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REGULATION OF HOST LARVAL DEVELOPMENT BY THE EGG-LARVAL ENDOPARASITOID *CHELONUS INSULARIS* [*HYM. : BRACONIDAE*](1)

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In laboratory studies the effect of parasitism by the egg-larval endoparasitoid *Chelonus insularis* CRESSON on the resulting larvae of 2 host species, *Heliothis virescens* (F.) and *Spodoptera ornithogalli* GUÉNÉE) were determined by comparing daily measurements of larval weights. Growth of parasitized larvae of both host species was slower than growth of unparasitized larvae. Injections of fluids from the female parasitoid's calyx or poison gland into *H. virescens* eggs retarded subsequent larval growth. However, a combination of fluids from these 2 organs produced the most significant reduction in the host larval growth rate. The growth reducing factor(s) was also effective when injected into 5-day-old host larvae.

Insect parasitoids, through coevolution with their hosts, have developed numerous mechanisms to maximize their chances of successful parasitism (VINSON, 1975). After parasitism the growth, physiology and behavior of the host are often altered. In some parasitoid-host relationships the host gains weight faster than non-parasitized controls while in other cases weight gain is decreased in parasitized host (VINSON & IWANTSCH, 1980). These effects have been attributed to the parasitoid egg, the developing parasitoid larvae and/or factors injected by the ovipositing female (VINSON & IWANTSCH, 1980). JONES & LEWIS (1971) demonstrated that a fluid from the calyx of a ♀ braconid larval endoparasitoid resulted in reduced weight gain of its host. Similar results have been reported for another braconid larval endoparasitoid and ichneumonid larval endoparasitoid (GUILLOT & VINSON, 1972 ; VINSON, 1972).

We report here results of a study conducted to : (1) determine whether parasitism by an egg-larval endoparasitoid, *Chelonus insularis* CRESSON, would result in reduced host weight gain, (2) determine whether the effects are similar in different hosts [the tobacco budworm, *Heliothis virescens* (F.), and the yellow striped armyworm, *Spodoptera ornithogalli* (GUÉNÉE)] and, (3) determine the source of the factor(s) responsible. Additionally we wanted to determine if the responsible factor(s) was effective in suppressing host weight gain if iniected into larvae, a stage not initially attacked by *C. insularis*.

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MATERIALS AND METHODS

Adult parasitoids were held in small cages at 21° C, 70 % RH, and a 14-h photophase. Food was provided via a cotton plug satured with a honey and water mixture (1:1). Host larvae were reared at 27° C and a 14-h photophase and were fed an artifical diet similar to that of VANDERZANT *et al.* (1962).

COMPARISONS BETWEEN PARASITIZED AND UNPARASITIZED HOST

Eggs (≥ 24-h old) of each host species were exposed to ♀ parasitoids for 4-5 h and then held separately from unparasitized eggs of the same age until eclosion. Upon eclosion, parasitized and unparasitized host larvae were placed singly into 28-ml plastic rearing cups with artificial diet. In order to minimize physical injury, the small larvae were not handled again until 4 days post eclosion, when measurements of host larva weight were begun. Larval weight was measured daily to the nearest 0.1 mg on a precision balance until 13 days post eclosion, when parasitoid larvae emerged from their hosts. Weights were compared for 25 parasitized and unparasitized larvae each for both host species.

EFFECTS OF CALYX AND POISON GLAND FLUIDS

The poison gland and calyx portion of the ovary were dissected from 10 to 12 ♀ parasitoids and concentrated in saline by methods similar to those of VINSON (1972). Evans blue dye was dissolved in the glandular fluid ; this served as a tracer which indicated whether or not a successful injection had been achieved.

We fashioned glass micro-needles by drawing out capillary tubes on an electrode puller, and the micro-needles were then attached to a 10-μl syringe. To obtain adequate pressure for injection, we had to first fill the syringe and needle with mercury. Dosages were dispensed with a microapplicator at ca. 0.006 μl or ca. 0.015 ♀ gland equivalents per injection. The microapplicator with electrical control was calibrated with a spectrophotometric dye dilution method. With this procedure, we injected *H. virescens* eggs (≥ 24 h old) and 2nd instar larvae with fluid from the poison glands, ovarian calyces, or a combination of the 2 fluids for a total of 3 treatments.

Thirty-five to 50 eggs were successfully injected for each of the 3 treatments as well as for an injected control consisting of saline plus dye. A second control consisted of untreated eggs. Eclosing larvae were placed singly into rearing cups with artificial medium. Five days after eclosion, 25 larvae were randomly selected from each group of treatments and controls and their respective weights were measured. Larval weights were recorded at 2- to 3-day intervals until 14 days post eclosion. We later recorded the number of days required to reach 100 % pupation, pupal weights and % adult emergence for each group.

In order to determine if fluids administered after larval eclosion would affect host development, we used the same procedures to inject 5-day-old larvae (0.007 ♀ gland equivalents per injection). Injections were made immediately behind the head capsule of CO_2-narcotized larvae and weights were recorded as above.

RESULTS

WEIGHT GAIN OF PARASITIZED AND UNPARASITIZED LARVAE

The effects of parasitism by the egg-larval parasitoid on weight gain of *H. virescens* and *S. ornithogalli* larvae are shown in figure 1. Although both host species gained weight at different rates, parasitism reduced the weight gains of both host species. Differences in the weights between parasitized and unparasitized larvae were evident by day 5 for *H. virescens* but only became evident at day 7 for *S. ornithogalli.*

The parasitoid larvae apparently developed at similar rates within both host species as they began emerging from both hosts 13 days after parasitism.

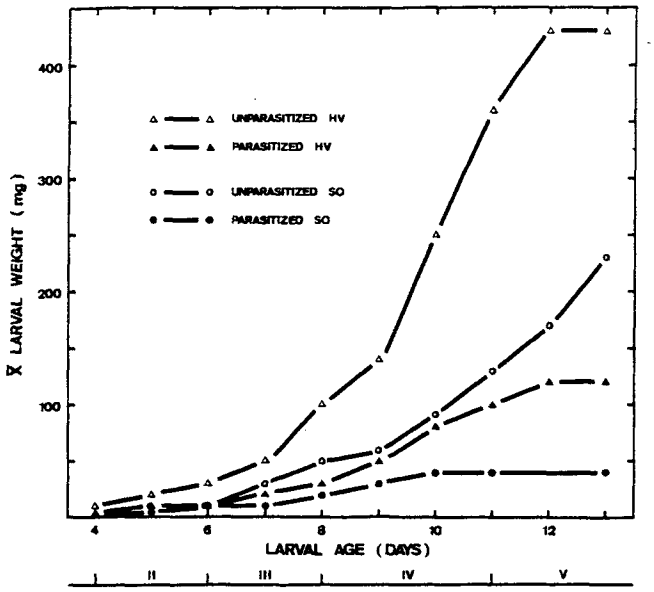

FIG. 1. Changes in mean larval weight of *Heliothis virescens* (HV) and *Spodoptera ornithogalli* (SO) larvae parasitized by *Chelonus insularis* and unparasitized larvae. Roman numerals indicate instars of unparasitized larvae.

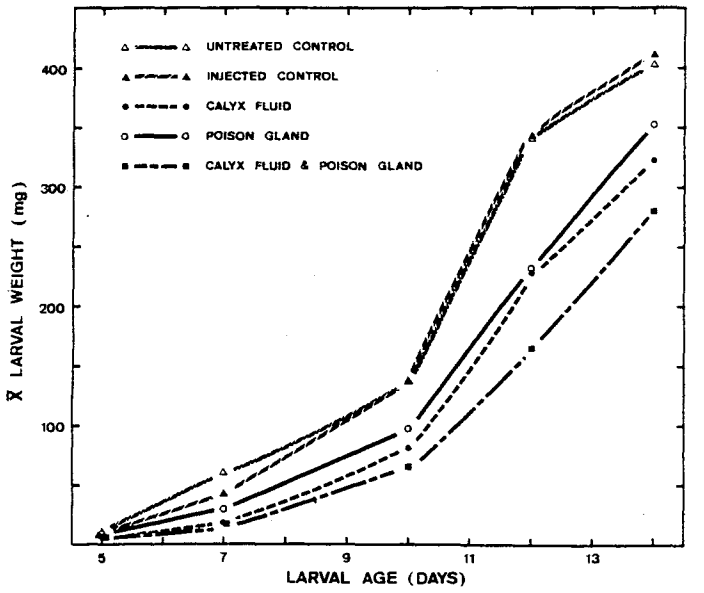

FIG. 2. Changes in mean larval weight of *Heliothis virescens* larvae following injections of eggs with various glandular fluids of *Chelonus inuslaris*.

EFFECT OF THE CONTENTS OF REPRODUCTIVELY ASSOCIATED GLANDS ON HOST WEIGHT GAINS

The injection of glandular fluids resulted in the collapse of ca. 2 % of the eggs. The remaining eggs, controls as well as injected, hatched at the same rate. However, the injections of eggs apparently produced some trauma in the ensuing larvae because the mean weight of larvae from the injected controls, as well as from the treatments, was significantly less ($P < 0.01$) than that of larvae from uninjected eggs at 7 days post injection (fig. 2). Ten days after injections into eggs there was no difference in the weights of uninjected and injected control larvae but larvae from all 3 treatments weighed significantly less than the controls. These significant differences persisted for the remainder of the test. Furthermore, the combinations of the calyx fluid and poison gland resulted in a significantly lower weight gain than the other treatments. Furthermore, the controls pupated in 16 days while the larvae injected with calyx fluid or poison gland fluid took 1 to 2 days longer to pupate while larvae from eggs injected with a combination of the poison gland and calyx contents required an extra 4 days to pupate ; however, these differences were not significant. No differences in pupal weights or percentage moth emergence were noted (table 1).

Injections of the calyx fluid or poison gland contents into 5-day-old budworm larvae produced a reduction in weight gain similar to those obtained by injection into eggs (fig. 3). The combination of the calyx and poison gland contents apparently lost its effectiveness prior to pupation when injected in larvae. Due to a shortage of material we had to refrigerate the fluid combinations overnight, and this may have resulted in some loss of activity.

TABLE 1

Days to pupation, pupal weight and adult emergence following injections of Heliothis virescens *eggs with* Chelonus insularis *reproductive gland fluids*

Treatment	Days 100 % Pupation[a]	$\bar{X}$ (± SD) pupal wt (mg)[a]	% adult emergence
Calyx fluid	18	305.2 ± 24.5	93.8
Poison gland	17	321.3 ± 38.4	96.2
Calyx fluid and poison gland	20	324.5 ± 35.1	94.7
Injected Control[b]	16	330.1 ± 20.1	94.7
Untreated Control	16	313.4 ± 31.8	95.5

(a) Means were not statistically different.
(b) Injection of saline and Evans blue dye.

DISCUSSION

There are a number of parasitoid-host relationships in which the weight gain of the host is reduced after parasitism (VINSON & IWANTSCH, 1980). In 3 larval endoparasitoid species, an ichneumonid and 2 braconids, the calyx fluid appears responsible (JONES & LEWIS, 1971 ; GUILLOT & VINSON, 1972 ; VINSON, 1972). Even though the contents of the calyx appears responsible there are differences in each relationship. In *Campoletis sonorensis* (CAMERON) a unique virus prevents weight gain from which the hosts never recover (VINSON *et al.*, 1979). In *Microplitis croceipes* (CRESSON) the calyx fluid causes a temporary reduction in host weight gain while *Cardiochiles nigriceps* VIRECK calyx fluid and poison gland contents cause a reduction in host weight gains from which the hosts fail to

recover (JONES & LEWIS, 1971 ; GUILLOT & VINSON, 1972). It is yet unknown whether the effects produced by the calyx fluid of the braconids is due to a unique baculovirus present in the calyx fluid (STOLTZ & VINSON, 1979) or other factors.

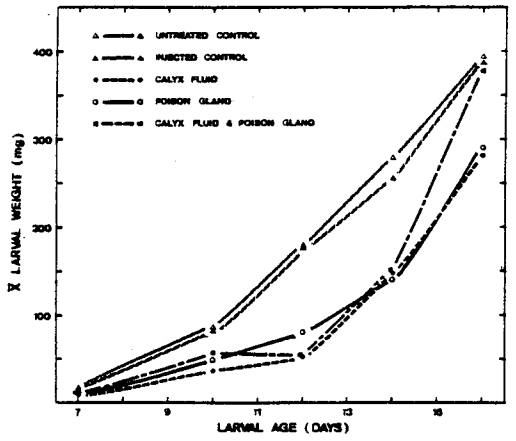

FIG. 3. Changes in mean larval weight of *Heliothis virescens* injected on day 5 posthatch with various glandular fluids from *Chelonus insularis*.

The results of this study show that parasitism of the egg stage by *Chelonus* can reduce the larval weight gain of 2 host species and that this change appears to be related to the hosts normal weight gain. In other studies with egg-larval parasitoids, reduced weight gain by parasitized hosts has been similarly demonstrated. For example, RECHAV & ORION (1975) determined than *C. inanitus* (L.) moderately reduced the weight of parasitized *S. littoralis* (BOISDUVAL) larvae between 2 and 5 days posthatch and significantly thereafter ; host development essentially ended on the 9th day. Furthermore, the weight of unparasitized hosts increased 10 fold. In other studies, HAWLITZKY (1970) and HAWLITZKY & CHEVIN (1979) demonstrated that *Phanerotoma flavitestacea* FISHER reduced the weight of newly hatched *Anagasta kuehniella* (ZELLER) larvae and that the weight ratio of parasitized larvae to healthy larvae was 1:2 by the time the parasitoid spun its cocoon. Our results show a similar ratio.

The results of our study also show that both the calyx fluid and poison gland contents are responsible for the reduced weight gain and thus appear similar to *Cardiochiles* while the observation that the larvae escape these effects resembles the results reported for *Microplitis* (JONES & LEWIS, 1971 ; GUILLOT & VINSON, 1972). The ability of the larvae injected with the reproductive gland fluids to escape the effects suggests that the changes in the hosts' physiology induced by these fluids are temporary. The difference in weight gain between parasitized and gland injected hosts particularly after 9 days, suggests that the developing larvae within the host may contribute to some of the differences observed between parasitized and unparasitized hosts. It is also noteworthy that the delay in the onset of the reductions in weight gain is different in both hosts attacked by *Chelonus* and that the delay is greater with *Chelonus* in both hosts than that reported for the other parasitoid species.

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RÉSUMÉ

Régulation du développement de la larve-hôte par l'endoparasitoïde ovolarvaire *Chelonus insularis* [*Hym. : Braconidae*]

Au laboratoire les effets du parasitisme par l'endoparasitoïde ovolarvaire *Chelonus insularis* CRESSON chez les larves des espèces hôtes : *Heliothis virescens* (F.) et *Spodoptera ornithogalli* (GUÉNÉE) ont été déterminés par la comparaison journalière des poids des larves. La croissance des larves parasitées des 2 espèces hôtes a été plus faible que celle des larves non parasitées. L'injection dans des œufs de *H. virescens* des sécrétions du calyx ou de la glande à poison de la femelle du parasitoïde a entraîné un retard dans le développement de la larve. Toutefois, un mélange des sécrétions de ces 2 organes a produit la réduction la plus significative de la vitesse du développement de la larve hôte. Le ou les facteurs ralentissant la croissance sont également actifs par injection dans des larves hôtes de 5 jours.

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