N O T E

Unusual Responses of Indianmeal Moth Larvae (Lepidoptera: Pyralidae) to Envenomation and Parasitization by a Braconid Ectoparasitoid[1]

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Two unusual physiological responses by host larvae were noted during studies on trophic interactions of the ectoparasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae) with the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Baker and Fabrick, 2000, Insect Biochem. Molec. Biol. 30: 937-946). In the first case, we found a pseudo decrease in moisture content in paralyzed host larvae when moisture content was determined by heating at 60°C. In the second case, an unusual immune response occurred when small wasp larvae were transferred from one host larva to a second host larva paralyzed by a different adult wasp. Experiments describing these observations and brief discussions of their possible biological significance are presented below.

Water relations. Moisture content of paralyzed larvae of *P. interpunctella* was significantly reduced compared with that of unparalyzed larvae when moisture content was calculated from gravimetric data obtained at 60°C. The apparent moisture loss at 60°C was ultimately found to be a measure of an apparent physical change in paralyzed larvae and not an actual loss of total body water content as first thought. Tests describing the effect of envenomation on water relations in the host larvae are described below.

Groups of 20 host larvae of *P. interpunctella* (El Paso strain) were removed from the laboratory diet and placed in 100 × 15 mm plastic Petri dishes with 5 adult *H. hebetor*. Larvae that were paralyzed within 3 h were removed and held at 25°C and 55% RH. Moisture contents of 3 replicates of 5 larvae per replicate were determined at 0, 20, and 48 h after paralyzation by weighing the larvae in groups of 5 before and after heating for 3 h in a forced air oven at 60°C. Results are based on three separate tests.

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The mean initial weight of larvae used in these studies was 7.7 ± 0.2 (S.E.) mg and there was no significant fresh weight change in stung or unstung (control) larvae after 48 h ($F = 0.26$, df = 4, $P = 0.90$). The initial calculations indicated a dramatic loss in moisture content by 20 h in the paralyzed larvae at 60°C (Fig. 1). This loss increased through 48 h. Concomitantly, the calculations indicated a corresponding dramatic increase in dry matter content in these larvae, from about 40% at 0 time to 67% at 48 h. However, because these larvae had no access to food and there was no significant change in fresh weight during the time period, an increase in dry matter in these larvae was not possible. To help explain this unusual result, we conducted identical tests but measured the larval moisture content at 100°C rather than 60°C. In these tests, the results (Fig. 1) indicated that there was no significant difference in actual water content between paralyzed and unparalyzed larvae.

One hypothesis to explain these disparate results is that stinging and envenomation of host larvae by adult *H. hebetor* may result in a change in the amount of bound or free water in hemolymph or may result in changes in colligative properties of hemolymph. One measure of these changes is that water is less able to be driven off when the paralyzed larvae are heated at 60°C; whereas, the water can still be re-

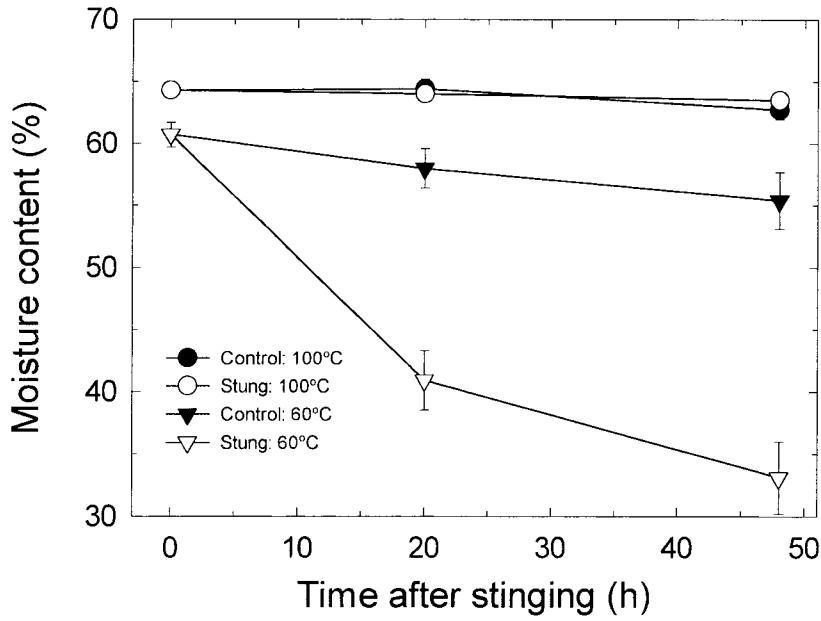

Fig. 1. Comparison of moisture content of paralyzed (open symbols) and control (solid symbols) larvae of *P. interpunctella* stung by *H. hebetor.* Moisture content was calculated from data obtained gravimetrically at 60°C (triangles) or 100°C (circles). [Note: Less water is driven off when stung larvae are dried at 60°C, giving the appearance of a reduced moisture content compared to that of control larvae that were not stung]. Means ± S.E. of 9 groups of 5 larvae/group for each treatment at each time interval.

moved by heating at 100°C. It is known that hemolymph becomes more difficult to collect beginning approximately 48 h after stinging (Fabrick, unpubl. data). During these latter studies, hemolymph did not appear to become more viscous with time after stinging, at least by 48 h, but fat body particles in the hemolymph were more predominant.

A second hypothesis is that envenomation changes cuticle structure in a manner that makes it somewhat more resistant to water loss. Injection of the neuromuscular toxin present in the venom of *H. hebetor* (Piek et al., 1982, Comp. Biochem. Physiol. 72C: 303-309; Quistad et al., 1994, Insect Biochem. Molec. Biol. 24: 955-961) results in flaccid host larvae with almost no muscular control, although the heart can continue to beat for 4 to 5 wks. Envenomation probably also results in loss of spiracular control which could also conserve host water content.

It is not known if these physical changes in host physiology facilitate subsequent feeding by larval parasitoids. After an adult wasp paralyzes the host, the time required for oviposition and egg hatch would exceed 24 h, so when newly-hatched larvae initiate feeding the changes in host physiology are already occurring. Paralyzed pyralid larvae are suitable hosts for adult feeding and progeny production by *H. hebetor* for periods up to 4 to 5 wks after stinging (Hagstrum and Smittle, 1978, Environ. Entomol. 7: 596-600). Physical changes induced by venom components after envenomation may play an important role in maintaining host suitability during these extended time periods.

Immune response. In previous studies (Fabrick et al., unpubl. data) we observed an interesting immune response by paralyzed *P. interpunctella* larvae to feeding by larval *H. hebetor*. We routinely obtain larval parasitoids by placing groups of wandering stage *P. interpunctella* larvae into Petri dishes along with adult *H. hebetor*. Parasitization occurs and larval parasitoids are soon obtained. We have reared hundreds of parasitoids on host larvae in this manner and only occasionally do we observe any immune response of hosts to the feeding parasitoid larvae. However, it was noted that when paralyzed host larvae were removed from these dishes with forceps, placed in a separate dish, and a single small wasp larva obtained from a different group of parasitized hosts is picked up with a single-hair brush and placed on each "surrogate" host, a strong immune response to feeding by these wasp larvae was noted in a high percentage of surrogates (Fig. 2A).

The typical immune response of the surrogate host to feeding by the parasitoid larva consists of a circular melanized area that contains a central unmelanized or clear zone that is also circular (Fig. 2B,C). A fine black slit (Fig. 2C,D) is located on the cuticle in the center of the unmelanized region. This slit apparently marks the location where the larval mouthparts penetrate the host cuticle and host hemolymph is ingested. Mean ± S.D. size of circular immune response marks was determined to be 0.229 ± 0.075 mm diam with a mean area of 0.046 ± 0.027 mm^2 (n = 58). The mean diam of the clear inner circle was 0.093 ± 0.051 mm with an area of 0.009 ± 0.008 mm^2. The ratio of areas of outer/inner circle was 7.8 ± 6.9. Of 58 feeding marks examined, 6 were solid black, with no clear zone in the center. Not all feeding marks were symetrical, and some marks were crescent-shaped (Fig. 2D).

To determine if prior feeding by the wasp larva is required to initiate the immune response in the surrogate host, we compared the effects of transfer of wasp larvae to that of transferring a wasp egg onto the surrogate and allowing the newly-hatched wasp larva to feed. In this test, surrogate larvae paralyzed within a 2-h period were isolated. Small wasp larvae (that were feeding on a previously paralyzed host) were

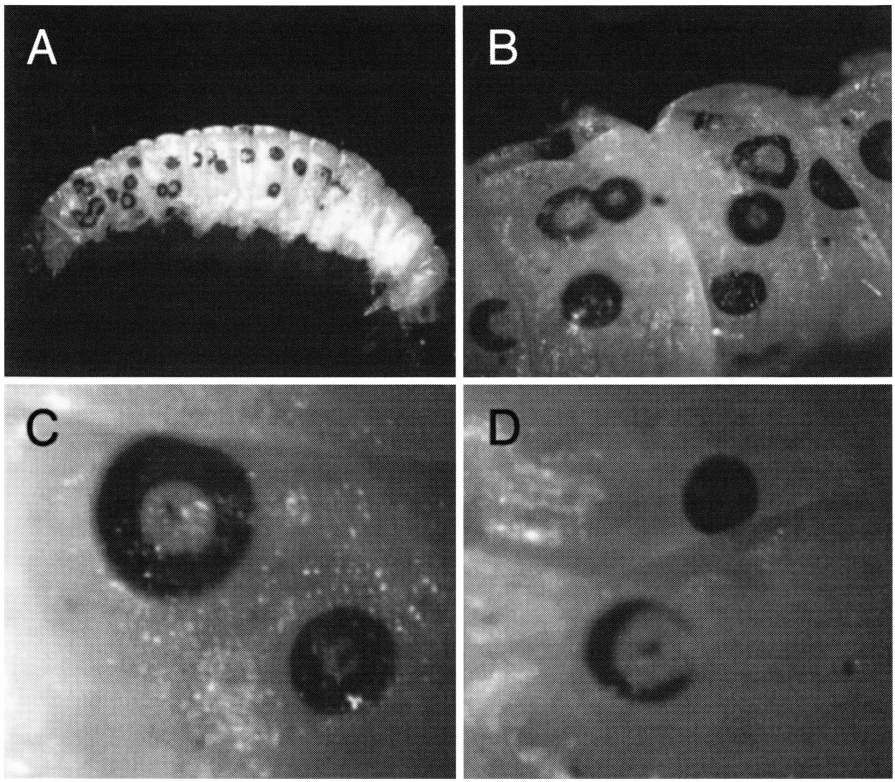

Fig. 2. Immune response of paralyzed surrogate hosts (*P. interpunctella*) to feeding by a small *H. hebetor* larva transferred from a primary host: (A) surrogate host larva with immune response; (B & C) close-up views of black circular immune response; (D) crescent immune response. Black feeding slits in the center of clear zones can be seen in C and D.

removed and transferred onto 20 surrogate hosts. Single wasp eggs were also removed from previously paralyzed hosts and placed on each of 63 surrogate hosts. Results indicated that 13/20 (65%) surrogate hosts initiated an immune response to transfer of larvae while 27/57 (47%) surrogate hosts initiated an immune response to larvae hatched from eggs placed directly onto the paralyzed surrogates. One surrogate in this experiment had 40 feeding marks induced by a newly-hatched wasp larva. These results provide no evidence that ingested food from a prior host is subsequently regurgitated into the surrogates and is implicated in producing the immune response.

In separate tests, we found that 28/53 hosts paralyzed during a discrete 1 h period and held for 7 d at 25°C and 55% RH were still capable of producing the immune response when fed upon by transferred larvae. In addition 15/18 surrogate hosts that were from a dish in which adult wasps were exposed to the larvae for 48 h, rather than the usual 1 to 2 h exposure, initiated the immune response. Finally, we found no

evidence that handling of the surrogate hosts with forceps was a factor involved in activation of the immune response. In these latter tests, 20/35 hosts that were handled gave an immune response to the wasp larvae, while 23/34 hosts that were not handled also gave an immune response.

As mentioned above, we only occasionally observe an immune response in hosts (*Plodia*) being fed upon by *H. hebetor* larvae in normal rearing cultures. Because we know that the prophenoloxidase (PPO) system in paralyzed larvae can be activated by wounding (unpubl. data), the lack of an immune response (melanization) by host larvae to feeding wounds during routine culturing indicates that for some reason the immune system is not activated. It also provides evidence that an immunosuppressant may be present as a venom component in the adult wasp and/or that larval parasitoids inject an immunosuppressant during feeding. The question remains as to how the mere transfer of a wasp larva or egg initiates the immune response in a surrogate host.

Our initial attempts to characterize parameters that activate the immune response in surrogate hosts have not been successful. It is likely that an interaction of biochemical elicitors in both adult and larval parasitoids, as well as the host larva, is involved. Adult *H. hebetor* are often observed probing already-paralyzed larvae. We do not know if they are injecting additional venom during this time or how much the titer of venom components varies among paralyzed larvae. Not all surrogate hosts give the immune response and perhaps differences in levels of both venom components and PPO activity in these hosts may be involved. In addition to toxins, hymenopterous venoms contain a number of enzymes, including phospholipase A and hyaluronidase, as well as several smaller biologically-active peptides (Schmidt, 1982, Ann. Rev. Entomol. 27: 339-368). Although the neuromuscular toxins in *H. hebetor* venom have been characterized, there is no information on the presence or activity of specific enzymes or peptides, or other venom components in this ectoparasitoid which may affect the immune response of the host.

Although the larva of a eulophid ectoparasitoid has been shown to secrete proteins during host feeding (Richards and Edwards, 2001, Arch. Insect Biochem. Physiol. 46: 140-151), we have no electrophoretic evidence that proteins from larval *H. hebetor* are regurgitated into the host (Baker and Fabrick, 2000). Otherwise, virtually nothing is known of any physiological aspects of feeding in this braconid ectoparasitoid and whether there is any fluid exchange between parasitoid and host while host hemolymph is being ingested. We also do not know if the black circular immune response in surrogate hosts results from prophenoloxidase activity in hemocytes attracted to the feeding wound or if a prophenoloxidase present in the cuticular epidermis is activated. The central clear zone, similar to the "bullseye" associated with tick bites and lyme disease, may be the result of an immunosuppressant injected by a feeding wasp larva. The distinct borders that differentiate the melanized from the unmelanized regions are certainly intriguing and indicate tight control of factors responsible for the response.

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