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Parasitism by *Microplitis demolitor* Induces Alterations in the Juvenile Hormone Titers and Juvenile Hormone Esterase Activity of its Host, *Pseudoplusia includens*

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***Microplitis demolitor* is a polydnavirus-carrying parasitoid that attacks the larval stage of *Pseudoplusia includens* and other noctuids. Parasitism or injection of wasp components like polydnavirus and teratocytes has a juvenilizing effect on *P. includens* development. Here we measured hemolymph juvenile hormone (JH) titers and juvenile hormone esterase activity in *P. includens* larvae after parasitism or injection of wasp components. Results were compared to nonparasitized larvae. During the fifth stadium, JH titers of nonparasitized larvae fluctuated between 0.08 and 0.50 ng/ml, whereas titers in parasitized larvae never fell below 2 ng/ml. *P. includens* larvae injected with calyx fluid plus venom or calyx fluid plus venom and teratocytes exhibited JH titers intermediate between parasitized and nonparasitized larvae. Nonparasitized larvae exhibited two peaks of JH esterase activity. The first occurred between 12 and 48 h in association with cessation of larval feeding, while the second occurred immediately before pupation. In contrast, levels of JH metabolism remained at low levels in parasitized larvae and larvae injected with calyx fluid plus venom or calyx fluid, venom and teratocytes. Only trace amounts of JH were detected in *in vitro* assays of teratocytes or *M. demolitor* larvae suggesting neither released JH into the hemolymph of parasitized larvae. Although calyx fluid plus venom and teratocytes did not elevate JH titers to the levels measured in parasitized larvae, their effects on formation of larval–pupal intermediates by *P. includens* were similar to those generated by exogenous application of methoprene or JH.**

Parasitoid Endocrine Polydnavirus Lepidoptera

INTRODUCTION

Microplitis demolitor (Hymenoptera: Braconidae) is a solitary, larval endoparasitoid of the soybean looper *Pseudoplusia includens* and several other noctuid moths (Shepard *et al.*, 1983). *P. includens* undergoes five stadia before pupating, and *M. demolitor* successfully parasitizes the second through fifth stadia (Strand *et al.*, 1988). Larvae parasitized prior to the ultimate stadium exhibit reduced weight gain, larval–larval molts at subthreshold sizes, and do not pupate. Larvae parasitized in the fifth stadium also exhibit reduced weight gain, but never undergo a supernumerary molt or exhibit any characteristics associated with initiation of wandering or metamorphosis (Strand *et al.*, 1988; Strand and Dover, 1991). Such individuals are referred to as developmentally arrested.

At oviposition *M. demolitor* introduces several factors into hosts that may play a role in causing these alterations (Strand *et al.*, 1988; Strand and Dover, 1991). These include *M. demolitor* polydnavirus (MdPDV) and a proteinaceous venom produced in the poison gland. MdPDV replicates in the calyx region of the wasp's ovary and is stored in the lumen of the lateral oviducts, forming a suspension of virions and protein called calyx fluid. Calyx fluid and venom are then coinjected into the host along with an egg at oviposition (Strand *et al.*, 1992; Strand, 1994). The *M. demolitor* egg also produces teratocytes which are a specialized group of cells derived

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from the serosal membrane that envelops the parasitoid during embryogenesis. These cells are liberated, at hatching, into the host's hemocoel where they increase in size and persist until the wasp larva completes its development (Strand and Wong, 1991). Venom alone has no measurable effect on host development, whereas injection of calyx fluid, purified MdPDV and/or teratocytes induce dose-dependent alterations including supernumerary molting, formation of larval–pupal intermediates, or developmental arrest (Strand and Dover, 1991; Strand and Wong, 1991; Dover *et al.*, 1995).

Collectively, these changes indicate that *M. demolitor* has a juvenilizing effect on its hosts. Other larval endoparasitoids have also been reported to alter development of their hosts (summarized by Beckage, 1985; Lawrence and Lanzrein, 1993). In some experimental systems, arrested development or production of larval–pupal intermediates is associated with both increased production and decreased metabolism of juvenile hormone (JH) (Beckage and Riddiford, 1982; Hayakawa, 1990; Zhang *et al.*, 1992). Reciprocally, some parasitoids induce precocious metamorphosis of their hosts by causing endocrine changes similar to those observed in the ultimate stadium (Jones, 1985; Grossniklaus-Buergin and Lanzrein, 1990). Here we report that parasitized *P. includens* exhibit an elevated hemolymph JH titer and greatly reduced metabolism of JH relative to nonparasitized hosts. Metabolism of JH was similarly reduced in larvae injected with calyx fluid plus venom or calyx fluid plus venom and teratocytes. JH titers in these hosts were higher than than those of nonparasitized larvae but lower than those of parasitized larvae.

MATERIALS AND METHODS

Insect rearing, staging, and collection of wasp factors

M. demolitor was maintained at 27°C as described in Strand *et al.* (1988). *P. includens* larvae were reared in 30 ml plastic cups filled with artificial diet at 27°C and a photoperiod of 16L : 8D (Strand, 1990). Adult moths and wasps were fed a 20% honey solution. Nonparasitized *P. includens* larvae were physiologically staged by previously described methods (Strand *et al.*, 1988; Strand, 1990). The first–fourth stadia are each 2 days long with larvae feeding on the first day and undergoing apolysis and ecdysis on the second day. The duration of the fifth stadium is 4 days with larvae feeding from approx. 1–44 h, wandering behavior at 50–60 h, cocoon spinning from 60–72 h, and pupating at approx. 92 h. All times within stadia were measured in hours after ecdysis to the stadium.

Calyx fluid and venom were collected by established methods (Strand and Noda, 1991; Strand, 1994), while teratocytes were collected from *M. demolitor* eggs cultured *in vitro* (Strand and Wong, 1991). Briefly, *M. demolitor* eggs were collected by dissecting parasitized hosts at 24 h after parasitism. Eggs were hatched in 50 μl drops of Excell 400 medium (JRH Scientific). The teratocytes were then separated from the parasitoid larva and cultured for 24 or 120 h in Excell 400 medium using 96-well culture plates (Corning). Since an average of 600 teratocytes are produced per egg (Strand and Wong, 1991), we referred to injection of this number of cells into a host as injection of one wasp equivalent.

For all experiments, *P. includens* larvae were parasitized by *M. demolitor* at 1–3 h of the fifth stadium. Hosts were parasitized individually in petri dishes to avoid superparasitism. For injections, larvae were anesthetized with CO_2 and injected through a proleg using a glass needle mounted on a micromanipulator. Larvae were injected with 0.1 wasp equivalents of calyx fluid plus venom or 1.0 wasp equivalent of teratocytes. Larvae injected with calyx fluid plus venom only were injected at 1–3 h of the fifth stadium. Larvae injected with all three wasp factors were first injected with calyx fluid plus venom at 1–3 h followed by injection of teratocytes 24 h later. This simulated the timing of when virus and teratocytes normally enter hosts when parasitized. Although venom itself does not affect host development, its presence synergizes the effects of MdPDV; a feature noted with other microgastrine braconids (Stoltz *et al.*, 1988). In addition, the developmental alterations induced by calyx fluid plus venom are indistinguishable from injection of gradient-purified MdPDV plus venom (Strand and Dover, 1991). Parasitized and injected larvae were transferred to individual diet cups and reared as described above. Larvae were observed daily until parasitoid emergence, pupation, or death.

JH radioimmunoassay

Hemolymph JH titers were measured using a modified radioimmunoassay (RIA) procedure as described by Goodman *et al.* (1990). Glassware used for RIA procedures was prepared as detailed by Granger and Goodman (1988). Titers were determined for nonparasitized larvae from ecdysis to the completion of cocoon spinning in the fifth stadium (72 h). Titers for parasitized and injected larvae were measured through 96 h. Three samples were collected for each time point examined. Staged larvae were anesthetized with CO_2, bled from a cut proleg, and the hemolymph pooled to a volume of 10–100 μl. One to three larvae were used per sample to assure a sufficient volume of hemolymph for analysis. Samples were stored at −80°C until use in the assay.

Frozen hemolymph samples were thawed at 4°C and immediately transferred to centrifuge tubes with acetonitrile. The tubes were warmed in a 60°C water bath for several minutes and then vortexed for 1 min and centrifuged (800 *g*, 10 min). The supernatants were transferred to 250 ml separatory funnels and extracted in a pentane–brine (4% NaCl in H_2O) solution to separate lipophilic compounds from proteins. The pentane phase (40 ml) was reduced to 4 ml, and the resulting sample was stored at −20°C. The pentane extract was chromatographed on silica TLC plates using a chloroform: hexane: ethyl acetate (7 : 7 : 1) solvent to isolate JH from contaminating

lipids. Controls were conducted to assure that JH ran in between the two dyes used for this study. Under these conditions, individual JH homologs could not be distinguished. The hormone containing fraction was scraped from the plate, eluted with ethyl acetate and stored in 4 ml reactivials at −20°C. Hormone recovery was determined after TLC (Goodman *et al.*, 1990) and ranged across all samples from 22 to 60%. JH titers reported in the results were corrected for recovery.

The rabbit polyclonal antibody designated 676 was used in all RIAs conducted during the study (Goodman *et al.*, 1990). Antibody 676 was generated toward racemic JH III; crossreacting with the major JH homologs (0, I, II, III) but not JH diols or acids. Displacement studies on JH I, II and III using racemic ^{3}H-JH III as a tracer (NEN Research Products, 9–15 Ci/mmol) indicated an order of relative binding affinity of JH III < JH II < JH I (1.0 : 1.5 : 2.0). This leads to a small underestimation in titer of the higher homologs in a complex mixture. However, total JH determinations on hemolymph extracts from *Manduca sexta* were in close agreement with GC/MS data (Goodman *et al.*, 1990). Based on these background results, all titers reported here were expressed as JH III equivalents. The raw numerical data were transformed using the four parameter logistic program and software developed by D. Rodbard (NIH) and modified by P. Licht (University of California, Berkeley).

JH metabolic activity

JH metabolism was measured by partition assay (Hammock and Sparks, 1977). One hundred μl of appropriately diluted hemolymph was incubated for 20 min at 30°C with 5 μl of racemic ^{3}H-JH III in ethanol (5 μM) from NEN Research Products (17 Ci/mmol). Reactions were stopped with 40 μl of methanol, water, NH_4OH (10 : 9 : 1) and samples extracted with iso-octane to remove unmetabolized JH III. The aqueous phase was counted in a scintillation counter to determine the amount of JH acid present. General esterase assays were conducted by the method of Sparks *et al.* (1979) using 3 ml of α-naphthyl acetate (αNA) (2.5 mM) in 0.1 M phosphate buffer (pH 7.2) for 30 min at 30°C. Reactions were stopped with 500 μl of 0.4% *o*-dianisidine diazotate and 3.4% SDS in water. Samples were diluted with phosphate buffer and absorbance read at 600 nm on a spectrophotometer. Total protein was measured by Bradford assay (Bradford, 1976) using a commercially available kit (Biorad) and bovine serum albumen as a standard.

JH release by teratocytes and M. demolitor larvae

In vitro assays were conducted to determine whether teratocytes or *M. demolitor* larvae release JH during development. Teratocytes were obtained as described previously and cultured for 24–120 h in Excell 400 medium in 96-well culture plates (Corning). First and second instar *M. demolitor* larvae were collected by dissecting *P. includens* larvae at 48 and 96 h after oviposition. For assays, 1200 teratocytes (2 larval equivalents) or two *M. demolitor* larvae were placed into individual wells of a 24-well culture plate containing 200 μl of Grace's medium plus 0.1% BSA (Fraction V, Boehringer-Mannheim). Cells and larvae were then cultured for 4 h at 27°C in a humidified incubator. Grace's medium plus BSA without cells or larvae served as a control. All samples were replicated 3–6 times. After removal of teratocytes or larvae, the culture medium was stored at −80°C until used in assays. Samples were assayed by RIA as outlined by Granger *et al.* (1986) with titers expressed in JH III equivalents as described previously. Viability of teratocytes and wasp larvae under these culture conditions was monitored in independent samples by vital staining using propidium iodide (Strand and Wong, 1991).

Methoprene and JH application

Solutions of 1 or 100 pmol/μl of (*RS*)-methoprene (Zoecon, Palo Alto, CA) and 10 nmol/μl of JH II (Sigma, St Louis, MO) were prepared in acetone. Synchronized cohorts of nonparasitized *P. includens* were treated topically on the dorsum of the thorax with 1 μl of solution per day. Control larvae were treated with 1 μl of acetone alone. Larvae were treated beginning at 1 h after ecdysis to the fifth stadium. Larvae were allowed to develop to their terminal stage (larva, larval–pupal intermediate, pupa) and were assigned to specific categories as described by Strand and Dover (1991). Briefly, this 7 point scoring system is based on the number of larval characters retained. A rank of 1 indicates formation of a normal pupa, ranks between 2 and 6 indicate formation of a larval–pupal intermediate with higher values being more larval in character, and a rank of 7 indicates a supernumerary molt that results in formation of a normal larva.

RESULTS

JH titers

The JH titer in nonparasitized larvae increased to 0.50 ng/ml by 24 h after ecdysis but then fell to 0.08 ng/ml at 48 h immediately prior to wandering at 60 h (Fig. 1). The JH titer thereafter increased to 0.52 ng/ml at 72 h in association with cocoon formation that preceeded metamorphosis. JH titers in parasitized larvae were higher than those of nonparasitized larvae at all time points (Fig. 1). Titers rose to 7.3 ng/ml by 24 h after ecdysis followed by subsequent rises in titer at 64 and 96 h. With the exception of the 1 h time point, titers never fell below 2 ng/ml. Parasitized larvae remained developmentally arrested throughout the assay period, exhibiting no characters associated with wandering or preparation for metamorphosis. The *M. demolitor* offspring were still in the egg stage at the 24 h timepoint. The parasitoid larvae were first instars at 48 h, second instars at 72 h and third instars at 96 h.

The JH titers of *P. includens* larvae injected with calyx

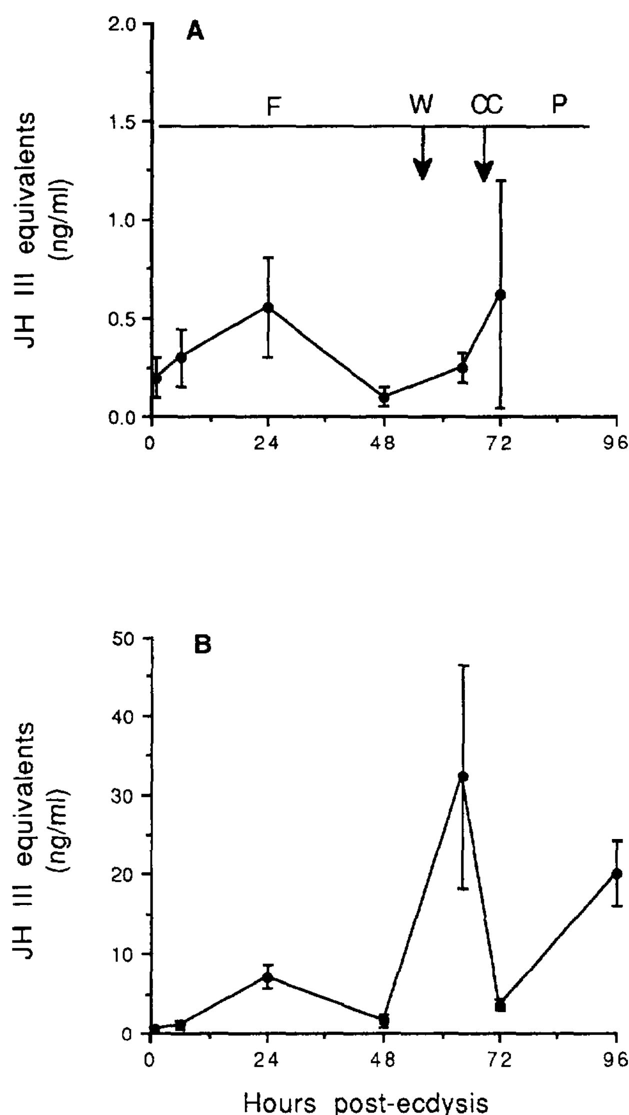


FIGURE 1. Temporal fluctuation of juvenile hormone in nonparasitized (A) and *M. demolitor* parasitized (B) *P. includens* larvae during the fifth stadium. Each datum point (± SD) is the mean of three samples, each pooled from 1–3 larvae. Larvae were parasitized at 1–3 h after ecdysis and staged by established criteria (see Methods). For (A) the horizontal line above the figure developmental stages of nonparasitized *P. includens* indicates: F, feeding stage; W, initiation of wandering; CC, initiation of cocoon spinning; P, pupation.

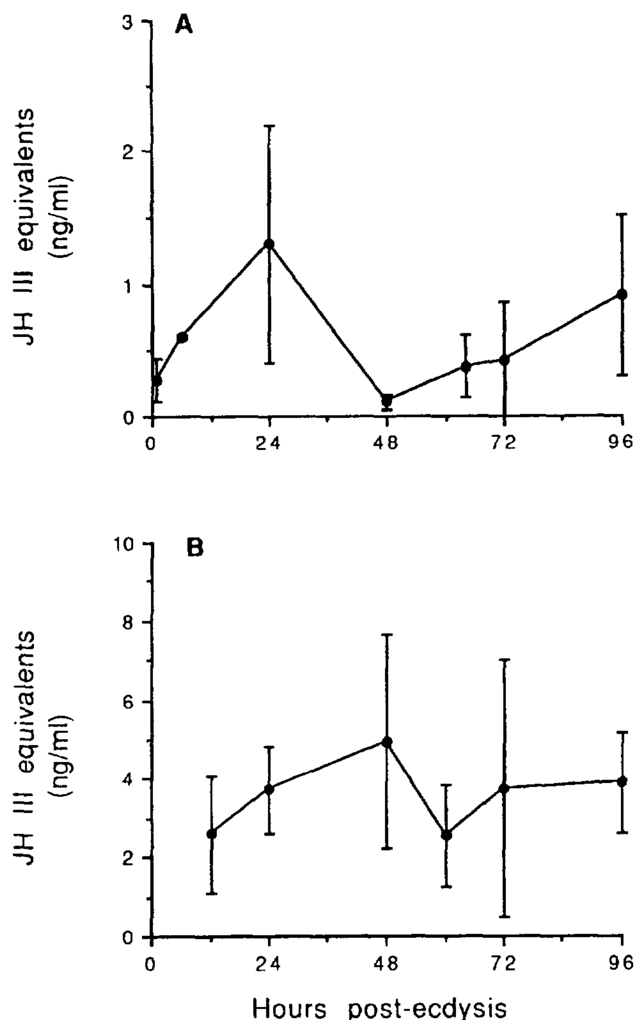


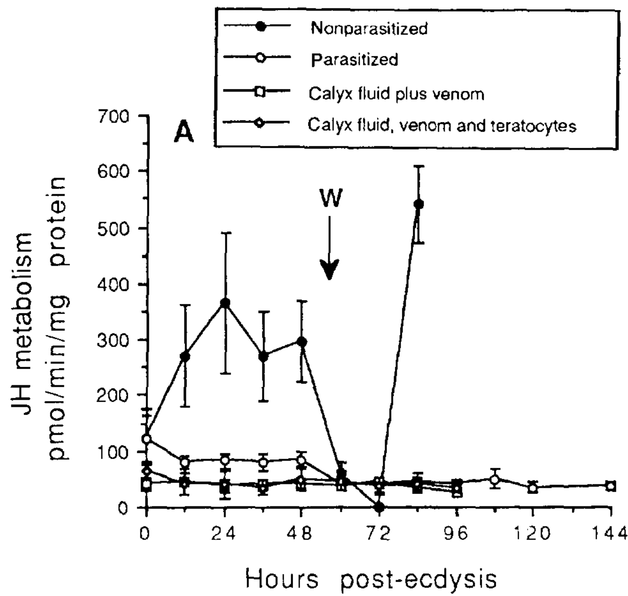
FIGURE 2. Temporal fluctuation of juvenile hormone in calyx fluid plus venom (A) and calyx fluid plus venom and teratocytes-injected (B) *P. includens* larvae during the fifth stadium. Each datum point (± SD) is the mean of three samples, each pooled from 1–5 larvae. Newly ecdysed larvae were injected with 0.10 wasp equivalents of calyx fluid plus venom. Selected larvae were then injected with 1 wasp equivalent of teratocytes 24 h later.

fluid plus venom fluctuated in a manner similar to nonparasitized larvae (Fig. 2). Titers peaked at 24 h (1.4 ng/ml) followed by a decline at 48 h and subsequent rise at 96 h (0.8 ng/ml). *P. includens* injected with calyx fluid plus venom did not initiate wandering after 48 h or exhibit any characteristics associated with metamorphosis during the assay period. Larvae injected with 0.10 wasp equivalents of calyx fluid plus venom remained in the fifth stadium for 10–15 days followed by molting to a larval–pupal intermediate with a mean rank of 3.6 ± 1.4 (SD) ($n = 25$) (Strand and Dover, 1991). Such intermediates typically exhibited antennae and mouthparts of an adult but possessed a head capsule, thorax and abdomen that were larval in appearance. Some pupal cuticle formed on abdominal tergites and while these intermediates retained their prolegs, crochets were usually absent.

The JH titers of larvae injected with calyx fluid, venom and teratocytes were higher than those of nonparasitized larvae but were lower than those of parasitized larvae (Fig. 2). Titers increased to 5 ng/ml at 48 h followed by a drop to 2.25 ng/ml at 60 h. Titers then rose to greater than 3 ng/ml at subsequent time points. Larvae injected with all three wasp components remained developmentally arrested in the fifth stadium for 10–14 days before dying. Larvae showed no signs of wandering or metamorphosis during this period.

JH metabolism

Metabolism of JH increased sharply from 0–24 h before declining to low levels in association with wandering (Fig. 3). Activity then increased at 84 h immediately preceeding pupation. In comparison to nonparasitized larvae, metabolism of JH was greatly reduced in



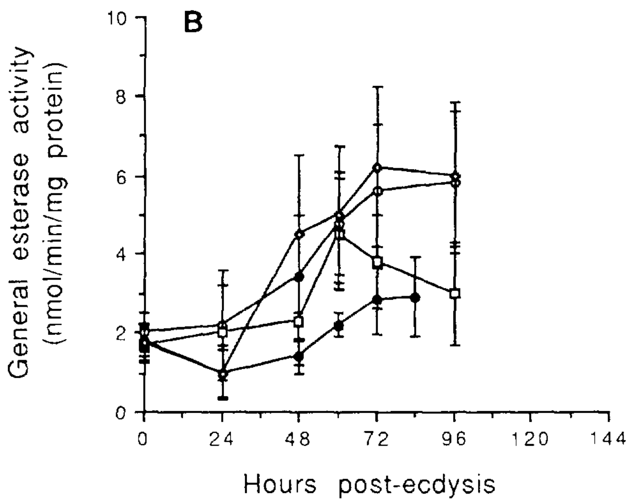


FIGURE 3. Metabolism of JH III (A) and general esterase activity (B) in *P. includens* during the fifth stadium. Newly ecdysed larvae were parasitized, injected with calyx fluid plus venom or injected with calyx fluid plus venom and teratocytes as described in Figs 1 and 2. Nonparasitized larvae served as controls. Each point is the mean (± SE) of three samples from individual larvae. The mg of hemolymph protein for each time point were calculated from the data in Fig. 4. In (A) W designates when the wandering stage begins in nonparasitized larvae.

parasitized hosts, hosts injected with calyx fluid plus venom, or hosts injected with calyx fluid, venom and teratocytes (Fig. 3). The profile of αNA esterase activity in nonparasitized larvae progressively increased in nonparasitized larvae (Fig. 4). In parasitized larvae and larvae injected with calyx fluid plus venom or calyx fluid, venom and teratocytes, αNA esterase activity also progressively increased to levels generally higher than those of nonparasitized larvae (Fig. 4). Hemolymph protein content increased rapidly during the feeding phase of nonparasitized larvae followed by a decline after wandering (Fig. 4). Protein levels in parasitized hosts and hosts injected with specific wasp factors did not change substantially over the 96 h assay period (Fig. 4).

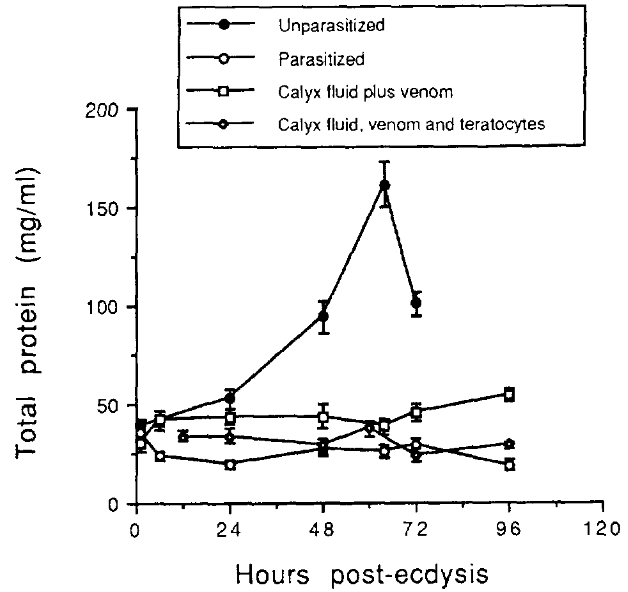


FIGURE 4. Hemolymph protein concentration in *P. includens* during the fifth stadium. Newly ecdysed (1 h) larvae were parasitized, injected with calyx fluid plus venom or injected with calyx fluid, venom and teratocytes as described in Figs 1 and 2. Nonparasitized larvae served as controls. Each point is the mean (± SD) of three samples from individual larvae.

JH release by teratocytes and M. demolitor larvae

Only trace levels of JH were detected in Grace's medium when teratocytes or *M. demolitor* larvae were cultured *in vitro* (Table 1). Culturing of teratocytes and larvae over longer periods (12–24 h) likewise did not result in any increase in the amount of JH detected in the culture medium (data not presented). Samples stained with propidium iodide confirmed that greater than 98% of teratocytes and all larvae were viable during the assays.

Effects of methoprene and JH on P. includens development

Responses by *P. includens* to topical application of methoprene or JH II varied both with dose and when

TABLE 1. JH release by *M. demolitor* teratocytes and larvae *in vitro*

Source	JH III equivalents (ng/ml ± SD)
Teratocytes	
12 h old cells	0.20 ± 0.06
120 h old cells	0.11 ± 0.02
Larvae	
24 h old (first instar)	0.05 ± 0.02
72 h old (second instar)	0.06 ± 0.04
Medium alone (control)	0.07 ± 0.03

Data represent the mean from three independent samples collected as described in the Materials and Methods

treatment began. Larvae treated with acetone only or 1 pmol of methoprene daily pupated without delay (Table 2). Larvae treated with 100 pmol beginning prior to the onset of wandering (2–48 h after ecdysis) exhibited delays in development and formed intermediates. The combined mean rank of these intermediates was significantly higher than that of controls treated with acetone only (Mann-Whitney U-test, $P < 0.05$). It was also evident that the earlier methoprene treatment began, the more larval characters the intermediate retained. For example, most larvae treated beginning at 2 h molted to a supernumerary sixth instar whose only abnormalities were the presence of pupal antennae and the outline of pupal eyes along the anterior margin of the head. In contrast, larvae treated beginning at 60 h (wandering stage) formed intermediates with primarily pupal characteristics. Daily treatment of larvae beginning at 2 h with 10 nmol of JH II did not have as great an effect as methoprene. Nonetheless, most individuals formed intermediates retaining some larval characters (Table 2).

DISCUSSION

The focus of this study was to determine whether the alterations in host development that occur after parasitism by *M. demolitor* were associated with changes in the hemolymph JH titer. We found in our control experiments that the fluctuations in JH titer and levels of JH metabolism in nonparasitized *P. includens* were similar to those reported for other Lepidoptera during the ultimate stadium (Sparks *et al.*, 1979; Baker *et al.*, 1987; Rembold and Sehnal, 1987; Zimowska *et al.*, 1989; Jones *et al.*, 1990; Grossniklaus-Burgin and Lanzrein, 1990; Strand *et al.*, 1991; Dover *et al.*, 1995). The initial increase in JH titer (12–24 h after ecydsis) was paralleled by an increase in JH metabolism. The JH titer then fell to very low levels prior to the critical period, followed by a second increase in titer preceding pupation. This increase in titer was similarly followed by an increase in JH metabolism. We recognize that the RIA used in this study does not distinguish between JH homologs, yet the pattern in which JH titers fluctuated in nonparasitized hosts indicate that our results accurately reflect the relative abundance of JH in the hemolymph of *P. includens*. Our results also indicate that JH titers of larvae parasitized by *M. demolitor* differed markedly from nonparasitized larvae. Over the course of parasitoid development, JH titers exhibited, on average, a 20-fold increase at all time points relative to the nonparasitized controls. Likewise, we found that metabolism of JH was greatly reduced in parasitized hosts. We suggest this response is specific for JH esterase (JHE) since general esterase activity in parasitized larvae was only slightly reduced relative to nonparasitized larvae, despite an overall large reduction in levels of protein present in the hemolymph. However, it is possible that metabolism of JH in *P. includens* is also influenced by epoxide hydrolase activity

TABLE 2. Developmental effects of methoprene and JH II applied daily to *P. includens*

Application time(h)*	Dose per day*	n	Delay in onset of wandering (days ± SD)†	Terminal stage formed (mean rank ± SD)‡
Methoprene				
2	1	15	0	1.0
	100	15	1.1 ±0.1	5.2 ± 0.5
18	1	15	0	1.0
	100	15	1.4 ± 1.0	4.8 ± 0.6
36	1	15	0	1.0
	100	20	1.0 ± 0.6	4.4 ± 1.2
48	1	15	0	1.0
	100	20	0.5 ± 0.2	3.2 ± 1.0
60	1	15	0	1.0
	100	15	0	1.5 ± 0.6
72	1	15	0	1.0
	100	15	0	1.0
JH II				
2	10	20	0.2 ± 0.1	2.8 ± 1.0
Solvent only (control)				
2	—	20	0	1.0

*All larvae treated at designated timepoints after ecdysis to the fifth stadium. Methoprene applied daily at a dosage of 1 or 100 pmol. JH II applied daily at a dosage of 10 nmol.
†Delay in wandering is compared to the time of wandering of larvae treated with solvent only.
‡Mean rank for terminal stage is based upon the ranking system of Strand and Dover (1991).

since this enzyme has also been implicated in JH metabolism in other Lepidoptera (Hammock, 1985).

Previous studies demonstrated that injection of calyx fluid plus venom or purified MdPDV plus venom induces *P. includens* to form larval–pupal intermediates while injection of calyx fluid or MdPDV plus venom and teratocytes results in developmental arrest (Strand and Dover, 1991; Strand and Wong, 1991). Inactivation of virus by pretreatment with psoralin and UV light eliminates its effect on host development (Strand *et al.*, 1992; Strand, 1994), indicating that viral transcription is probably necessary for the juvenilizing effects calyx fluid has on host development. Based upon these results, we originally hypothesized that calyx fluid and/or teratocytes disrupt host development by inducing an elevation in JH titer via suppression of JH metabolism or increased synthesis and release of hormone. Our methoprene and JH application experiments are consistent with such a hypothesis. *P. includens* exhibited a dose-dependent response to methoprene and consistently formed larval–pupal intermediates if treatment began before larvae initiated wandering. Indeed, the number of larval characters retained by intermediates treated with 100 pmol/day of methoprene (beginning at 2 h after ecdysis) are virtually identical to the intermediates that form when larvae are injected with a physiological dose of calyx fluid or MdPDV plus venom (Strand and Dover, 1991).

Results reported here for *P. includens* and elsewhere for the alternative host *Heliothis virescens* (Dover *et al.*, 1995) further indicate that calyx fluid plus venom or calyx fluid, venom and teratocytes suppress JH metabolism to the same degree as parasitized hosts. These factors also caused changes in how the JH titer fluctuated over time relative to nonparasitized larvae. However, contrary to expectation, these factors did not induce as large an increase in titer as observed in parasitized larvae. That JH titers differ between parasitized larvae and larvae injected with wasp factors could be due to several factors, including: (1) JH is released into the host by the *M. demolitor* larva; (2) true parasitism induces an elevation in synthesis and release of JH by the host's corpora allata (CA); or (3) parasitism induces increased JH binding protein activity. We have not yet compared binding protein levels between parasitized and injected larvae. However, we found no evidence in our *in vitro* assays that either *M. demolitor* larvae or teratocytes secrete JH into culture medium. In preliminary studies we also find no increase in *in vitro* production of JH by isolated CA–CC–brain complexes from parasitized hosts compared to complexes from nonparasitized hosts (Balgopal *et al.* unpublished).

Increased degredation of JH has been documented in hosts parasitized by other larval endoparasitoids (Beckage and Riddiford, 1982; Hayakawa, 1990; Zhang *et al.*, 1992; Schopf and Rembold, 1993) as has production and possible release of JH into the host by developing parasitoid larvae (Lawrence *et al.*, 1990; Schopf and Rembold, 1993). Our results suggest that the elevation in JH titer seen in parasitized *P. includens* is associated primarily with decreased metabolism of hormone rather than release of JH by *M. demolitor*, associated teratocytes or the host's CA. What remains unresolved is why injection of calyx fluid plus venom, or calyx fluid plus venom and teratocytes juvenilize hosts in a manner very similar to natural parasitism, reduce levels of JH metabolism, yet do not induce an elevation in JH titer comparable to naturally parasitized hosts. This calls into question whether an elevation in JH titer is the primary factor responsible for inducing developmental arrest or formation of larval–pupal intermediates in *P. includens* larvae injected with factors from *M. demolitor*.

The adult characters exhibited by pupae of Lepidoptera are produced by imaginal precursor cells (Chapman, 1982). Although some differences exist between species, these cells appear differentially responsive to juvenoids relative to other regions of the integument. For example, experiments with *Manduca sexta* indicate that eyes, mouthparts and wings often show pupal characteristics in supernumerary larvae, both when the JH titer is manipulated to precociously decline (Kiguchi and Riddiford, 1978) or when maintained at an artificially elevated level (Nijhout, 1975). Hormonal state is also influenced by nutrition, with starvation and other nutritional perturbations inducing supernumerary molting and/or formation of larval–pupal intermediates (Bhaskaran and Jones, 1980; Cymborowski *et al.*, 1982). Collectively, these types of studies suggest that tissue-specific differences exist in the timing of pupal commitment and in sensitivity to specific hormones; a conclusion supported by recent molecular studies on the ecdysone response hierarchy in *Drosophila* (Koelle *et al.*, 1991; Talbot *et al.*, 1993). They also suggest the possibility that proper coordination of metamorphic events could be influenced more by relative changes in JH titer over time than by the absolute concentration of hormone. Disruption of metamorphosis by parasitism, therefore, may be the result of changes in how hormone levels fluctuate or in the sensitivity of tissues to a hormonal stimulus rather than a quantitative increase in titer. Thus, even though calyx fluid plus venom from *M. demolitor* induce a relatively small increase in JH titer relative to nonparasitized larvae, their impact on how the JH titer fluctuates induces alterations similar to those observed in larvae treated with an exogenous source of juvenoids.

Polydnavirus, venom and teratocytes have been implicated in causing many of the developmental alterations that occur in host species parasitized by microgastrine braconids. In some systems, polydnavirus or polydnavirus plus venom are sufficient to disrupt pupation or induce developmental arrest (Tanaka, 1987; Tanaka and Vinson, 1991), while in others teratocytes (Zhang *et al.*, 1992; Pennacchio *et al.*, 1992), or polydnavirus plus teratocytes (Wani *et al.*, 1990; Strand and Wong, 1991), are required. Although we found no evidence that teratocytes from *M. demolitor* release JH, Joiner *et al.* (1973) reported JH-like activity in association with teratocytes

of *Cardiochiles nigriceps*. Like *M. demolitor*, however, calyx fluid and venom of *Apanteles kariyai* suppresses JH metabolism in *Pseudolatia separata* in the absence of teratocytes (Hayakawa and Yasuhara, 1993). Hayakawa (1990) identified a "growth blocking peptide" in *P. separata* that is normally present during the penultimate stadium but is absent during the final instar. This factor transiently suppresses JHE activity when injected into ultimate stadium *P. separata*, suggesting that calyx fluid from *A. kariyai* may influence the concentration of this factor in parasitized hosts. Recent studies with *M. demolitor*, however, suggest suppression of host JH metabolism is not due to a humoral factor in the alternative host *Heliothis virescens* (Dover *et al*., 1995). Neither injection of hemolymph from parasitized *H. virescens* larvae into nonparasitized larvae nor direct incubation of hemolymph from parasitized larvae with hemolymph from nonparasitized larvae had an effect on JH metabolic activity. Whether MdPDV inhibits synthesis or release of JHE in *P. includens* and *H. virescens* is currently under investigation.

REFERENCES

Baker F. C., Tsai L. W., Reuter C. C. and Schooley D. A. (1987) *In vivo* fluctuation of JH, JH acid, and ecdysteroid titer, and JH esterase activity during development of fifth stadium *Manduca sexta*. *Insect Biochem.* **17**, 989–996.

Beckage N. E. (1985) Endocrine interactions between endoparasitic insects and their hosts. *A. Rev. Ent.* **30**, 371–413.

Beckage N. E., and Riddiford L. M. (1982) Effect of parasitism by *Apanteles congregatus* on the endocrine physiology of the tobacco hornworm *Manduca sexta*. *Gen. Comp. Endocr.* **47**, 308–322.

Bhaskaran G. and Jones G. (1980) Neuroendocrine regulation of corpus allatum activity in *Manduca sexta*: the endocrine basis for starvation-induced supernumerary larval moult. *J. Insect Physiol.* **26,** 431–440.

Bradford M. (1976) A rapid and sensitive method for the quantitation of microgram quantitites of protein utlizing the principle of protein-dye binding. *Analyt. Biochem.* **72,** 218–254.

Chapman R. F. (1982) *The Insects: Structure and Function*. Harvard University Press, Cambridge.

Cymborowski B., Bogus M., Beckage N., Williams C. Riddiford L. (1982) Juvenile hormone titres and metabolism during starvation-induced supernumerary larval moulting of the tobacco hornworm, *Manduca sexta* L. *J. Insect Physiol.* **28,** 129–135.

Dover B. A., Menon A., Brown R. C. and Strand M. R. (1995) Suppression of juvenile hormone esterase in *Heliothis virescens* by *Microplitis demolitor* calyx fluid. *J. Insect Physiol.* **41,** 809–817.

Goodman W. G., Coy D. C., Baker F. C., Xu L. and Toong Y. C. (1990) Development and application of a radioimmunoassay for the juvenile hormones. *Insect Biochem.* **20,** 357–364.

Granger N. A. and Goodman W. G. (1988) Radioimmunoassay: juvenile hormones. In *Immunogical Techniques in Insect Biology* (Eds Gilbert L. I. and Miller T. A.), pp. 215–251. Springer Verlag, Berlin.

Granger N. A., Mitchell I. J. and Niemiec S. M. (1986) Biosynthesis of juvenile hormones I and III by the corpora allata of *Manduca sexta*: effect of *in vitro* conditions on gland activity. In *In vitro Invertebrate Hormones and Genes* (Eds Kurstak E. and Oberlander H.), pp. 1–15. Elsevier, New York.

Grossniklaus-Burgin C. and Lanzrein B. (1990) Qualitative and quantitative analyses of juvenile hormones and ecdysteroids from the egg to the pupal molt in *Trichoplusia ni*. *Arch. Insect Biochem. Physiol.* **14,** 13–30.

Hammock B. D. (1985) Regulation of juvenile hormone titer: degradation. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology* (Eds Kerkut G. A. and Gilbert L. I.), Vol 7, pp. 431–472. Pergamon Press, Oxford.

Hammock B. D. and Sparks T. C. (1977) A rapid assay for juvenile hormone esterase activity. *Analyt. Biochem* **82,** 573–579.

Hayakawa Y. (1990) Juvenile hormone esterase activity repressive factor in the plasma of parasitized insect larvae. *J. Biol. Chem.* **265,** 10,813–10,816.

Hayakawa Y. and Yasuhara Y. (1993) Growth-blocking peptide or polydnavirus effects on the last instar larvae of some insect species. *Insect Biochem. Molec. Biol.* **23,** 225–231.

Joiner R. L., Vinson S. B. and Benskin J. B. (1973) Teratocytes as a source of juvenile hormone activity in a parasitoid–host relationship. *Nature New Biol.* **246,** 120–121.

Jones D. (1985) The endocrine basis for developmentally stationary prepupae of larvae of *Trichoplusia ni* pseudoparasitized by *Chelonus insularis*. *J. Comp. Physiol.* **155B,** 235–240.

Jones G., Hanzlik T., Hammock B, Schooley D. A., Miller C. A., Tsai L. W. and Baker F. C. (1990) The juvenile hormone titre during the penultimate and ultimate larval stadia of *Trichoplusia ni*. *J. Insect Physiol.* **36,** 77–83.

Kiguchi K., and Riddiford L. M. (1978) A role of juvenile hormone in pupal development of the tobacco hornworm, *Manduca sexta*. *J. Insect Physiol.* **24,** 673–680.

Koelle M. R., Talbot W. S., Segraves W. A., Bender M. T., Cherbas P., and Hogness D. S. (1991) The *Drosophila* EcR gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. *Cell* **67,** 59–77.

Lawrence P. O. and Lanzrein B. (1993) Hormonal interactions between insect endoparasites and their host insects. In *Parasites and Pathogens of Insects* (Eds Beckage N. E., Thompson S. N. and Federici B. A.) Vol. 1, pp. 59–86. Academic Press, New York.

Lawrence P. O., Baker F. C., Tsai L. W., Miller C. A., Schooley D. A. and Geddes L. G. (1990) JH III levels in larvae of pharate pupae of *Anastrepha suspensa* (Diptera: Tephritidae) and in larvae of the parasitic wasp *Biosteres longicaudatus* (Hymenoptera: Braconidae). *Arch. Insect Biochem. Physiol.* **13,** 53–62.

Nijhout H. F. (1975) Dynamics of juvenile hormone action in larvae of the tobacco hornworm, *Manduca sexta* (L.). *Biol. Bull.* **149,** 568–579.

Pennacchio F., Vinson S. B. and Tremblay E. (1992) Host regulation effects on *Heliothis virescens* larvae induced by teratocytes of *Cardiochiles nigriceps*. *Arch. Insect Biochem. Physiol.* **19,** 177–192.

Rembold H. and Sehnal F. (1987) Juvenile hormones and their titer regulation in *Galleria mellonella*. *J. Insect Biochem.* **17,** 997–1001.

Schopf A. and Rembold H. (1993) Changes in juvenile hormone titer of gypsy moth larvae by parasitism of *Glyptapanteles liparidis*. *Naturwisssenschaften* **80,** 527–528.

Shepard B. M., Powell J. E. and Jones W. A. (1983) Biology of *Microplitis demolitor*, an imported parasitoid of *Heliothis* spp. and the soybean looper *Pseudoplusia includens*. *Environ. Entomol.* **12,** 641–649.

Sparks T. C., Willis W. S., Shorey H. and Hammock B. D. (1979) Haemolymph juvenile hormone esterase activity in synchronous last instar larvae of the cabbage looper, *Trichoplusia ni*. *J. Insect Physiol.* **25,** 125–132.

Strand M. R. (1990) Characterization of larval development in *Pseudoplusia includens* (Lepidoptera: Noctuidae). *Ann. Ent. Soc. Am.* **83,** 538–544.

Strand M. R. (1994) *Microplitis demolitor* polydnavirus infects and expresses in specific morphotypes of *Pseudoplusia includens* haemocytes. *J. Gen. Virol.* **75,** 3007–3020.

Strand M. R. and Dover B. A. (1991) Developmental disruption of *Pseudoplusia includens* and *Heliothis virescens* larvae by calyx fluid and venom of *Microplitis demolitor*. *Arch. Insect Biochem. Physiol.* **18,** 131–145.

Strand M. R. and Noda T. (1991) Alterations in the haemocytes of

Pseudoplusia includens after parasitism by *Microplitis demolitor*. *J. Insect Physiol.* **37,** 839–850.

Strand M. R. and Wong E. A. (1991) The growth and role of *Microplitis demolitor* teratocytes in parasitism by *Pseudoplusia includens*. *J. Insect Physiol.* **37,** 503–515.

Strand M. R., Goodman W. G. and Baehrecke E. H. (1991) The juvenile hormone titer of *Trichoplusia ni* and its potential role in embryogenesis of the polyembryonic wasp *Copidosoma floridanum*. *Insect Biochem.* **21,** 205–214.

Strand M. R., Johnson J. A. and Culin J. D. (1988) Developmental interactions between the parasitoid *Microplitis demolitor* (Hymenoptera: Braconidae) and its host *Heliothis virescens* (Lepidoptera: Noctuidae). *Ann. Ent. Soc. Am.* **81,** 822–830.

Strand M. R., McKenzie D. I., Grassl V., Dover B. A. and Aikan J. M. (1992) Persistence and expression of *Microplitis demolitor* polydnavirus in *Pseudoplusia includens*. *J. Gen. Virol.* **73,** 1627–1635.

Stoltz D. B., Guzo D., Belland E. R., Lucarotti C. J. and Mackinnon E. A. (1988) Venom promotes uncoating *in vitro* and persistence *in vivo* of DNA from a braconid polydnavirus. *J. Gen. Virol.* **69,** 903–907.

Talbot W. S., Swyryd E. A. and Hogness D. S. (1993) *Drosophila* tissues with different metamorphic responses to ecdysone express different ecdysone receptor isoforms. *Cell* **73,** 1323–1337.

Tanaka T. (1987) Calyx and venom fluids of *Apanteles kariyai* as factors that prolong larval period of the host, *Pseudolatia separata*. *Ann. Ent. Soc. Am.* **80,** 530–533.

Tanaka T. and Vinson S. B. (1991) Depression of prothoracic gland activity of *Heliothis virescens* by venom and calyx fluids from the parasitoid *Cardiochiles nigriceps*. *J. Insect Physiol.* **37,** 139–144.

Wani M., Yagi S. and Tanaka T. (1990) Synergistic effect of venom and teratocytes of *Apanteles kariyai* on the inhibition of larval pupal ecdysis of the host, *Pseudolatia separata*. *Ent. Exp. Appl.* **57,** 101–104.

Zhang D., Dahlman D. L. and Gelman D. B. (1992) Juvenile hormone esterase activity and ecdysteroid titre in *Heliothis virescens* larvae injected with *Microplitis croceipes* teratocytes. *Arch. Insect Biochem. Physiol.* **20,** 231–242.

Zimowska G., Rembold H. and Bayer G. (1989) Juvenile hormone identification, titer, and degradation during the last larval stadium of *Spodoptera littoralis*. *Arch. Insect Biochem. Physiol.* **12,** 1–14.

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