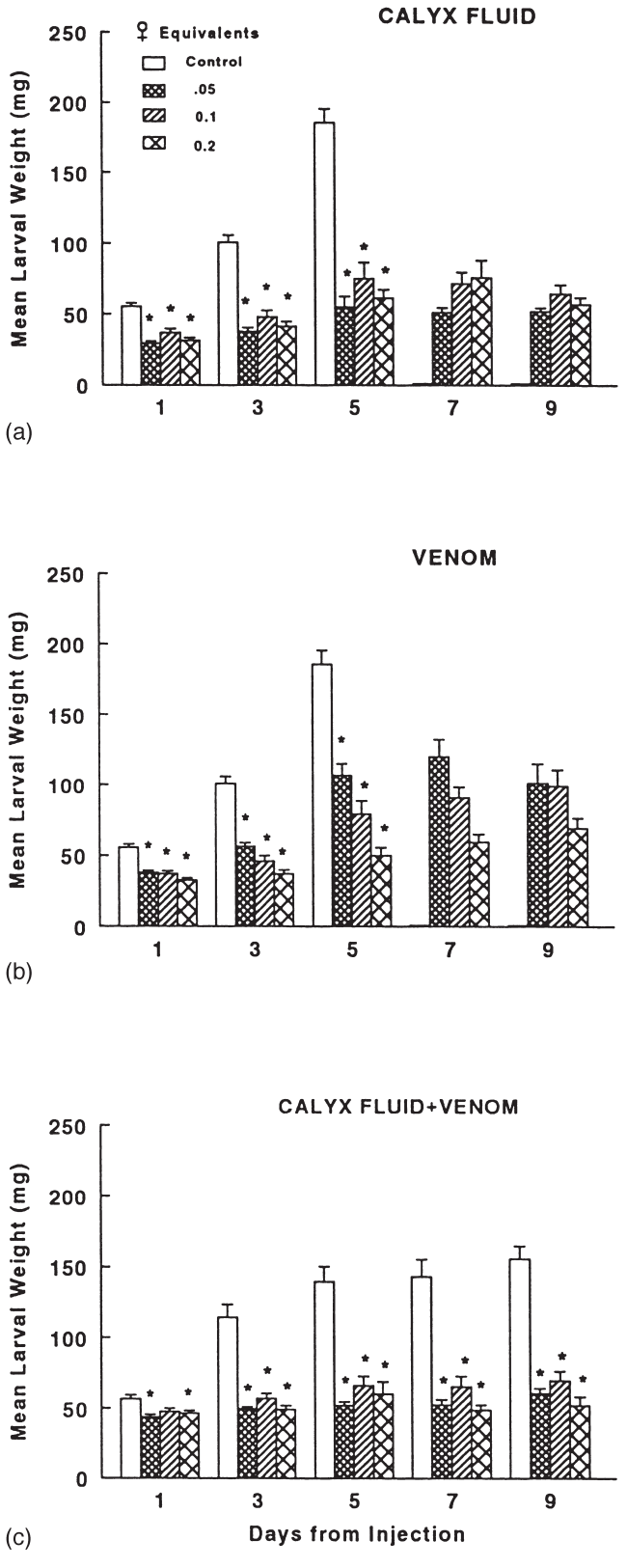

Fig. 2. Mean daily weights (mg ± SE) of *Galleria mellonella* larvae injected with female equivalents of *Microplitis croceipes* calyx fluid and venom separately and in combination. Control larvae (sham) were injected with Pringle's saline; data for the calyx fluid and venom treatments on days 7 and 9 ($n = 4$ and $n = 2$, respectively) are not presented because most of the larvae pupated. Injections were done on day 0. *Significantly different from the control at $P < 0.05$.

at specific doses. Pupation was also altered by venom at 0.2 FE. Adult emergence was completely inhibited at 0.02 FE of calyx fluid plus venom.

3.3. Effects of calyx fluid and venom in S. exigua

3.3.1. Larval growth

The sham-injected control larvae only gained weight during the first 24 hrs after injection (day 0 through to day 1). The larvae then started pupation after day four. None of the three treatments had a significant effect on growth although there appeared to be a small dose-dependent effect on day one.

3.3.2. Developmental responses

The effect of the injections on the developmental responses was minimal in *S. exigua*. A significant delay in pupation occurred at the 0.1 and 0.2 FE of calyx fluid plus venom. Adult weight was significantly reduced at the 0.1 FE dose of the combination of fluids.

4. Discussion

Previously, we successfully reared *M. croceipes* on *G. mellonella*, but not on *S. exigua*, both of which are atypical hosts (Blumberg and Ferkovich, 1995). One explanation of this result is that the polydnavirus was partially effective in suppressing the immune system *of G. mellonella*, but not effective in *S. exigua* since all of the oviposited eggs were encapsulated in parasitized *S. exigua* larvae. Thus, we hypothesized that the polydnavirus associated with *M. croceipes* affects the development of the natural host, *H. zea*, but was partially effective in *G. mellonella*, and ineffective in *S. exigua*. Our results clearly showed that injections of venom alone had no effect, but calyx fluid alone and in combination with venom reduced growth and development in the natural host, *H. zea*, and that these effects were similar to the effects of the parasitoid on host larvae (Blumberg and Ferkovich, 1995). Thus, our results with calyx fluid and venom of *M. croceipes* and *H. zea* are in agreement with earlier reports on braconid parasitoids and their natural hosts. In general, braconids with polydnavirus all reduce the growth and development of their typical host and prolong the larval stage. Furthermore, the virus is generally synergized by venom while venom alone rarely has

an effect (Vinson et al., 1979; Tanaka and Vinson, 1991; Fleming, 1992; Dushay and Beckage, 1993; Fathpour and Dahlman, 1995; Fleming, 1992).

In the atypical host, *G. mellonella*, calyx fluid, venom, and calyx fluid plus venom reduced larval growth, percent pupation, and adult weight and prolonged the developmental period from injection to pupation. Moreover, calyx fluid alone or injected with venom prevented emergence. However, additional injections of calyx fluid plus venom in combination with parasitism did not improve emergence of parasitoids from *G. mellonella* (unpublished data). We have no explanation of how the injections of calyx fluid plus venom in the absence of parasitoid may alter the growth and physiology of atypical hosts, but it is reported that the virus may be interfering with endocrine or metabolic functions (Beckage, 1985; Davies et al., 1987; Lawrence, 1991; Strand and Noda, 1991; Strand and Dover, 1991; Fleming, 1992). Similar observations are made when polydnavirus from *Cotesia congregata* are injected into larvae of *M. sexta* (Beckage et al., 1994). Thus, these studies and our findings reported here support the conclusion that our earlier success in rearing *M. croceipes* on *G. mellonella* (Ferkovich and Oberlander, 1991; Ferkovich et al., 1994; Blumberg and Ferkovich, 1995) was due not only to the host meeting the nutritional needs of the parasitoid, but also to the ability of the polydavirus to partially suppress the host's immune response.

The effects of the injections on the growth of atypical host, *S. exigua*, were inconclusive since the sham injected control larvae only grew from the day of injection (day 0) to day one and thereafter did not gain additional weight through day four when the insects began pupation. Specifically, fifth instar larvae weighed an average of 73 mg in the first set of experiments (Fig. 3A and B) and 98 mg in the second of experiments (Fig. 3C) at the time of injection. By 24 hours after injection (day 1), the control larvae weighed an average of 130 to 150 mg and thus should have been capable of further growth. In the colony of *S. exigua* used in this study, larvae that stopped feeding prior to wandering weighed an average of 216 mg. However, the combination of calyx fluid plus venom had a significant effect on the host development at the two highest doses (0.1 and 0.2 FE) by increasing the developmental period from injection to pupation and also reducing the weight of the adult moths at the intermediate dose (0.1 FE).

Since the calyx fluid of *M. croceipes* does have an effect, although minimal, on the development of *S. exigua*, these results do not support the hypothesis that *M. croceipes* cannot successfully develop in *S. exigua* because the host is refractory to the virus of parasitoid (Blumberg and Ferkovich, 1995). In related studies, the virus of *Campolitis sonorensis* is not compatible with *Trichoplusia ni* larvae which results in poor protection of the parasitoid's eggs and subsequent death of both

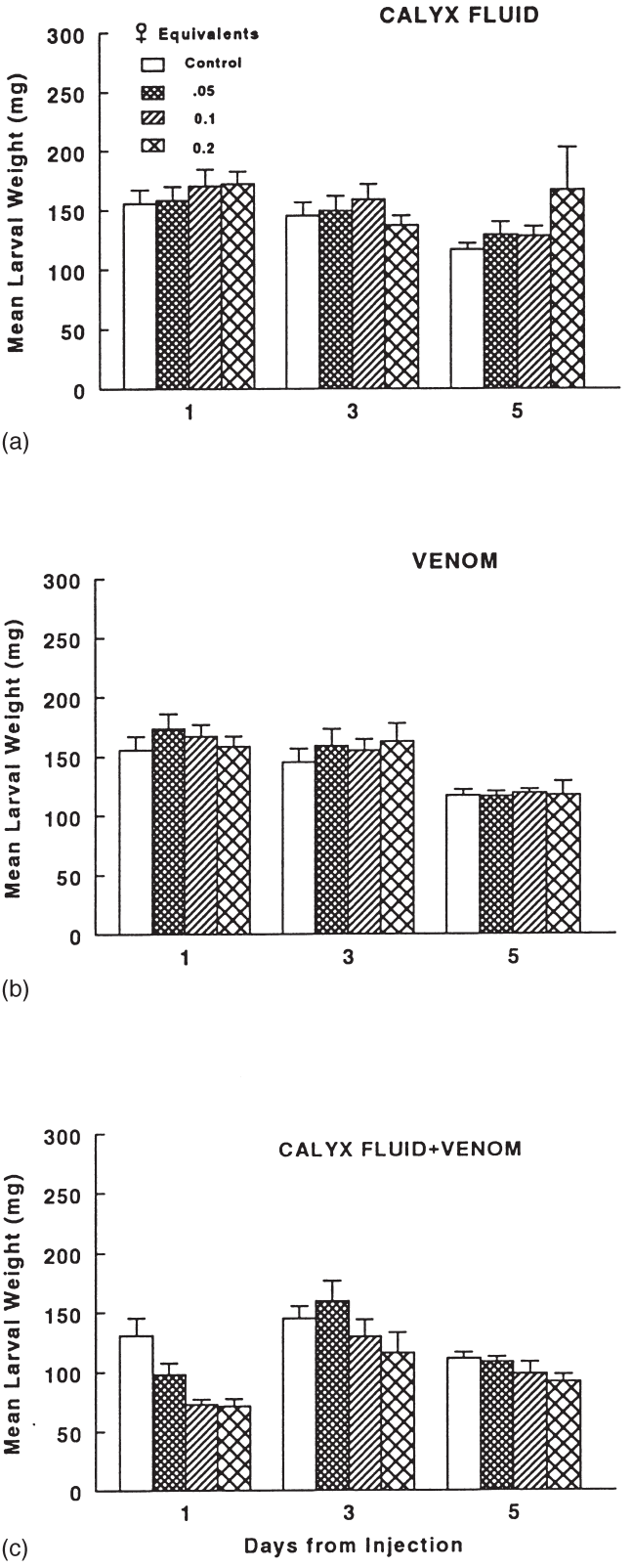

Fig. 3. Mean daily weights (mg ± SE) of *Spodoptera exigua* larvae injected with female equivalents of *Microplitis croceipes* calyx fluid and venom separately and in combination. Control larvae were injected with Pringle's saline. Injections were done on day 0. *Significantly different from the control at $P < 0.05$.

hosts and parasitoid larvae (Vinson and Stoltz, 1986). Vinson and Scott (1974) report that eggs of *Cardiochiles nigriceps*, undergo different surface structural changes in the natural host, *H. virescens* from those oviposited into an unsuitable host, *H. zea*, and that 90% of the eggs are encapsulated within 24 hrs in the latter species. Vinson (1977) also finds that polydnaviruses from *M. croceipes* also confers protection to eggs of *C. nigriceps in H. zea*.

In conclusion, these data indicate that calyx fluid and venom from *M. croceipes* can differentially interfere with the growth and development of the atypical hosts, *G. mellonella* and *S. exigua*, in addition to its natural host, *H. zea*.

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