Behavioral Interactions Between the Hyperparasitoid *Mesochorus discitergus* (Hymenoptera: Ichneumonidae) and Four Species of Noctuid Caterpillars: Evasive Tactics and Capture Efficiency[1]

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J. Entomol. Sci. 29(3): 420-427 (July 1994)

ABSTRACT The hyperparasitoid, *Mesochorus discitergus* (Say), oviposits into larvae of primary parasitoids while the latter are developing inside various lepidopteran hosts. To accomplish hyperparasitism, *M. discitergus* must capture the lepidopteran larvae. We studied the evasive behaviors of 2nd-, 3rd-, and 4th instar *Helicoverpa zea* (Boddie) (corn earworm), *Pseudoplusia includens* (Walker) (soybean looper), *Spodoptera frugiperda* (J. E. Smith) (fall armyworm), and 2nd- and 4th-instar *Plathypena scabra* (Fabricius) (green cloverworm), as well as the effectiveness of these tactics in eluding capture by *M. discitergus* females. Most individuals of all instars of corn earworm and fall armyworm made minimal efforts to escape. Although not commonly used by corn earworm and fall armyworm, their occasional vigorous responses were effective in deterring *M. discitergus* females. Dropping from a leaf, either unattached or attached by a thread, was also effective for preventing capture of those two noctuid species. Soybean loopers and green cloverworms responded much more actively. Second-instars of both species dropped off the leaf on a thread, while 3rd- and 4th-instar soybean loopers commonly used vigorous responses. Fourth-instar green cloverworms used vigorous responses and dropping off the leaf. Overall, 68% of the caterpillars using the vigorous response were captured, while 39% of those dropping on a thread and about 26% of those dropping off a leaf, unattached by a thread, were captured. Green cloverworms and soybean loopers were more difficult to capture than corn earworms and fall armyworms.

KEY WORDS *Cotesia marginiventris, Helicoverpa zea, Plathypena scabra, Pseudoplusia includens, Spodoptera frugiperda.*

The capture efficiency of a parasitoid may be influenced both by its tactics and the defensive behavior of a potential host. Lepidopteran caterpillars use an array of behaviors to elude capture by generalist parasitoids (Gross 1993, Witz 1990), but the effectiveness of the behaviors is not well documented. Some parasitoids with limited host ranges use specialized behaviors to capture hosts (Yeargan and Braman 1986), but generalist parasitoids must overcome many different defensive behaviors.

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Members of the Mesochorinae (Ichneumonidae) are hyperparasitic on parasitoids in the families Braconidae, Tachinidae, and Ichneumonidae (Dasch 1971). *Mesochorus discitergus* (Say) attacks many species of primary parasitoids, including the generalist parasitoid, *Cotesia marginiventris* (Cresson) (Dasch 1971). The set of lepidopteran larvae attacked by both *C. marginventris* and *M. discitergus* includes: *Helicoverpa zea* (Boddie) (corn earworm), *Spodoptera frugiperda* (J. E. Smith) (fall armyworm), *Plathypena scabra* (F.) (green cloverworm), and *Pseudoplusia includens* (Walker) (soybean looper) (Carlson 1979, Marsh 1979). Therefore, to parasitize *C. marginiventris* residing in any of these caterpillars, *M. discitergus* females must first overcome the defensive behavior(s) of that caterpillar. We studied the evasive behaviors of these four lepidopteran species and the effectiveness of these behaviors in eluding capture by *M. discitergus*.

Materials and Methods

Soybean loopers were obtained from the Southern Field Crop Insect Management Laboratory, Stoneville, MS. Corn earworms were obtained from the Georgia Coastal Plain Experiment Station, Tifton, and fall armyworms were obtained from Louisiana State University, Baton Rouge. All three caterpillar species were reared in the laboratory over multiple generations. A colony of green cloverworms was maintained in our laboratory and periodically augmented with individuals collected from alfalfa fields in Fayette Co., KY. Colonies of both *C. marginiventris* and *M. discitergus* were maintained in the laboratory and supplemented periodically with field collected adults. The colony of *C. marginiventris* was maintained using green cloverworms as hosts (as described by Yeargan and Braman 1986) and the colony of *M. discitergus* was maintained using *C. marginiventris* as hosts within green cloverworms (as described by Yeargan and Braman 1989).

Caterpillars of all four species were allowed to develop to 2nd-, 3rd-, and 4th instars in the laboratory on greenhouse-grown, 'Pella' variety soybeans. In the field, these are the instars most likely to contain stages of *C. marginiventris* susceptible to hyperparasitism by *M. discitergus* (Yeargan and Braman 1989). All caterpillars exposed to *M. discitergus* were exposed to parasitism by *C. marginiventris* three days earlier. Therefore, all caterpillars used in the bioassays presumably contained 3-day-old *C. marginiventris* larvae. The day before experiments, *M. discitergus* females were allowed to capture the noctuid species with which they were to be tested under the following conditions. A capture was defined as a female *M. discitergus* inserting its ovipositor into a caterpillar. A single *M. discitergus* female, 5 to 10 days old, was released from an aspirator into an acrylic cage (31 × 31 × 41 cm; l × w × h) covering a soybean plant [growth stage V3 (Fehr and Caviness 1977)] infested with 10 to 15 caterpillars of the same instar and species. After 5 successful captures by a female, she was removed and used the following day. If females did not capture 5 caterpillars within 30 minutes, they were removed and not used in subsequent bioassays.

Female *M. discitergus* were observed in no choice tests with caterpillars of the same instar and species. One female was released into an acrylic cage (described above) containing a growth stage V3 soybean plant stocked with caterpillars as described above. After an encounter (antennal contact by *M. discitergus*), we recorded the evasive behavior of each caterpillar and the response of *M. discitergus* to that behavior.

Evasive behaviors of caterpillars were placed in one of three categories: minimal response, vigorous response, or dropping off the leaf. We classified a behavior as minimal response if the caterpillar either did not move, attempted to crawl away, or simply curled up. When caterpillars forcefully swung their head and thorax back and forth, regurgitated, attempted to bite the hyperparasitoid, or flipped violently over the surface of the leaf but did not fall from the leaf, a vigorous response was recorded. The last behavioral category was dropping off the leaf, which was divided into two subcategories: cases in which caterpillars dropped but remained attached to the leaf by a silk thread, or cases where the caterpillar dropped without a silk thread to another leaf or off the plant completely.

We used a log-linear analysis (Proc Catmod) (SAS Institute 1988) to determine whether or not different instars of a given noctuid species differed in the use of minimal response, vigorous response or dropping response. A log-linear model is appropriate when testing multidimensional contingency tables (Sokal and Rohlf 1981). We used a linear model for categorical data (Proc Catmod) (SAS Institute 1988) to analyze the effectiveness of different caterpillar behaviors in avoiding capture by *M. discitergus*.

The observations of defensive behaviors of the different noctuid species were not blocked in time because it was not possible to record precise defensive behaviors of caterpillars of all four species at the same time. However, to adequately compare the ease of capture among the four noctuid species, it was necessary to record capture data of all four species blocked in time. Thus, differences in capture efficiency between female *M. discitergus* that may result from random colony effects were minimized.

We tested for differences in ease of capture among caterpillar species by blocking replicates in time (four cages run simultaneously). Ten to twenty 2nd-instar green cloverworms, soybean loopers, corn earworms, and fall armyworms were placed on separate V3 soybean plants in four adjacent cages (dimensions as above). As before, all caterpillars were exposed to parasitism by *C. marginiventris* three days before an experiment. A single experienced *M. discitergus* female was released into each cage, and the number of encounters and the number of successful captures were recorded. The experiment was repeated with 4th-instar caterpillars of all four species.

We analyzed differences in number of captures by *M. discitergus* of different species of noctuid (identical instars) in a two-way ANOVA (SAS 1988, Snedecor and Cochran 1980). Number of captures was divided by the total number of encounters; the proportional values were arcsin, square-root transformed for analysis. The comparison, within instar, of percentage capture by *M. discitergus* between the groupings of sessile (minimal response used in > 50% of the encounters) and active (minimal response used in < 50% of the encounters) caterpillar species was made with an orthogonal contrast.

Results

Defensive behaviors of a given species varied with instar. Differences in capture rates by *M. discitergus* between instars of a given species were not significant. Capture rates varied significantly between different defensive behaviors, but this may be due to the ease with which *M. discitergus* capture caterpillars showing the minimal response. Thus, within each species we compared capture rates among the three remaining behavioral categories, omitting the capture rates of caterpillars showing minimal response.

Encounters between *M. discitergus* and corn earworms or fall armyworms elicited minimal response behaviors from the caterpillars (Table 1). In 153 encounters between corn earworms and M. discitergus females, 111 larvae showed minimal response. The minimal response behavior resulted in a 95% capture rate, while vigorous response (50% capture rate) and dropping from a leaf attached by a thread (44% capture rate) and unattached (45% capture rate) were more effective in evading capture. The last three defensive behaviors did not differ ($x^2 = 2.01$; df = 2; $P = 0.57$) in effectiveness of preventing capture.

We observed 202 encounters between fall armyworm and *M. discitergus;* 127 larvae showed minimal response (Table 1). *M. discitergus* females captured 99% of the caterpillars that responded minimally, while 64% of the caterpillars that responded vigorously, 71% of the caterpillars that dropped on a thread and 55% of the caterpillars that dropped without a thread were captured. Omitting the minimal response behavior from analysis, we find capture rates did not differ significantly among the remaining defenses ($x^2 = 1.95$; df = 2; $P = 0.38$).

Second-instar soybean loopers responded to encounters with *M. discitergus* primarily with either a minimal response or dropping from the leaf (Table 1). The 3rd- and 4th-instar soybean loopers commonly reacted with vigorous responses. More than 94% of the caterpillars using the minimal responses were captured. Seventy-two percent of the caterpillars that responded vigorously, 22% of those that dropped on a thread and 18% of those that dropped without a thread were captured. Omitting the minimal response behavior from analysis, we find significant differences in capture rates among the remaining behavioral categories ($x^2 = 22.79$; df = 2; $P < 0.001$).

In 63% of the encounters between 2nd-instar green cloverworms and *M. discitergus,* the larvae dropped on a thread, while 4th-instar caterpillars used all behaviors in roughly equal proportions (Table 1). Ninety-five percent of the caterpillars exhibiting minimal responses were captured while 81% of those using vigorous responses, 63% of those that dropped on a thread, and none of those that dropped without a thread were captured. Omitting the minimal response category from analysis, we found significant differences in capture rates among the remaining behavorial categories ($x^2 = 35.05$; df = 2; $P < 0.001$).

Mean proportion of successful captures by *M. discitergus* varied significantly among species (Table 2). It appears that the caterpillars form two groups: sessile species (corn earworm and fall armyworm) and active species (green cloverworm and soybean looper). Using orthogonal contrasts, we found that sessile caterpillars were caught more frequently by *M. discitergus* females (Table 2).

Table 1. Numbers of four species of noctuid caterpillars displaying various behaviors in response to an encounter with the hyperparasitoid, *M. discitergus*, and numbers of caterpillars that were caught by the hyperparasitoid.

			Minimal response		Vigorous response		Drop w/ thread		Drop w/out thread	
		Total No	Number		Number		Number		Number	
Species*	Instar	of larvae	responding	caught	responding	caught	responding	caught	responding	caught
CEW	2	63	53	51	4	3	5	2	1	1
	3	45	26	24	11	5	4	2	4	2
	4	45	32	31	7	3	0	0	6	2
FAW	2	81	52	51	9	6	11	9	9	3
	3	42	27	27	11	7	2	1	2	2
	4	69	48	48	11	7	1	0	9	6
SL	2	87	39	37	13	9	21	5	14	3
	3	50	6	5	38	28	2	0	4	1
	4	52	13	13	24	17	0	0	15	2
GCW	2	111	27	25	1	1	70	46	13	0
	4	55	15	15	15	12	14	7	11	1

*Corn earworm (CEW), fall armyworm (FAW), soybean looper (SL) and green cloverworm (GCW).

Table 2. Capture efficiency (± S. E.) by *M. discitergus* of 2nd- and 4th-instar corn earworm (CEW), fall armyworm (FAW), soybean looper (SL) and green cloverworm (GCW) larvae. Ten to 15 encounters per hyperparasitoid female were observed. Two-way ANOVA was used to compare mean percentage capture among species within instar

	No. of females	Percent Capture						
		CEW	FAW	SL	GCW	df	F	*P**
2nd-instar	9	89.9 ± 3.6	87.1 ± 4.2	42.8 ±10.9	57.1 ± 8.5	3,24	9.23	< 0.001
4th-instar	6	92.8 ± 4.0	81.0 ± 4.8	69.0 ± 4.9	70.7 ± 3.8	3,15	5.84	<0.01

* Orthogonal contrasts comparing the combined means of corn earworm and fall armyworm to the combined means of soybean looper and green cloverworm were significant for 2nd- (F = 9.18; df = 1,24; P < 0.001) and 4th-(F = 4.68; df = 1, 15; P < 0.02) instar larvae.

Discussion

Corn earworm and fall armyworm were less active than green cloverworm and soybean looper. The difference in behaviors may be related to the feeding habits of the different species. Corn earworms often feed in protected sites on soybean plants (Eckel et al. 1992); fall armyworms feed on fully and partially expanded leaves (M. E. B., pers. observ.), soybean loopers feed on expanded leaves in unprotected areas (Herzog 1980), and green cloverworms feed on partially and fully expanded soybean leaves (M. E. B., pers. observ.). We observed both corn earworms and fall armyworms displaying the vigorous responses and dropping on a thread, but these behaviors were not commonly used. Furthermore, Terry et al. (1989) suggest that dropping on a thread (which they called spin down behavior) is not a defensive behavior of corn earworm. The same may be true for fall armyworm. Soybean loopers and green cloverworms frequently used the vigorous responses and dropping off the leaf in encounters with *M. discitergus* females.

Alternatively, the lack of response by corn earworm and fall armyworm larvae to *M. discitergus* females may not reflect normal defensive behaviors of these species in the field because individuals of both species were obtained from cultures maintained in the laboratory for many generations. In laboratory cultures, individuals would not be under selective pressure to maintain parasitoid avoidance behaviors. The soybean looper larvae, however, also were obtained from laboratory cultures and these did demonstrate defensive behaviors which were effective in deterring or avoiding capture by the hyperparasitoid. In the latter case, loss of defensive behaviors in the absence of parasitoid pressure was not evident. Therefore, there is no obvious correlation between defensive behaviors, or lack thereof, and the length of time a colony was maintained in the laboratory.

More than 95% of the caterpillars that used minimal response were captured by *M. discitergus* females. Sixty-eight percent of the caterpillars using the vigorous responses were captured, and 39% of those that dropped on a thread were captured by *M. discitergus* females. Twenty-six percent of the caterpillars that dropped without a thread were captured. *Mesochours discitergus* females either reeled in the thread, walked down the thread, or used a combination of both behaviors to capture suspended caterpillars (also described by Yeargan and Braman 1989); within the group of caterpillars that dropped on a thread, percentage capture varied among species. Seventy-seven percent of the suspended fall armyworms and 63% of the suspended green cloverworms were captured, but only 22% of the suspended soybean loopers and 44% of the suspended corn earworms were captured.

Our data indicate that the minimal response behaviors are easily overcome by *M. discitergus,* and caterpillars that predominantly use these behaviors are more easily captured. Similarly, capture efficiency by the hyperparasitoid, *Alloxysta (Charips) victrix* (Westwood), depends upon the species of aphid attacked and the species of primary parasitoid within the host (Gutierrez 1970). Gutierrez and van den Bosch (1970) observed that the pea aphid, *Acyrthosiphon pisum* (Harris), responds minimally to encounter with *A. victrix,* but the spotted alfalfa aphid, *Therioaphis trifolii* (Monell), jumps from the plant, often dislodging the hyperparasitoid. Therefore, rates of hyperparasitism of a given primary parasitoid species can be affected by the behavior of the primary parasitoid's host.

Acknowledgments

W. E. Barney, G. C. Brown, R. Pfannenstiel, and L. H. Townsend reviewed earlier drafts of the manuscript. The investigation reported in this paper (93-7-128) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

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