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Effects of parasitization of *Trichoplusia ni* by *Chelonus* sp.

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ABSTRACT. Parasitization of *Trichoplusia ni* (Huebner) (Lepidoptera: Noctuidae) by *Chelonus* sp. (Hymenoptera: Braconidae), an egg–larval parasitoid, leads to precocious cocoon spinning of the host in the fourth (penultimate) stadium followed by parasitoid emergence from the prepupa. We have investigated the mechanism by which *Chelonus* sp. disrupts host development. The developing larva and fluids injected by the adult female separately from the egg, are not the source of these effects, but it remains a possibility that the teratocytes, originating from the trophamnion of the parasitoid egg, are responsible.

The titre of the juvenile hormone esterase activity in the haemolymph of the parasitized fourth instar host is similar to that in the initial period of the final instar of normal *T.ni*, but lacks the postwandering peak of activity. The increased JHE activity leads to a reduced JH titre early in the fourth stadia. This indicates that disruption of host development occurs within 12 h after apolysis to the fourth stadium, if not before. Anti-juvenile hormone activity is not detected in extracts of parasitized *T.ni*. The morphological and behavioural changes associated with precocious development of the *T.ni* host are prevented by applications of juvenile hormone I, juvenile hormone II and the juvenoid, Ro 10-3108, but not juvenile hormone III and the juvenoid R 20458. However, these applications fail to prevent the onset of juvenile hormone esterase activity, another marker of precocious development. These observations indicate that simple anti-juvenile hormone activity may not be the mechanism of disruption of host development. Development of the parasitoid is disrupted by application of Ro 10-3108 and juvenile hormones I, II and III, but timing of eclosion is only affected by application of juvenile hormone I, juvenile hormone II and Ro 10-3108. This observation may indicate a discrimination by the parasitoid between its own juvenile hormone III and the host's juvenile hormone II.

Key words. Juvenile hormone, juvenoid, *Chelonus, Trichoplusia ni* anti-juvenile hormone, parasitization.

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Introduction

Vinson & Iwantsch (1980) review numerous reports of disrupted host development due to parasitization of Lepidoptera by endoparasitic hymenopterans. However, the induction of precocious spinning of a cocoon by the host one or more larval instars ahead of normal development as observed after parasitization by the egg–larval parasitoid *Chelonus* (Hymenoptera: Braconidae) (Butler, 1966; Broodryk, 1969) represents a very successful effect in the sense of biological control. It not only causes a reduction of the overall number of adult hosts emerging but, as observed with *Trichoplusia ni* (Huebner) (Lepidoptera: Noctuidae) it also leads to a significant decrease in food consumption mainly due to absence of the actively feeding last larval stadium (Jones *et al.*, 1981). Therefore, elucidation of the mechanisms underlying the disruption could lead towards new ideas for insect control.

A previous study on the same host–parasitoid system showed that the induction of precocious cocoon spinning in the penultimate fourth instar of the host followed the same temporal patterns and showed the developmental changes as observed with normal last instar *T.ni* (Jones *et al.*, 1981; Jones, 1985). Thus, this model may provide new information on the processes of moulting and metamorphosis in the lepidopteran order. Using several approaches, we investigated both the source and mechanism of the disrupted development following parasitization of *T.ni* by *Chelonus* sp.

Though the origins of many of the effects described as consequences of parasitization remain undetermined, previous work on different host–parasitoid systems concentrate on three sources affecting the host (for review see Vinson & Iwantsch, 1980): namely the adult parasitoid female; the developing parasitoid larva; and the teratocytes, cells originating from the serosa membrane surrounding the developing parasitoid embryo that are liberated into the haemocoel of the host upon its hatching. We investigated the potential role of these sources by selectively removing or implanting them.

From a knowledge of the endocrinological events leading to the onset of the pupation process in *T.ni* (see Sparks, 1984), the mechanism of disrupted development may involve the parasitoid lowering the host juvenile hormone (JH) titre in some manner and thus causing premature development towards the pupa. This possible anti-JH mechanism (AJH) was explored by four ways: parasitized larvae were extracted in various media and the presence of a putative AJH factor tested using two different bioassays; the titre of juvenile hormone esterase (JHE) in the haemolymph during the last two stadia was monitored because this hydrolytic pathway is largely responsible for the metabolism of JH in *T.ni*; JHs and juvenoids were applied topically to determine those times sensitive to the presence of JH; and JH titres were determined in parasitized and control animals using the *Galleria* bioassay (de Wilde *et al.*, 1968).

The effects of different JHs and juvenoids upon this host/parasite relationship were also examined: it may be only the Lepidoptera that possess all the known homologues of JH (JH 0, JH I, JH II, JH III) whilst the hymenopteran and other insect orders seem to have only JH III in their systems (Trautman *et al.*, 1974; Schooley *et al.*, 1984).

Materials and Methods

Chemicals and solutions

If not otherwise stated, TMK-buffer (Tris -HCl, 0.01 M, $MgCl_2$, 0.005 M, KCl, 0.15 M, pH 7.4) was used as physiological saline for all purposes. JH I [methyl (2E,6E, 10-cis)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate, MW=294], JH II [methyl (2E,6E, 10-cis)-10,11-epoxy-3,7,11-trimethyl-22,6-tri-decadienoate, MW=280] and JH III [methyl (2E,6E)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate, MW=266] were purchased from Calbiochem. The juvenoid, Ro 10-3108, (1-(4′-ethlyphenoxy)-6,7-epoxy-3-ethyl-7-methyl nonane, MW=303) was a gift from Dr Peter Masner, Dr Maag AG, Switzerland, while R 20458 [(2E)-1-(4′-ethyl phenoxy)-6,7-epoxy-3,7-dimethyl-2-octene was synthesized in this laboratory (Mumby & Hammock, 1979).

Insects

Mass rearing of *T.ni* followed the procedure of Roe *et al.* (1982) using diet number 1. The

parasitoid was collected in Ethiopia by E. F. Legner (University of California, Riverside) and thought to be closely related to *Chelonus curvimaculatus* Cameron. A separate *T.ni* culture was used for the rearing of the parasitoid. Paper towels upon which *T.ni* had oviposited were collected daily from the adult cages and were exposed to adult *Chelonus* females held in a plexiglass cage and fed honey–water (1:1). Time allowed for stinging ranged from 2 to 6 h depending on the number of adult parasites and host eggs. Thereafter, the egg-sheets were treated similarly to unparasitized ones. Adult parasitoids were collected daily by aspiration. Care was taken to have a high percentage of parasitized hosts, ranging above 80% of the developing larvae. The rate of parasitization was determined by setting aside fifty larvae and checking them for premature spinning in the late fourth stadium (L4). Parasitized host insects for all experiments were separated based on the easily recognizable stage of apolysis (D0) of their larval moults (i.e. L2D0, L3D0, L4D0). Our culture shows a high degree of synchrony in that >80% of the larvae apolyse and then ecdyse on the same day. Characteristically, there is one full day of feeding between these moults (i.e. L1D1, L2D1, L3D1, L4D1). The time-course of precocious development observed in fourth instar parasitized *T.ni* shows the same morphological markers as observed in fifth instar nonparasitized *T.ni* (Jones *et al.*, 1981), with wandering and spinning behaviour on the third full day (L4D3) and parasitoid emergence on the fourth day (L4D4) at *c.* 8 h after lights on (ALO).

Parasitoid larvae were implanted in or removed from host larvae anaesthetized with carbon dioxide. Larvae were removed from their hosts by making a small lateral incision into the abdominal region. To reduce the encapsulation rate of implanted parasitoids, reduced glutathione (SIGMA) was added to the transferring buffer (Brewer & Vinson, 1971). A small amount of gentamycin sulphate (SIGMA) was then added and the wound allowed to dry before removing the insects from the carbon dioxide source.

For transfusions, 10 μl of haemolymph were collected on L3D1 and L4D0 in glass microcapillaries through small cuts in the abdominal prolegs and immediately injected into the dorsal vessel of nonparasitized larvae anterior to the Malphigian tubules. Simultaneously, a comparable amount of haemolymph was withdrawn by cutting one of the last pair of prolegs. For the collection of teratocytes, insects were immobilized in ice-water on L2D1, L3D1 and L4D0, carefully cut longitudinally and the haemocoel washed out with buffer containing 125 mM KCl. After removal of coarse material and of the parasitoid larvae, the solution was centrifuged at 400 *g* and 4°C for 45 min. The pellet was resuspended in buffer so that 1 μl contained the equivalent 'wash out' of four parasitized *T.ni*: each recipient larva received the haemocytes and teratocytes recovered from four parasitized larvae. Control larvae received either haemocytes from unparasitized animals or 1 μl of buffer. All injected larvae were of the same age as the donor.

Extractions

The following media and extraction procedures were used on parasitized and unparasitized *T.ni* collected in batches of 500–700 on L4D0 and immediately frozen at −70°C: glass distilled water containing 0.01% 1-phenyl-2-thiourea (PTU, Aldrich), 0.1 mM phenylmethylsulfonyl fluoride (PMSF, Calbiochem) and 0.5 mM diisopropyl fluorophosphate (DFP, Aldrich); glass distilled water containing 0.01% PTU; 0.1 M ammonium bicarbonate with 0.01% PTU, 0.5 mM DFP and 0.1 mM PMSF; and 1 M acetic acid with 2 mM hydrochloric acid, 0.1 mM PMSF, 0.01 mM Pepstatin A and 0.1% mercaptoethanol. Also, insects were extracted using the method outlined for proctolin (Brown, 1977), and in methanol. Further work-up procedures varied according to the initial extraction medium used. Extractions of JH for the *Galleria* bioassay were done as described by Bergot *et al.* (1981).

Developmental assay

About 5–10% of developing *T.ni* host eggs stung by *Chelonus* produce larvae which spin a cocoon in the penultimate fourth stadium and also follow all other behavioural patterns described for parasitized *T.ni* (Jones *et al.*, 1981; Jones, 1985), but no parasite emerges. Dissection of these 'pseudoparasitized' larvae showed

no signs of a parasitoid larva or any obvious signs of encapsulation. The observation of effects similar to these larvae, referred to as pseudoparasitized, was used as bioassay for the above biological extracts.

The extracts of parasitized and unparasitized L4D0 *T.ni* were tested by application to or injection in normal larvae on L3D1, L4D0 and L4D1. Lipid fractions in petroleum ether/ether or in pentane were applied in 5 μl of acetone to the dorsal surface of normal larvae. The highest dosage tested in each case was five larval equivalents per normal larva. The treated larvae were observed for changes in coloration and behaviour as well as for the time taken to reach the pupal stage. The more polar fractions were injected in 2 μl of glass distilled water at five larval equivalents per normal larva. The pellets were washed with water and similarly assayed.

Anti-JH assay

To monitor possible AJH properties, extracts were assayed using *T.ni* following the procedure outlined by Sparks (1984). Larvae were set apart on L5D0 and on L5D3, all wandering larvae were used for the assay before 4 h ALO. The next day, L5D4, starting at 5 h ALO all treated larvae were scored every hour for pupation, sclerotization or larval–pupal intermediates. The time when 50% of the treated larvae of each assay sclerotized either as pupae or as larval–pupal intermediates was noted for a comparison of the effects of the different extracts with the controls. The same extracts were each tested by topical application or injection using ten larvae. Under these conditions, the known AJH, fluoromevalonate, causes a 8.5 h delay in pupation when applied at 200 nmol/larva.

With both the developmental and the AJH assay, each extract was tested at a minimum of two doses with at least ten larvae tested at each level. An important component of the assay was the use of analogous extracts prepared from unparasitized larvae as well as solvents or buffers as controls.

Activity of juvenile hormone esterase

Parasitized and unparasitized control insects on L3D0 and L4D0 at 6 h ALO were put into separate containers with diet. At specified times, haemolymph was collected from randomly picked larvae by clipping one of the anal prolegs and collecting exuded haemolymph in a calibrated micropipette. The haemolymph was pooled ($n > 15$ for each point), immediately diluted 1:1 with phosphate buffer pH 7.4, I=0.2, 0.01% PTU) and frozen at −70°C until assayed for JHE activity according to the method of Hammock & Sparks (1977) using 5×10^{-6} M ^{3}H-JH III (NEN, specific activity 11.6 Cl/mmol).

Hormone applications

JH I, JH II, JH III, Ro 10-3108 and R 20458 were applied in ethanolic solutions to the dorsal thorax of various stages of isolated *T.ni* larvae with a Hamilton micro-syringe. The solutions contained doses of 30, 3 and 0.3 μg of juvenoid per μl of solution. The amount of ethanol solution used ranged from 0.2 μl for the first two instars, 0.5 μl for the third instar and 1 μl for the fourth instar. Topically applied ethanol had no observed effect on any stage of *T.ni.* Larvae were staged as outlined for the JHE titre. If not otherwise stated, treatments were conducted at 11 h ALO on groups of larvae ($n > 10$). Observations were made during the photophase with parasite emergence and larval development being scored at 12 h ALO.

The effect of an artificially maintained high level of JH on the JHE activity in the haemolymph of parasitized and non-parasitized fourth stadium larvae was tested by a single topical application of Ro 10-3108 to staged larvae on L3D0, L3D1 and L4D0. JHE activity was assayed on L4D1 13 h ALO.

Results

Injection of substances by the adult female

During oviposition, the adult parasitoid female injects fluids with the egg that originate from either the calyx or glands associated with the genital tract. They could either precede or follow the actual egg-laying or they could coat the egg. We have studied the first two possibilities by interrupting the egg-laying, a process that lasts 15–30 s, after 5 and 10 s. An increasing percentage of pseudoparasitized larvae,

TABLE 1. Dependence of the percentage of parasitized *T.ni* on the time allowed for oviposition.

Time allowed for oviposition (s)	Percentage parasitized larvae	Percentage pseudoparasitized larvae	*n*
5	7	0	30
10	40	0	25
Uninterrupted*	85	0	48

*Normal oviposition requires 22±8 s (mean ±SD, *n*=23).

and a decreasing percentage of parasitized hosts with decreasing stinging time would be expected if the fluid from the adult female preceded the actual egg-laying and were responsible for the disrupted development. Assuming that fluids are injected after the deposition of the egg, parasitoid larvae should be expected without any disruption of the development of the host. The results (Table 1) show that the interruption of oviposition after only 5 s leads to a very low percentage of parasitized hosts, but none of the developing *T.ni* developed into an pseudoparasitized larva. A higher percentage of successful parasitization was observed when the adult female was removed from the host egg after 10 s but, again, no pseudoparasitized larvae developed. Without interruption, the number of hosts, from which a parasitoid emerged, was 85%, and no pseudoparasitized larvae were observed during the experiment. It therefore seems unlikely that fluids injected separately from the egg, i.e. not coating it, are the cause of the disrupted development.

Parasitoid larva

Two experimental approaches were taken to evaluate the role of the parasitoid larvae as a source of the factor disrupting development. First, parasitoid larvae were dissected out of their host on L3D0, L3D1 and L4D0 and implanted into unparasitized *T.ni* of the same age. Second, the first instar parasitoid larvae on L2D1, L3D0, L3D1 and L4D0 of the host were removed, and the development of the former host observed.

Although two out of the seven implanted parasitoid larvae developed and finally emerged out of their second host, the effects associated with normal parasitization were not observed. None of the hosts started spinning in the fourth instar, nor did they show the colour change typical of normal parasitized larvae that stop feeding. All of the twelve surviving larvae from which the parasitoid was removed developed into pseudoparasitized larvae further indicating that the parasitoid alone cannot induce precocious development after the first instar.

Teratocytes

In host larvae parasitized by *Chelonus* sp., teratocytes are found in the haemolymph from the first instar of the host up to L4D1. At later stages than this, no teratocytes have been found (unpublished observations). To investigate their possible role in the disruption of host development, two experiments were undertaken. A sample of haemolymph (10 μl) from parasitized and nonparasitized control larvae was collected on L4D0 and L4D1 and injected into nonparasitized *T.ni* of the same stage from which an equal volume of haemolymph was simultaneously withdrawn by bleeding. Experimental and control insects developed with considerable delay (up to 4 days) but all surviving larvae moulted to the fifth instar without resembling pseudoparasitized larvae. The same result was observed in experiments where cellular material from L2D1, L3D1 and L4D0 was enriched by centrifugation and the equivalent of four *T.ni* injected into non-parasitized *T.ni* of the same age.

Effects of parasitization on the activity of juvenile hormone esterase and juvenile hormone in the haemolymph

Haemolymph JH hydrolytic activity is manifested periodically at apolysis throughout the early stadia of both parasitized and nonparasitized *T.ni* larvae (Fig. 1). These peaks of JHE activity are referred to as 'apolysis peaks'. The

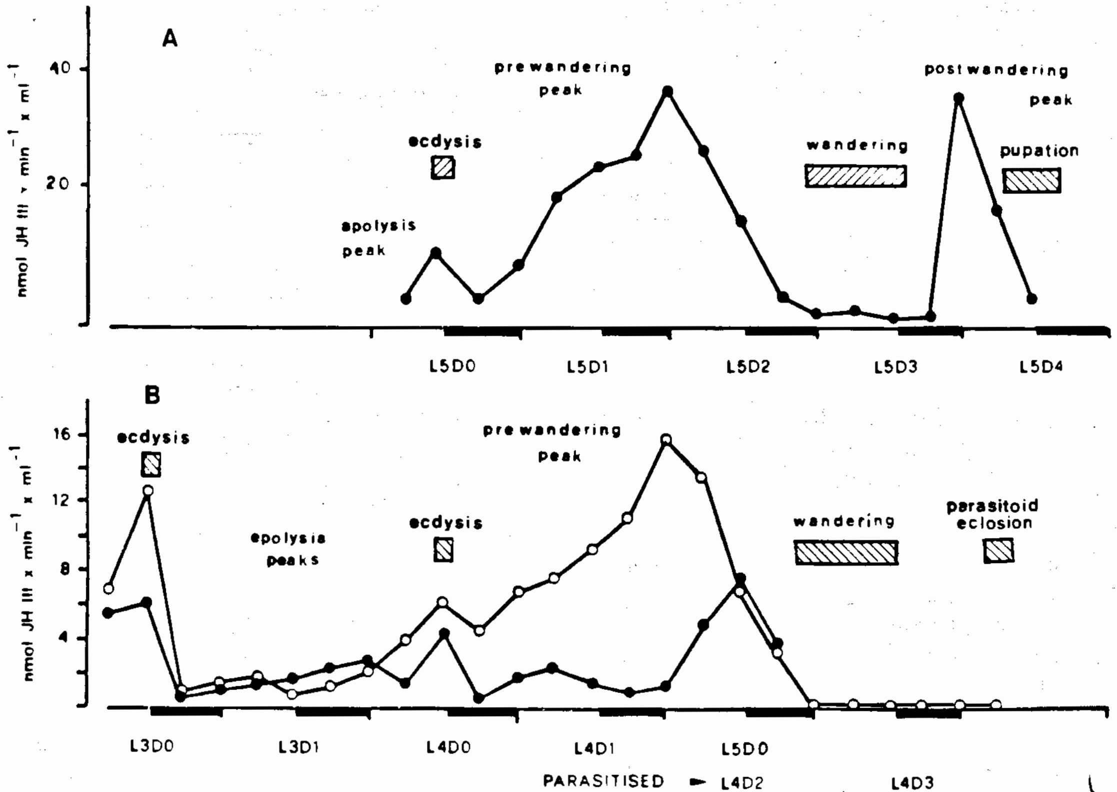

FIG. 1. Haemolymph JHE titres from the third through to the last larval stadium of parasitized and non-parasitized *T.ni*. Fig. 1B allows comparison of JHE titres in normal *v*. parasitized third and fourth stadia larvae of the same chronological age. Fig. 1A is arranged to facilitate comparison of JHE titres in non-parasitized fifth stadium larvae (A) with titres in precocious fourth stadium parasitized larvae of similar physiological age (B). Data were obtained on haemolymph pools from ten to forty individual larvae. Assay variation is less than 3% of the illustrated values. Data for non-parasitized fifth instar *T.ni* were adapted from Sparks *et al.* (1979) and Wing *et al.* (1981).

JHE activity in the parasitized fourth instar follows a similar course at approximately half the specific activity of that of the prewandering peak in the fifth and final stadium of non-parasitized *T.ni* (Sparks *et al.*, 1979; Wing *et al.*, 1981). The parasitized *T.ni* JHE titre does not have a postwandering peak of activity on L4D4, the day of parasitoid emergence. As expected, the increased JHE activity leads to a greatly reduced JH titre in parasitized early fourth instar larvae. For instance, the average JH titres by the *Galleria* assay are 2840±390 *Galleria* units per ml (GU/ml) in early L4 control larvae but only 700±325 GU/ml in early L4 parasitized larvae (average of three different times from L4D0 and L4D1). The difference in the profiles of JHE activity in parasitized and non-parasitized fourth instar larvae indicates that the parasitoid has disrupted host development on or before the scotophase of L4D0.

Extraction experiments

The AJH bioassay never showed consistent evidence for factors interfering with the JH titre. Regardless of extraction method, injection or topical application of up to five larval equivalents of parasitized *T.ni* per treatment failed to delay the time of 50% pupation in comparison with identically prepared extracts from non-parasitized control insects. Similarly, the developmental bioassay never yielded pseudoparasitized larvae using the same extracts on non-parasitized larvae on L3D1, L4D0 or L4D1. In some cases, extracts from

both parasitized and non-parasitized larvae led to developmental aberrations.

Effect of exogenous treatment with juvenoids

Up to 60 μg of JH III or R 20458 applied on any day of the fourth stadium had no observed effect on the parasitized *T.ni* larvae. The parasitoids contained within these larvae emerged with the control parasitoids. However, JH III applied at any dose on L4D1 caused a high percentage (48%; n=10 out of 21) of parasitoids to display normal development of head and thorax but retarded development of the abdomen.

Topical applications of JH I, JH II or Ro 10-3108, on the other hand, had similar effects in producing a variety of responses in both the parasitoid and its *T.ni* host. Precocious development, as measured by the morphologically distinct wandering form of larval development, was delayed or prevented by Ro 10-3108 and (to a lesser extent) by JH I and JH II if present before L4D2, 11h ALO (Table 2). *T.ni* treated at dosages of 3 μg or greater prior to this time remained in the larval form, moulted

TABLE 2. Effect of JH I and Ro 10-3108 on precocious development of parasitized *T.ni.**

Stage of treatment	Compound	Dose (μg)	*n*	Effect on precocious development of *T.ni*
L3D0	JH I	0.15	13	None
		1.5	14	Delayed 1 day
	Ro 10-3108	0.15	12	Delayed 1 day
		1.5	10	Delayed 3 days
		15	10	Overcome†
L3D1	JH I	0.15	9	None
		1.5	9	Delayed 1 day
	Ro 10-3108	0.15	10	Delayed 2 days
		1.5	9	Delayed 3 days
		15	10	Overcome†
L4D0	JH I	0.3	15	None
		3.0	15	Delayed 1 day
		30	15	Overcome†
	Ro 10-3108	0.3	25	Delayed 1 day
		3.0	23	Overcome†
		30	26	Overcome†
L4D1	JH I	0.3	15	Delayed 1 day
		3.0	16	Overcome†
		30	9	Overcome†
	Ro 10-3108	0.3	20	Overcome†
		3.0	22	Overcome†
		30	22	Overcome†
L4D2	JH I	3.0	10	None
	Ro 10-3108	3.0	10	None
		30	10	None

*Compounds were applied topically once in ethanolic solution at 11 h ALO. *T.ni* were observed daily at 12 h ALO for the time required for >50% to reach the wandering stage of development or until parasitoid emergence or mortality occurred. Mortality was observed only in situations of arrested development. Ethanol-treated parasitized larvae wandered on L4D3. Delays are expressed as days after control larvae wandered. In some cases treated larvae wandered as L5 rather than as L4. JH III applied at these doses had no effect.

†Refers to situations in which the larvae moulted to L5 and were never observed to wander. In most of these cases the prewandering hosts were killed when the parasites emerged.

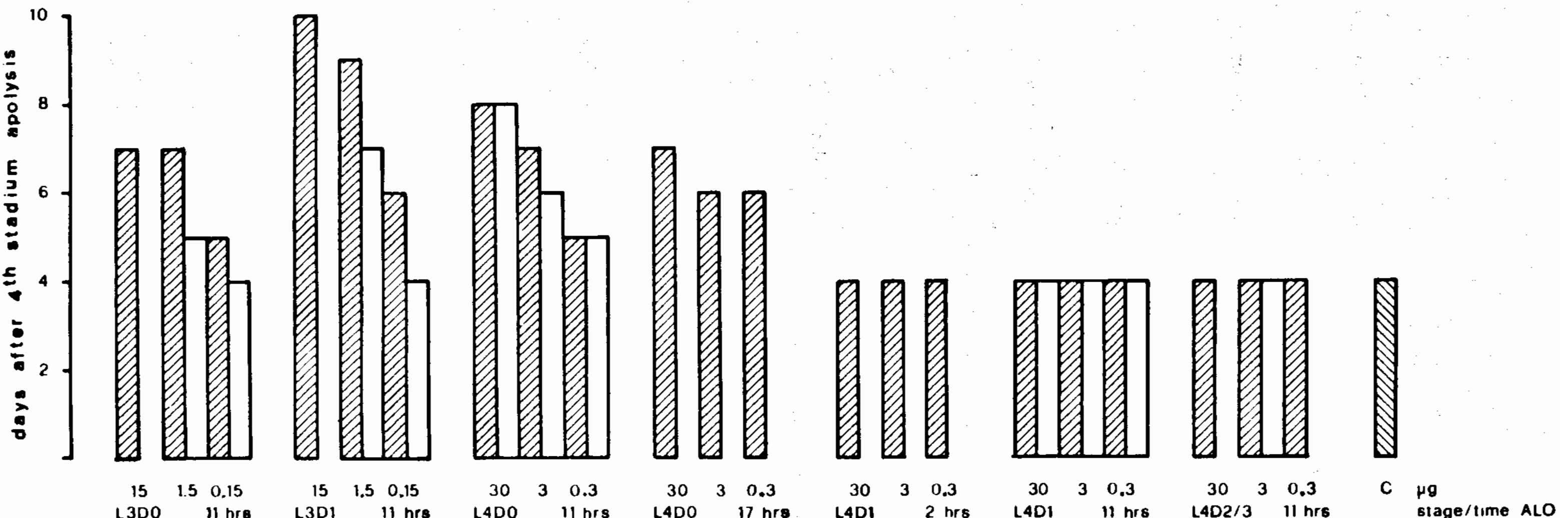

FIG. 2. Emergence pattern of *Chelonus* sp. from their *T.ni* hosts after a single topical treatment of the host *T.ni* with three different doses (expressed in μg) of JH I (open columns) and Ro 10-3108 (hatched columns) in ethanolic solution. Emergence (ordinate) is expressed as days when >50% of the parasitoid larvae have emerged by 12 h ALO after apolysis of the *T.ni* larvae into the fourth stadium (n=10–26, two replicates). The juvenoids were applied on seven different days from L3D0 through L4D3. Controls with and without ethanol application were performed at each time and always resulted in emergence after 4 days. No delay was observed following application on L4D1 until L4D3. Some mortality was observed in *T.ni* hosts prior to parasitoid emergence only after >3 days in arrested development (6 days after apolysis). JH experiments were repeated with JH II (data not shown) which proved to be only slightly less active than JH I. When experiments were repeated with JH III and R 20458 (data not shown), no delays in parasitoid emergence were observed.

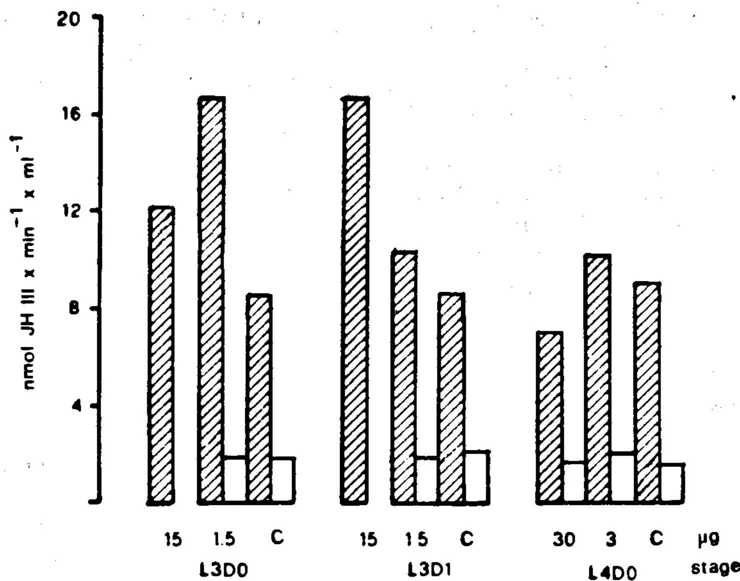

FIG. 3. Activity of JHE on L4D1 11 h ALO in the haemolymph of parasitized (hatched columns) and non-parasitized (open columns) *T.ni* after a single topical treatment at 11 h ALO with Ro 10-3108 in ethanol. Doses of Ro 10-3108 were used which prevent the parasitoid induced precocious morphological and behavioural changes associated with development towards the pupa.

on L4D2 and continued to feed as if they were non-parasitized. In this group, no further moults were observed and either parasitoids emerged from the hosts while the host larvae were still in the non-wandering larval form or, in a small minority of cases in which the parasitoids were evidently not viable, the wandering stage of the host was delayed in a dose-dependent manner with no subsequent development. Parasitized *T.ni* that were treated on L4D2 and subsequently, behaved as the controls.

Parasitoids associated with *T.ni* that had been topically treated with JH I, JH II or Ro 10-3108 displayed a critical time in the late scotophase of L4D0 (Fig. 2): before this they are sensitive to the presence of these compounds as evidenced by a dose-dependent delay in emergence, but subsequent to this critical time their emergence is unaffected by the doses applied. As with JH III, developmental aberrations occurred in the parasitoids if hosts were treated immediately before L4D2. Daily topical applications of a low but biologically active amount of JH I (0.3 µg, n=20), from the first stadium of parasitized *T.ni* (L1D1) to the beginning of the final stadium (L4D0), failed to prevent precocious development. Topical treatment at this dose of JH I from L3D0 to L4D2 caused *T.ni* to remain as larvae in the fourth stadium until the parasites emerged 2 days later than normal from the prewandering larval form of *T.ni.*

Onset of JHE activity

Topical application of Ro 10-3108 to the penultimate parasitized instar (at two different doses known to overcome the precocious development and to cause a larval moult on the second day of the fourth stadium, L4D2) failed to prevent the onset of JHE activity in this instar as measured on L4D1 (Fig. 3). These effects signify that while the overt morphological and behavioural changes associated with precocious development are prevented by the exogenous application of Ro 10-3108, precocious appearance of JHE still occurs.

Discussion

Using the *T.ni–Chelonus* sp. system, we have studied the mechanisms used by the parasitoid to induce premature spinning of its host in the

penultimate stadium. Similar studies have been carried out with other systems. Rectal and salivary glands of developing larvae of *Hyposoter exiguae* apparently secrete proteinaceous material causing pathological changes mainly in the fat body of the host, *Trichoplusia ni* (Iwantsch & Smilowitz, 1976), and the salivary glands of *Diaplazon pectoratorius* are thought to be responsible for precocious termination of diapause of the syrphid host, *Epistrophe bifasciata* (Schneider, 1950). The release of a factor by the endoparasitoid *Apanteles glomeratus* at the moult from the first to the second instar is thought to decrease the weight gain of the host, *Pieris brassicae,* thereafter (Fuehrer & Keja, 1976).

In the *T.ni–Chelonus* sp. system, our results do not point to the developing parasitoid as the source of the disrupted development. Removal of the first instar parasitoid from its host on L2D1 or later resulted always in pseudoparasitized larvae spinning in the penultimate fourth stadium with subsequent arrest of development. Considering the degree of development reached by the parasitoid at this stage (also described as a waiting stage; Broodryk, 1969), we believe it is unlikely to be the source of the factor which causes precocious development. Ultimate proof is lacking for this hypothesis since the parasitoid could have released a factor or caused the effect before L2D1.

Furthermore, it is unlikely that the feeding of the endoparasite leads to the disrupted development. First instar parasitoid larvae implanted into non-parasitized *T.ni* on L3D1 developed without ever disrupting host development before their emergence.

It has been claimed that development of the host *Heliothis virescens* is regulated by the adult female of *Chelonus insularis* injecting calyx and poison gland fluid during oviposition (Ables & Vinson, 1981). However, because the experiments described by these authors led to 100% pupation and never induced premature spinning with subsequent arrested development, as observed for our host–parasitoid system as well as for *H.armigera* parasitized by *Chelonus curvimaculatus* (Broodryk, 1969), it cannot be assumed that the effect of parasitization is due to this source. Our results show that decreasing the time allowed for oviposition is followed by a decrease in the percentage of parasitized hosts. *T.ni* that were not parasitized, developed without delay into fully viable pupae showing none of the characteristic changes observed after parasitization. From all the observed insects, regardless of stinging time, no pseudoparasitized larvae developed, giving strong evidence against the existence of a factor other than the egg (that may also be coated with substances from the genital system) which leads to arrested development.

Finally, several functions have been claimed for the teratocytes originating from the serosa membrane of the developing parasitoid embryo and liberated into the host haemocoel after the parasitoid hatches. Teratocytes were thought to be a food source for the developing parasitoid (Jackson, 1928), but in some species of parasitoids they were shown to interfere with the host's defence mechanisms (Vinson, 1972) or they were claimed to delay development of the host (Vinson, 1970). Joiner *et al.* (1973) claimed that the teratocytes of *Cardiochiles nigriceps* have JH-like activity without giving subsequent explanations for possible functions of the contained JH. There is no conclusive evidence for the claimed roles of the teratocytes in the above host–parasitoid systems, nor were we able to provide positive evidence for the function of the teratocytes in our host–parasitoid system. Possibly, transfusion of larger numbers of teratocytes, or use of younger recipient larvae, may be required to duplicate the effect observed in the normal *T. ni–Chelonus* sp. system.

It is difficult to exclude the presence of a factor by negative data. However, the extraction procedures used included ones which would extract putative factors of varying polarity and charge. Several different bioassays were used based upon both induction of precocious development in several stages and prevention of the actual pupation process. Extracts also were analysed by both topical application which reduces artefacts, and injection which allows the detection of polar materials. Thus our data indicate that discovery of such a factor may not be simple and certainly would not have detected multiple factors.

Along with the negative results from our efforts at extracting a putative AJH factor, certain observations lead us to conclude that the mechanism of disruption of development by *Chelonus* may not be due to an induction of

an allatoinhibin which lowers the JH titre to levels that allow premature development. Daily topical treatment with a low but active dose of JH I from the first through the third stadia, failed to prevent the parasitoid's effect.

Higher doses applied after the end of the second stadium prevents or delays the overt precocious morphological development associated with the prepupal stage (Table 2). However, such application does not prevent the precocious, sustained appearance of JHE, an event unique to development towards the pupa. Thus only a part of the phenomena associated with the precocious development are influenced by JH. It appears that the mechanism by which *Chelonus* disrupts its host's development works at a more fundamental level which both induces JHE production in the fat body (Wing *et al.*, 1981) and inhibits JH synthesis in the corpora allata. Simply stated: the mechanism of the disrupted development may not be so much 'anti-Juvenile Hormone' as it is 'pro development'. The possibility that a simple induction of JHE occurs to lower the JH titre and thus cause precocious development is unlikely as the timing and activity profile of JHE in the initial 3 days of the final instar of both parasitized and non-parasitized host larvae are identical, thus indicating endogenous control by *T.ni.* Experiments to test this hypothesis are now being undertaken.

The results from the topical applications of the hormones and juvenoids are intriguing in that they indicate the potency of JH I, JH II and Ro 10-3108 in effecting behavioural and developmental aberrations on both organisms, while JH III affects only the subsequent development of the hymenopteran parasitoid after a normal emergence time from its lepidopteran host. Doses of JH III as high as 60 μg applied on L4D0 caused stunted growth but with a normal development time. *T.ni* has been shown to have predominantly JH II, with minor amounts of JH I and JH III, while its hymenopteran parasitoid is believed to have only JH III (Jones, 1984). These observations reveal a possible discrimination by the parasitoid between its own JH III and those of its host, i.e. the parasitoid cues its emergence on the absence of its host's JH II but not its own JH III. This is not simply due to failure of JH III to reach target tissues for some reason as developmental aberrations occurred in the parasitoid with JH III application on L4D1 thus indicating JH associated activity in the parasitoid. Another explanation is that the cue for emergence is secondary to a host JH associated event.

These data indicate that the development of the parasitoid is closely associated with that of its host. However, implantation and juvenoid treatment result in parasitoids emerging from prewandering L4 or L5 larvae, thus parasitoid development and emergence are not irreversibly tied to the host reaching the prepupal stage.

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