

Life History of the Hyperparasitoid *Mesochorus discitergus* (Hymenoptera: Ichneumonidae) and Tactics Used to Overcome the Defensive Behavior of the Green Cloverworm (Lepidoptera: Noctuidae)

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ABSTRACT *Mesochorus discitergus* (Say) is an endogenous, solitary hyperparasitoid with a broad range of hosts. Its developmental period from oviposition to adult emergence with *Cotesia marginiventris* (Cresson) as its host ranged from about 16 d at 27°C to about 25 d at 21°C. When hyperparasitism occurred 3 d after primary parasitism, the first two instars developed within the *C. marginiventris* larva, and the third instar developed within the *C. marginiventris* larva and pupa. Hyperparasitoid pupation occurred within the cocoon spun by the primary parasitoid. *M. discitergus* must overcome the defensive responses of green cloverworm larvae to oviposit in larval primary parasitoids located inside the caterpillars. Young green cloverworm larvae often drop when disturbed and hang from foliage on silken threads. To capture a young (second instar) caterpillar, the hyperparasitoid usually hangs by its hind tarsi from the edge of the leaf from which the larva is suspended and reels in the caterpillar by pulling upward on the caterpillar's silken thread. Capturing suspended third and fourth instars often requires that the parasitoid walk part or all the way down the thread toward the caterpillar. By probing briefly with the ovipositor, hyperparasitoid females distinguish between green cloverworms parasitized by *C. marginiventris* and those that are unparasitized. Unparasitized larvae are quickly rejected ($\bar{x} = 5.0$ s), whereas parasitized green cloverworms are held and probed longer ($\bar{x} = 83.4$ s).

KEY WORDS Insecta, *Cotesia marginiventris*, *Plathypena scabra*, development

HYPERPARASITIDS IN THE GENUS *Mesochorus* attack many species of primary parasitoids in a variety of lepidopteran, coleopteran, and other hosts. Several species, including *Mesochorus discitergus* (Say), hyperparasitize hymenopteran primary parasitoids of the green cloverworm, *Plathypena scabra* (F.), an occasionally serious pest of leguminous crops in the United States (Hill 1925, Barry 1970, Lentz 1973, Soteres et al. 1984, Daigle et al. 1988). Despite their potentially serious effect on primary parasitoids involved in biological control, as reported by Simpson et al. (1979), there have been few biological studies of *Mesochorus* species (e.g., Coseglia et al. 1977, Ellsbury & Simpson 1978).

Mesochorus discitergus hyperparasitizes a variety of hymenopteran primary parasitoids that attack caterpillars in at least 12 lepidopteran families (Dasch 1971). To oviposit in these endoparasitic primary parasitoids, *M. discitergus* must overcome the defensive behaviors (if any) of caterpillars that contain the primary parasitoids. To our knowledge, there is no published information concerning the tactics used by this parasitoid to capture lepidopteran larvae and little information on its biology. Here we report aspects of the life history of *M. discitergus* when the braconid *Cotesia marginiventris* (Cresson) is its host, as well as behavioral adaptations employed by *M. discitergus* to over-

come the defensive responses of different instars of the green cloverworm. Voucher specimens are deposited in the insect collection of the Department of Entomology, University of Kentucky, Lexington.

Materials and Methods

Colonies of *C. marginiventris* and *M. discitergus* were started in 1986 and supplemented in 1987 with parasitoids reared from green cloverworms collected from alfalfa in Fayette County, Ky. Similarly, a green cloverworm colony was started and periodically augmented with field-collected moths during 1986 and 1987. These colonies were maintained as described by Yeorgan & Braman (1986).

Development of the Parasitoid. First instars of the green cloverworm were parasitized by *C. marginiventris* by placing 35 larvae on a soybean plant (growth stages V4 to V6 [Fehr & Caviness 1977]) inside a ventilated acrylic cylinder (10.5 cm diameter, 35.0 cm high) and introducing two mated female *C. marginiventris* for 6 h. These larvae were incubated for 3 d at 24°C and a 15:9 (L:D) photoperiod. Three-day-old *C. marginiventris* then were hyperparasitized by *M. discitergus* by placing two mated female parasitoids of this species in

the acrylic cylinders with the green cloverworm larvae for 4 h. Following parasitism by *M. discitergus*, green cloverworm larvae were reared at constant temperatures of 21, 24, and 27°C (all \pm 1°C) and 15:9 (L:D). Temperatures were chosen to represent a range of those which occur in Kentucky when *M. discitergus* is active.

The periods of time required from oviposition to cocoon formation by the host (i.e., the primary parasitoid) and from cocoon formation until adult emergence were ascertained by observations made at 12-h intervals (0800 and 2000 hours EDT) to determine the time of cocoon formation and adult emergence from the host cocoon. Only those hyperparasitoids that became adults and emerged from the host cocoon were included in calculations of required periods for oviposition to cocoon formation. Developmental periods for males and females were compared (Student's *t* test). Similar data were collected at 24°C for those primary parasitoids that escaped hyperparasitism to determine if the hyperparasitoid caused the developmental period (oviposition to cocoon formation) of the primary parasitoid to be extended. Statistical comparisons of developmental periods from oviposition to cocoon formation (hyperparasitized versus non-hyperparasitized *C. marginiventris*) and from cocoon formation to adult emergence (*C. marginiventris* versus *M. discitergus*) were based on Student's *t* test.

First instars of the green cloverworm were parasitized by *C. marginiventris* and hyperparasitized by *M. discitergus* in the manner described above and were reared at 24°C to obtain specimens for measurements of *M. discitergus* larvae at progressive stages of development. Samples of these larvae were then dissected at 24-h intervals until primary parasitoids emerged from the green cloverworm and spun cocoons. Dissections made after the primary parasitoid had emerged from its lepidopteran host were standardized by collecting those primary parasitoid cocoons formed between 1600 hours on the 10th day and 1100 hours on the 11th day after oviposition by *M. discitergus*. We had observed that *C. marginiventris* that contained male or female *M. discitergus* hyperparasitoid larvae required about 10 and 11 d, respectively, after oviposition by the hyperparasitoid to complete development to cocoon formation. Those cocoons and their contents were dissected on day 11 and thereafter at 48-h intervals until the adult hyperparasitoid emerged. The instars of green cloverworms and primary parasitoids were determined at the time of each dissection. Length and width (at widest point) of freshly dissected specimens were recorded for hyperparasitoid eggs, larvae, and pupae.

Attack Behavior of *M. discitergus*. The behavior of *M. discitergus* females was observed directly while the hyperparasitoids searched for and captured young green cloverworms on soybean plants in the laboratory. Direct observations were aug-

mented by videography which allowed further study of behavioral events replayed in slow motion.

Defensive responses of first to sixth instars of the green cloverworm were described by Yeargan & Braman (1986). *M. discitergus* hyperparasitizes three indigenous braconids—*C. marginiventris*, *Diolcogaster facetosa* (Weed), and *Rogas noloophanae* Ashmead—which in turn have attacked young larvae of the green cloverworm (Barry 1970, Lentz 1973; K.V.Y., unpublished data). These three braconids attack primarily the following green cloverworm instars: *C. marginiventris*, first and sometimes second instars (Kunnalaca & Mueller 1979); *D. facetosa*, second and third instars (Yeargan & Braman 1986); *R. noloophanae*, third instars (Lentz & Pedigo 1974). By direct observation in the laboratory, we documented the methods by which *M. discitergus* captures second-, third-, and fourth-instar green cloverworms, because these are the instars most likely to contain the appropriate stage of the above-mentioned primary parasitoids.

Second-, third-, and fourth-instar green cloverworms were exposed to mated female *M. discitergus* by placing 25–50 larvae of a particular instar on a soybean plant (growth stage V3) inside an acrylic cage (31 by 31 cm, 41 cm high) and introducing one female hyperparasitoid at a time. The behavior of approximately 30 individual female hyperparasitoids was observed in this manner, and the tactics they employed to capture at least 25 caterpillars of each instar were recorded.

Differences in behavior of *M. discitergus* females in response to encountering unparasitized versus parasitized green cloverworm larvae were quantified by recording the length of time individual females spent probing the green cloverworm with the ovipositor. First instars of the green cloverworm were observed in the presence of *C. marginiventris* females. Those larvae observed to have been attacked by *C. marginiventris* were housed together on a soybean plant (growth stage V4), and a similar group of unparasitized first instars was placed on a similar soybean plant in a separate rearing cylinder. All larvae were incubated at 24°C for 3 d. Both groups of larvae were then exposed to *M. discitergus* females, and the amount of time spent by each parasitoid female in probing with the ovipositor was recorded. Different females were used in timing probing for parasitized versus unparasitized larvae; 12 female parasitoids were used in obtaining 43 individual observations of attempted parasitism. Green cloverworm larvae that had been observed to be parasitized by *C. marginiventris* were subsequently dissected to confirm the presence of a primary parasitoid larva.

Results and Discussion

Development of the Parasitoid. Female *M. discitergus* required longer to develop than males at 21°C and at 24°C but not at 27°C (Table 1). Overall development from oviposition to adult emergence

Table 1. Time required for development ($\bar{x} \pm SE$ in days) by *M. discitergus* using *C. marginiventris* as the p parasitoid host within the green cloverworm

Temp. °C	n		Oviposition to cocoon formation ^a		Cocoon formation to adult emergence	
	♂	♀	♂	♀	♂	♀
21	44	39	13.4 ± 0.4	13.6 ± 0.2	10.9 ± 0.1	11.8 ± 0.1 ^b
24	74	34	10.1 ± 0.2	11.2 ± 0.3 ^b	9.5 ± 0.1	10.0 ± 0.1 ^b
27	58	46	9.3 ± 0.3	10.1 ± 0.4	6.6 ± 0.1	6.7 ± 0.1

^a Cocoon formation by primary parasitoid.

^b Developmental periods for males and females were significantly different ($P < 0.05$, Student's *t* test) before host cocoon formation at 24°C and after host cocoon formation at 21 and 24°C.

ranged from about 16 d at 27°C to about 25 d at 21°C. Adult parasitoids emerged from 87.3, 92.7, and 91.4% of the cocoons formed at 21, 24, and 27°C, respectively.

Cotesia marginiventris that had not been hyperparasitized required the following mean ($\pm SE$) number of days to develop at 24°C: oviposition to cocoon formation, males 11.4 ± 0.2 ($n = 41$), females 11.8 ± 0.3 ($n = 44$); cocoon formation to adult emergence, males 5.9 ± 0.2 ($n = 41$), females 6.1 ± 0.1 ($n = 44$). When hyperparasitized by *M. discitergus*, *C. marginiventris* required longer ($P < 0.05$) to develop from oviposition to cocoon formation at that same temperature: males required 13.1 ± 0.1 d ($n = 74$) and females required 14.4 ± 0.3 d ($n = 34$). The developmental period within the host cocoon is considerably longer ($P < 0.05$) for *M. discitergus* than that required by *C. marginiventris* that are not hyperparasitized: 9.5 versus 5.9 d for males and 10.0 versus 6.1 d for females.

The total developmental period from oviposition to emergence of the adult of *M. discitergus* appears to be similar to developmental periods of three common primary parasitoids of the green cloverworm which it hyperparasitizes (*C. marginiventris*, *D. facetosa*, and *R. nolophanae*). As reported here, *C. marginiventris* requires 17–18 d to mature at 24°C, whereas *M. discitergus* requires 20–21 d

at that temperature. Lentz (1973) found that *R. nolophanae* completed development in 18–19 d at temperatures ranging from 21.1 to 26.7°C ($\bar{x} = 23.8$), and Yearcan & Braman (1986) showed that *D. facetosa* required 21–22 d at 24°C. Thus, at comparable temperatures, *M. discitergus* and three of its common primary parasitoid hosts in the green cloverworm all require 2.5–3 wk to develop. This indicates that generations of the hyperparasitoid could be well synchronized with generations of these primary parasitoids.

Dissection of host larvae revealed the presence of three larval stages of *M. discitergus*, as was reported for *Mesochorus agilis* Cresson (Ellsbury & Simpson 1978). The egg stage lasted 2 d at 24°C (Table 2). First instars are caudate-mandibulate with large head capsules. The first stadium lasted 2 d at 24°C.

The slender second instar of the hyperparasitoid has a more reduced head capsule than the first instar. Second instars were present from day 5 to day 10 after oviposition at 24°C, although some third instars also were found by day 8 (Table 2).

The third instar is grublike. Guppy & Miller (1970) showed that the mouthparts of this final instar can distinguish this species from other secondary parasitoids that emerge from braconid pupae. Third instars were present within some *C.*

Table 2. Sizes and stages of development of *M. discitergus* as determined by dissection of larvae, pupae, and cocoons of the primary parasitoid (*C. marginiventris*) reared in green cloverworm larvae at 24°C

Green cloverworm instar no. at dissection	Primary parasitoid instar no. at dissection	Days after hyperparasitoid oviposited	n	Hyperparasitoid stage or instar no.	Size range of hyperparasitoid	
					Length, mm	Width, mm
2	1	1	3	Egg	0.16–0.22	0.09
2	1	2	3	Egg	0.18–0.27	0.09–0.18
3	1	3	3	1	0.47–0.76	0.13–0.16
3	1	4	4	1	0.63–0.67	0.18
4	1 and 2	5	6	2	0.45–0.81	0.09–0.13
4	1 and 2	6	4	2	0.40–1.13	0.09–0.23
4	2	7	3	2	1.32–1.69	0.26–0.39
5	3	8	3	2 and 3	1.32–2.04	0.38–0.57
5	3	9	3	2 and 3	1.13–2.07	0.38
5	3	10	5	2 and 3	1.17–2.26	0.22–0.34
— ^a	Pupa	11	3	3	2.26–2.64	0.45–0.68
—	Pupa	13	3	3	2.07–2.49	0.68–0.90
—	— ^b	15	3	Pupa	3.39–3.92	0.90–1.13
—	—	17	4	Pupa	3.39–3.77	0.75–1.13

^a Primary parasitoids had emerged from lepidopteran hosts by this date.

^b Hyperparasitoids had consumed primary parasitoids by this date.

marginiventris larvae on days 8 through 10, but other *C. marginiventris* larvae contained second instars at those times (Table 2). On days 11 and 13 (no dissections were made on day 12), however, the primary parasitoid had pupated, and those pupae contained only third-instar hyperparasitoids.

Mesochorus discitergus pupae were found when host cocoons were opened 15 and 17 d following hyperparasitism. On day 19, adult parasitoids were found inside the cocoons; thus the pupal stage required 4–5 d at 24°C. Adult emergence from host cocoons was completed by day 21 after oviposition by *M. discitergus*.

In summary, the hyperparasitoid places its eggs inside first-instar *C. marginiventris* which, in turn, are inside second-instar green cloverworms. The hyperparasitoid causes a lengthening of the larval developmental period of the primary parasitoid, and the hyperparasitoid does not complete its larval development until a few days after the primary parasitoid has emerged from a green cloverworm fifth instar, spun a cocoon, and pupated. Upon completion of its larval development (about 2 wk after oviposition at 24°C) and after destroying the primary parasitoid pupa, the hyperparasitoid pupates inside the cocoon of the primary parasitoid. It should be noted that this phenology may differ if the hyperparasitoid attacks older primary parasitoids; our unpublished observations indicate that *M. discitergus* can, in fact, parasitize older instars of *C. marginiventris*.

Attack Behavior of *M. discitergus*. The first three instars of the green cloverworm respond to disturbance from certain predators and parasitoids by dropping immediately from the plant and remaining suspended on a silken thread, which they later ascend to resume feeding (Yeargan & Braman 1986). Fourth instars of the green cloverworm respond by dropping with a thread about half the time and dropping without a thread on the remaining occasions. *M. discitergus* females, when exposed to green cloverworm larvae or to soybean plants fed upon by those larvae, immediately become agitated and elevate their wings, which they vibrate continuously while they capture caterpillars and probe them with their ovipositors. Muesebeck & Dohanian (1927) placed female *M. vitreus* (= *discitergus* [Krombein et al. 1979]) in vials with parasitized gypsy moth larvae, and they noted similar wing vibrations by this hyperparasitoid.

Mesochorus discitergus captures green cloverworm larvae by one of four methods (Table 3), the frequency of each depending on the instar of the larva. When the hyperparasitoid locates a small (second instar) larva suspended by a thread, *M. discitergus* usually hangs head downward from the edge of the leaf suspended by its hind tarsi (Fig. 1) and reels in the caterpillar's silken thread, thus lifting the larva toward the hyperparasitoid. The two front tarsi alternately grasp and pull up the thread, while the middle legs gather up the slack thread from the front tarsi. When the caterpillar

Table 3. Behavioral tactics used by the hyperparasitoid *M. discitergus* to capture second-, third-, and fourth-instar green cloverworm (GCW) larvae on soybean plants

Green cloverworm instar no.	No. hyperparasitoids that:			
	Captured GCW on leaf	Hoisted GCW by reeling in thread ^a	Combined reeling and walking down thread	Walked down thread ^b
2	2	19	8	0
3	10	4	9	2
4	24	1	10	19

^a Hyperparasitoid hung from leaf edge while reeling.

^b Hyperparasitoid walked down to caterpillar without reeling thread.

is within grasping distance (<1 cm), the wasp seizes it and both fall to the ground or remain suspended on the larva's silken thread while parasitism of the primary parasitoid within the cloverworm takes place.

Larger (third and fourth instar) larvae were captured on leaves more frequently than second instars, apparently reflecting a greater tendency for small larvae to drop on threads when approached by *M. discitergus* (Table 3). Although a few suspended third and fourth instars were captured by the reeling tactic described above, more were captured by techniques that involved the hyperparasitoid walking down the caterpillar's thread. Sometimes the wasp walked part way down the thread and then reeled the caterpillar upward. At other times, the wasp walked all the way down to the caterpillar without reeling the thread. In general, it seems that the larger the green cloverworm, the more important the role of walking (Table 3).

We previously reported the thread-descending behavior of *D. facetosa* (Yeargan & Braman 1986) and noted that only one other species, to our knowledge, had been reported to have that type of behavior. As described above, *M. discitergus* utilizes different tactics to solve the same problem.

Mesochorus discitergus females can distinguish quickly between parasitized and unparasitized cloverworm larvae, apparently on the basis of a cue detected by inserting the ovipositor into the body of the cloverworm. *M. discitergus* spent an average of only 5.00 ± 0.06 (SE) s ($n = 23$) with the ovipositor inserted in unparasitized green cloverworms. In contrast, these hyperparasitoids spent 83.40 ± 21.74 s ($n = 20$) with the ovipositor inserted in parasitized larvae; this difference in time was highly significant ($P < 0.001$). Because *M. discitergus* has so many hosts which, in turn, parasitize a wide variety of lepidopteran larvae, it seems reasonable to expect that some common chemical cue serves as an indicator of previous parasitism. Such a cue might be provided by material deposited in the lepidopteran larva by the primary parasitoid during oviposition (e.g., associated with the parasitoid's calyx fluid) or it might be a substance pro-

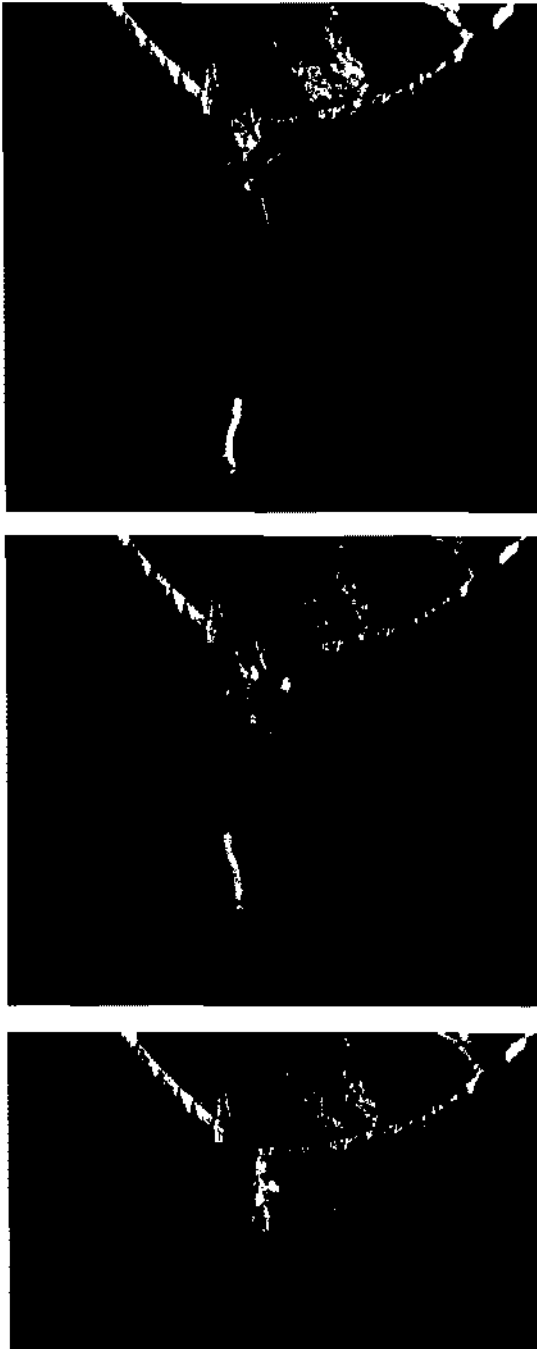


Fig. 1. The hyperparasitoid *M. discitergus* pulling up a green cloverworm by reeling in the caterpillar's silk thread (top and middle), and beginning to probe it with its ovipositor (bottom).

duced, or the concentration of which is altered, by the lepidopteran larva in response to primary parasitism.

This species has been reported to develop not only as a hyperparasitoid on hymenopteran species, but also as a primary parasitoid on certain lepi-

dopteran species (Dasch 1971, Pisica et al. 1979). However, these and similar reports of Mesochorinae as primary parasitoids have been questioned (Krombein et al. 1979). We have never observed *M. discitergus* behave as a primary parasitoid of the green cloverworm. Rather, an unparasitized green cloverworm seems to be rejected after a brief probing with the ovipositor. Parasitized larvae often are probed in several locations before oviposition occurs, whereas unparasitized larvae are probed only once. Usually only one egg is deposited by *M. discitergus* in the larva of a primary parasitoid. Only one adult hyperparasitoid was ever observed to emerge from a host cocoon. We have reared *M. discitergus* from cocoons formed by *C. marginiventris*, *D. facetosa*, and *R. nolophanae* that had emerged from field-collected green cloverworm larvae, but we have never reared it from green cloverworms in the absence of a primary parasitoid as host.

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