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BIOLOGICAL OBSERVATIONS OF *CENTISTES GASSENI* SHAW (HYMENOPTERA: BRACONIDAE), A PARASITOID OF *DIABROTICA* SPP. (COLEOPTERA: CHRYSOMELIDAE)[1]

R. F. W. Schroder and M. M. Athanas

(RFWS) Research Entomologist, retired, (MMA) Entomologist, Insect Biocontrol Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Bldg. 011A, Rm. 107, BARC-West, Beltsville, MD 20705, U.S.A. (e-mail: athanasm@ba.ars.usda.gov)

Abstract.—In 1992–1993, *Centistes gasseni* Shaw was imported into the United States, and in the laboratory successfully parasitized: southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber; banded cucumber beetle, *D. balteata* LeConte; western corn rootworm, *D. virgifera virgifera* LeConte; and striped cucumber beetle, *Acalymma vittatum* (F.). Males and females of *C. gasseni* lived an average of 15.4 and 12.9 days (with a maximum of 30 and 29 days), respectively. A single female oviposited in 383 host *Diabrotica* over her lifetime, from which 158 cocoons were recovered. Additional observations on the biology and rearing of the parasitoid are presented.

Key Words: *Acalymma vittatum*, biological control, *Centistes gasseni*, *Diabrotica balteata*, *Diabrotica barberi*, *Diabrotica speciosa*, *Diabrotica undecimpunctata howardi*, *Diabrotica virgifera virgifera*, host specificity

The northern corn rootworm (NCR), *Diabrotica barberi* Smith and Lawrence, and the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte are the most damaging and costly pests of corn in North America (Sutter and Lance 1991). To achieve effective control of these chrysomelids, soil insecticides routinely have been used on 50–60% of the corn acreage, or 12–16 million ha annually (Metcalf 1986). These insecticides are generally applied prophylactically and are frequently unnecessary (Lance and Sutter 1992). According to Metcalf (1986), the cost from crop loss and treatment due to the corn rootworm (CRW) complex is approximately one billion dollars per year.

In addition, southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi* Barber; striped cucumber beetle, *Acalymma vittatum* (F.); western striped cucumber beetle, *Acalymma trivittatum* (Mannerheim); and banded cucumber beetle, *Diabrotica balteata* LeConte cause 50–100 million dollars in damage to other crops (Cucurbitaceae, Fabaceae) (Metcalf et al. 1962).

Lance and Sutter (1992) cite health risks to growers, livestock and wildlife due to soil insecticides used for CRW control. They also refer to reports of these insecticides being detected in ground and surface water. Therefore, it is evident that there is a need for alternative control measures that are environmentally safe and cost effective.

In 1990, while searching for biocontrol agents of Colorado potato beetle (Coleoptera: Chrysomelidae) in Rio Grande do Sul,

[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

Brazil, we learned of the existence of an undescribed braconid parasitoid of the adult Neotropical leaf beetle, *Diabrotica speciosa* Germar (Gassen 1986). It was subsequently described by Shaw (1995) as *Centistes gasseni* Shaw, a solitary endoparasitoid of the adult. As there are no known effective parasitoids of the CRW complex in the United States, we were interested in acquiring and studying *C. gasseni* as a potential new biological control agent. We traveled to Brazil in 1992–93, and, in cooperation with Dirceu Gassen, EMBRAPA-CNPT, collected and imported adults of *D. speciosa* parasitized by the braconid. In this article, we present observations on the biology, rearing, mating behavior and host specificity of *C. gasseni,* obtained during initial attempts to colonize the parasitoid in quarantine.

Materials and Methods

Approximately 6,000 *D. speciosa* adults were collected from April 24–26, 1993, at the University of Passo Fundo, Passo Fundo, Rio Grande do Sul, Brazil. Collections were made primarily from potato plants, but some adults were also collected from nearby plots of pepper and sweet potato plants. Parasitoids were also recovered from beetles collected from flowering weeds and wild cucurbit plants, 98 km west of Passo Fundo. Sub-samples of field collected beetles were dissected to determine percent parasitism and the number of parasitoids per host. In subsequent collections, Heineck-Leonel and Salles (1997) recovered *C. gasseni* from *D. speciosa* collected in corn, beans, melon, cucumber, cabbage, broccoli, spinach and lettuce.

Field collected adult beetles were held in 30.5 cm collapsible aluminum screen cages (BioQuip® Products, Gardena, CA). Each cage was inverted and the hinged top served as the bottom. A fitted piece of moistened felt was placed on the bottom as a liner, and covered with a ca 0.5–0.6 cm layer of moistened sand with a 2.5 cm sand-free margin on all sides. The sand and felt served as a substrate in which the parasitoid larvae spun cocoons. Approximately one cm above the bottom of the cage, we fitted a pre-cut piece of number 12 mesh stainless steel screen, that separated the adult *Diabrotica* from emerging *C. gasseni* larvae, which passed through to pupate.

The adult beetles, and 31 parasitoid cocoons recovered prior to departure, were taken to the Maryland Department of Agriculture quarantine facility, Annapolis, MD, and held at 21.1°C, 60% RH, and a 16:8 LD photoperiod. The host beetles were kept in the modified cages which were serviced three times a week. Parasitoid cocoons were carefully removed from the sand and placed in petri dishes, along with a moistened, 3.8 cm cotton roll to maintain humidity.

The containers used for oviposition, and to house the *C. gasseni* adults, were plexiglass tubes (7.6 cm in diameter and 10.2 cm long) modeled after those used by Whistlecraft et al. (1984) in their braconid rearing program. To confirm mating, a single female was confined in an aspirator tube with multiple males until copulation was observed. With *Cotesia melanoscela* (Ratzeburg), Weseloh (1977) found that 20 seconds was necessary to successfully transfer sperm to the female. We therefore considered a successful mating session as one in which copulation was observed for 20 seconds or more. We used several of the methods discussed by Singh (1982) to induce successful mating, including shaking the insects to the bottom of the tube, increasing the number of males per female, and periodically chilling the females prior to exposure to the males.

The following procedures were used in quarantine to conduct studies on the biology, mating behavior, and host preferences of the parasitoid.

Host exposure methods.—*Individual exposure:* To expose host *Diabrotica* adults individually, we placed a parasitoid female into a 35 × 10 mm polystyrene culture dish and introduced a single *Diabrotica* adult. The host beetle was observed until ovipo-

sition occurred, then it was removed. Ten to twenty *Diabrotica* were exposed to a female per session. Following each session, the female was held for one hour in a tube or petri dish, and given access to water and honey solution. Each female had no more than two exposure sessions per day. A separate cage (#1–3) was set up for the *Diabrotica* exposed to each of the three, mated *C. gasseni* females used.

Group exposure with immediate removal: Approximately 35 *Diabrotica* beetles were placed in an oviposition tube, into which a *C. gasseni* female was then introduced. As soon as a host beetle was parasitized, it was aspirated out of the tube. As above, following each session females were permitted to rest, and limited to two sessions per day. A separate cage (#4–6) was set up for the *Diabrotica* exposed to each of the three, mated *C. gasseni* females used.

Group exposure for one hour: Six *C. gasseni* females were used to sting a total of 394 BCB over a 7-day span. The females were confirmed mated, and all had emerged in association with other males and females. Beetles were exposed in groups of 15–20 to individual females for one hour, then placed into a single cage (#7).

Group exposure for four hours: Six *C. gasseni* females, selected from a group of females, were used to sting a total of 140 BCB over a 4-day span. All the females had been confined with an equal number of males (17 ♂ & 17 ♀) for 72 hours preceding exposure to *Diabrotica,* but mating was not confirmed. As above, all had emerged in association with other parasitoid males and females. BCB were exposed in groups of 20–25 to single *C. gasseni* females, for four hours duration, then placed into a single cage (#8).

Host species preference.—Adult SCR and BCB were sexed, and three males and three females of each species were placed into an oviposition tube. A *C. gasseni* female reared from BCB host was introduced and exposed to the beetles for one hour, after which time she was removed and held as previously described. Using nine *C. gasseni* females, a total of 792 *Diabrotica* were exposed.

Host sex preference.—To determine if *C. gasseni* exhibited a preference for either male or female hosts, SCR reared parasitoids (n = 10) were exposed individually to six male and six female SCR for one hour. One group of hosts (204 ♀ & ♂, cages 1 & 2, respectively) was exposed to *C. gasseni* females 1–3 days old, another group of hosts (372 ♀ & ♂, cages 3 & 4, respectively) was exposed to *C. gasseni* females, the majority of which were 1–5 days old. Two cages of unexposed male and female *Diabrotica* were maintained as controls.

Host specificity.—Host specificity tests were conducted to determine whether SCR, WCR, BCB, the related SCB and *Cerotoma trifurcata* (F.) were suitable hosts for *C. gasseni.* The criteria for these tests were based on observing the parasitoids' ovipositional behavior for one hour after exposure to the hosts, and then holding parasitoid and hosts together (25–50 per exposure) over night. The female was then removed and the hosts held for parasitoid emergence for 30 days.

Additional non-target insects that may occur in the same habitat, including beneficial and phytophagous coccinellids were also exposed to *C. gasseni* to determine their suitability as hosts. In each test, a single *C. gasseni* female, less than seven days old, was placed in an oviposition tube. Five *Diabrotica* (BCB or SCR), which served as controls, and 25–50 adult host beetles (hosts were obtained from laboratory and field sources) were then introduced. Each tube was observed for one hour to confirm any oviposition that occurred. The *Diabrotica* were removed after they were stung and set up in separate parasitoid emergence containers. After one hour, observations were discontinued, and females were kept with the test beetles for 24 hours. Host beetles were then removed, and held for parasitoid emergence. Thirty days after expo-

Table 1. Emergence, sex ratios and hyperparasitism of *Centistes gasseni* from field collected *Diabrotica speciosa*.

Date	No. Cocoons	No. ♂	No. ♀	Sex Ratio ♂ : ♀	No. *Mesochorous*	% *Centistes* Emergence
4/92	108	11	19	1:2	12	28
4/93	31	5	6	1:1	2	35
5/93	338	138	64	2:1	3	60
5/94[a]	101	10	13	1:1	5	23
6/94[a]	86	8	8	1:1	14	19

[a] Data from cocoons collected at the ARS, South American Biological Control Laboratory, Buenos Aires, Argentina.

sure, host beetles were dissected to determine if they had been parasitized.

Results

From the 31 cocoons brought back from Brazil, 11 *C. gasseni* and two hyperparasitoids, *Mesochorous* sp. (Hymenoptera: Ichneumonidae) emerged. From the ~ 6000 *D. speciosa* collected in Brazil, we recovered 338 *C. gasseni* cocoons, 66 *C. gasseni* larvae, and 11 puparia of *Celatoria bosqui* Blanchard (Diptera: Tachinidae) (Table 1). As the number of host beetles collected was approximated, we could not accurately determine the percent parasitism. However, in one sub-sample (n = 111) 10.0% were parasitized. In Pelotas, RS, Brazil, Heineck-Leonel and Salles (1997) reported that parasitism, of *D. speciosa* by *C. gasseni*, ranged from 0–18.9% ($\bar{x}$ = 8.7, SD = 7.1) in samples obtained at monthly intervals from May 1994–April 1995.

Mating data were obtained on a sub-sample of P and F generation parasitoid females. Forty-one percent of the P sample (n = 17) successfully mated (Table 2). All *C. gasseni* pupae obtained from *D. speciosa* were set up individually, thus all females that emerged were isolated from other males and females. Of those that mated, 29% did so on the first attempt, 43% on the second, and 28% required three or more attempts to mate. Unsuccessful mating attempts were observed for a minimum of 10 minutes. Seventy-one percent of the F sample (n = 90) successfully mated (Table 2). Of these, 72% occurred on the first attempt, 20% on the second, and 8% required three or more attempts. In the majority of cases, the female would aggressively fight off the advances from any males after she had successfully mated. Nevertheless, 9% of the females did mate a second time, all within 2.1 minutes of the first. When we exposed previously mated females to new males, 8% mated a second time.

Parasitoid female progeny were obtained only from positively mated parental females. When confined with a suitable host, the female mounted the beetle from the side and thrust her ovipositor into the abdomen. The beetle responded to the attack by trying

Table 2. Reproductive behavior of *Centistes gasseni* reared from native and alternative host *Diabrotica*.

Host	Mean % ♀ Mated	Mean ♀ Age (Days)	% Iso.[a] ♀ Mated	% ♀ with ♀ Mated	% ♀ with ♂ Mated	% ♀ with ♀ & ♂ Mated	Mean Expos. (Min)	Mean Length (Sec)	Mean No. ♂ Used	Mean Age ♂ (Days)
Pgen[b]	41	2.1 ± 1.3	41	N/A	N/A	N/A	3.8 ± 3.8	33.6 ± 4.8	5.7 ± 2.0	4.3 ± 3.4
F1-9[c]	71	1.5 ± 0.9	78	86	68	57	3.9 ± 4.1	32.9 ± 11.8	5.8 ± 2.1	2.4 ± 0.4

[a] Isolated from other females and males.
[b] *C. gasseni* reared from *D. speciosa*.
[c] *C. gasseni* reared from North American *Diabrotica*.

Table 3. Parasitism by *Centistes gasseni* using various host exposure methods.

Cage[a]	Days ♀ in Use	♀ Emerged with ♂, ♀	Hosts Exposed	No. Cocoons	% Parasitism	No. ♂ Emerged	No. ♀ Emerged	% Emerged
1	1–5	None	103 SCR[d]	62	60	7	39	74
2	1–2	29♂ 1♀	61 SCR	45	80	37	0	82
3[b]	1–3	29♂ 1♀	47 SCR	28	60	14	1	54
4	2–13	3♂ 3♀	232 SCR	154	73	102	0	66
5	5–9	3♂ 3♀	129 BCB[e]	61	62	57	0	93
6[c]	2–12	1♀	383 BCB	158	52	93	0	59
7	1–9	—	394 BCB	163	52	68	14	50
8	4–6	—	140 BCB	67	64	36	5	61

[a] Cages 1–3, hosts exposed individually, cages 4–6, hosts exposed in groups with immediate removal, cage 7 hosts exposed for one hour, cage 8 hosts exposed for four hours.

[b] Female did not mate until 37 of the 47 hosts had been exposed.

[c] Female mated twice.

[d] Southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber.

[e] Banded cucumber beetle, *D. balteata* LeConte.

to brush the *C. gasseni* from its back, becoming highly active or agitated, or by dropping to the bottom of the container.

Dissections of field collected *D. speciosa* revealed that parasitized beetles contained a single *C. gasseni* larva per host. However, laboratory dissections indicated that superparasitism did occur, with only one larva completing development. At 21.1 ± 0.5°C, the parasitoid would complete its life cycle in 33–41 days ($\bar{x}$ = 34.7 ± 1.7 days). At 23.9 ± 0.5°C, it took 26–32 days ($\bar{x}$ = 27.8 ± 0.7 days). At 21.1 ± 0.5°C, *C. gasseni* females and males lived an average of 12.9 ± 5.0 days and 15.4 ± 2.8 days, respectively.

Host exposure methods.—*Individual exposure:* In the hosts exposed individually (cages 1–3), the mean percent parasitism, based upon the number of hosts exposed and the number of parasitoids (cocoons and/or larvae) recovered, was 66.7% (SD = 11.54), and the mean parasitoid emergence from the resultant cocoons was 70% (SD = 14.4) (Table 3). At 32 ± 5 days post exposure, 89.3% (SD = 5.03) of the host SCR were dead. The female of cage 1, which emerged alone, produced offspring with a sex ratio of 6 ♀:1 ♂, the highest ratio of females to males obtained in this study.

Group exposure with immediate removal: The females of cages 4 and 6 oviposited in 232 SCR and 383 BCB respectively, over a 12 day period (Table 3). These yielded 154 and 158 cocoons, respectively, and 102 males and 93 males, respectively. This was the largest number of hosts stung, and number of cocoons and progeny obtained from individual *C. gasseni* females.

Pre-cocoon mortality was higher in the BCB hosts, averaging 22.0% versus 8.5% in the SCR. Eliminating cage 6 from the computations, since this female was exposed to an excessive number of hosts to determine oviposition limits, percent parasitism using this method of exposure averaged 67.5% (SD = 7.78). The mean parasitoid emergence from the cocoons was 72.6% (SD = 18.0), and at 29 ± 4 days post exposure, 91.5% (SD = 3.5) of the exposed hosts were dead.

Parasitoid females 10–12 days old, were the oldest from which viable progeny were recovered. The oldest age attained by a *C. gasseni* male and female, was 30 and 29 days, respectively. Over the course of the female's life, she was exposed to 114 SCR, both in groups and individually. As she aged, all hosts were exposed individually. On her 24th day, she stung two SCR, this was the oldest age at which we observed attack behavior. A cocoon was recovered from a SCR parasitized by a female 14–24 days old. There was no emergence from the

Table 4. Host specificity of *Centistes gasseni*.

Host Species Tested	Para. Emerge from Host	Para. Dissected from Host	Para. Emerge from Control
Coccinellidae			
Coleomegilla maculata (DeGeer)	−	−	+
Hippodamia variegata (Goeze)	−	−	+
Propylea quatuordecimpunctata (L.)	−	−	+
Epilachna varivestis Mulsant	−	−	+
Cycloneda munda (Say)	−	−	+
Chilocorus stigma (Say)	−	−	+
Hippodamia convergens Guerin	−	−	+
Harmonia axyridis (Pallas)	−	−	+
Chrysomelidae			
Cerotoma trifurcata (F.)	−	−	+
Acalymma vittatum (F.)[a]	+		
Diabrotica balteata LeConte[a]	+		
D. undecimpunctata howardi Barber[a]	+		
D. virgifera virgifera LeConte[a]	+		

[a] The *Acalymma* and *Diabrotica* tested were suitable hosts, dissections and controls were therefore not used.

cocoon, and a male was subsequently dissected out. This proved to be the oldest *C. gasseni* from which both cocoon, and fully formed offspring have been recovered.

Group exposure for one and four hours: The percent parasitism obtained by exposure for one (cage 7) and four (cage 8) hours, was 52% and 64%, respectively (Table 3). From cage 7, 82 parasitoids with a sex ratio of 5 ♂:1 ♀, were recovered. From cage 8, we recovered 41 parasitoids with a 7 ♂:1 ♀ sex ratio. This was the first instance in which parental females that were not confirmed mated, produced female offspring. Pre-cocoon mortality averaged 23% for cages 7 and 8. Parasitoid emergence from the cocoons was 50% and 61% for cages 7 and 8, respectively.

Host species preference.—Although all parental *C. gasseni* used were BCB reared, they did not exhibit a preference for BCB hosts. The percentage parasitism averaged 32% in both the BCB and SCR. Fifty-seven parasitoids, including seven females, were recovered from SCR hosts, and 55 parasitoids, including three females, were recovered from BCB hosts. All *C. gasseni* female offspring were recovered from BCB females and SCR males. Pre-cocoon mortality was 14% for both SCR and BCB. Parasitoid emergence from cocoons was 51% for BCB males and females, 65% for SCR males and 42% for SCR females.

Host sex preference.—In cages 1 and 2, the percent parasitism for female and male hosts was 32% and 23%, respectively. In cages 3 and 4, it was 45% and 38% for the female and male hosts, respectively. Emergence averaged 70% from the cocoons obtained from female hosts, and 78% from cocoons obtained from the males. For both *C. gasseni* age groups, all female offspring were recovered from female SCR hosts.

Beetle mortality appeared to be excessive with this generation: pre-cocoon mortality averaged 46% for cages 1 and 2, and 53% and 60% for cages 3 and 4, respectively. Thus it is uncertain whether the recovery of female parasitoids from female hosts was due to parasitoid preference, or selective mortality.

Host specificity.—The SCR, WCR, BCB and SCB exposed to *C. gasseni* were successfully parasitized, resulting in the recovery of parasitoids from all four hosts. Neither *Cerotoma trifurcata* (F.), nor any of the exposed coccinellids were parasitized (Table 4). In every test, given the choice be-

tween the control *Diabrotica* and the test insect, *C. gasseni* always searched for, and attacked the *Diabrotica* first, indicating that the parasitoid was active. The time it took to parasitize all control *Diabrotica* varied from 10–30 minutes. *C. gasseni* females approached the test beetles numerous times during the observation period. Usually, they would rapidly tap their antennae on the beetle, then move on to another host. Occasionally the female would mount the test beetle and probe with the tip of the abdomen at the same location where she would normally oviposit.

Discussion

The survival of *C. gasseni* larvae found during the maintenance of the host cages was extremely low, regardless of whether they were returned to the original cage or set up in petri dishes containing moistened sand. Vance (1932) observed that any *Chelonus annulipes* Wesmael larvae that emerge from the host and completes its final feeding, is unable to construct a cocoon if it is disturbed in any way. Harrison et al. (1993) observed a similar situation with larvae of *Microplitis croceipes* (Cresson). Those larvae died within two hours of exiting the host if they had not spun cocoons by then.

When inducing *C. gasseni* to mate, most of the successful copulations occurred when modified, open-ended aspirator tubes were used, and an air current provided. This seemed to aid the males in orienting to the virgin females (Vinson 1978). This suggests the presence of a sex pheromone in *C. gasseni* (Matthews 1974). In several cases in which females did not mate, or mating occurred after multiple attempts, the males used were ≤24 hours old. Laing and Caltagirone (1969) observed that females of the braconid *Habrobracon lineatellae* (Fischer) will not mate until they are at least 24 hours old, and that insemination is more likely to occur when older males (5–6 days old) are used. As Schlinger and Hall (1960) reported that the sperm supply drops rapidly with each successful mating, whenever possible, we removed each male after it had mated.

The host beetle apparently dies soon after parasitoid emergence; when harvesting *C. gasseni* cocoons we would find almost an equal number of dead beetles. Loan and Holdaway (1961) observed that the curculionid, *Sitona* sp., would stop laying eggs 1–2 days after being stung, and usually die in 3–4 hours following emergence of the endoparasitic braconid larva, *Pygostolus falcatus* (Nees). No female parasitoid progeny were obtained using group exposure of hosts. The large host to parasitoid ratio (Grinberg and Wallner 1991), and rapid rate of oviposition (Flanders 1956) may have been factors. When exposed to hosts in groups, the females would oviposit at a rate of one host per 0.92 ± 0.38 min. When presented with hosts individually, the rate slowed to one host per 2.2 ± 0.78 min.

Centistes gasseni was successfully reared through nine generations. During this period, the sex ratio varied widely, ranging from all males to 6 ♀:1 ♂. An adequate number of females was obtained to conduct experiments and propagate the colony. By the tenth generation, only males were produced. Although our results showed that females contacting each other did not preclude the production of female offspring, it may have affected the sex ratio. With the scelionid *Trissolcus,* contact with other females of the same species (Viktorov 1968), or trace pheromones of other females (Viktorov and Kochetova 1973, Buleza 1975), resulted in production of significantly more male offspring. In addition, the fluorescent lighting utilized in quarantine may have hindered successful mating. Nealis and Fraser (1988), observed that the braconid *Apanteles fumiferanae* (Viereck), mated more frequently under natural rather than artificial light conditions.

The SCR, WCR, BCB, and SCB were readily attacked and parasitized by *C. gasseni,* whereas other phytophagous and beneficial beetles tested proved unsuitable as

hosts. Due to the small number of *C. gasseni* females and availability of host beetles, the specificity tests were limited in scope, but generic specificity was evident.

Centistes gasseni has potential for use as a biocontrol agent of the North American CRW complex, and the striped cucumber beetle, *A. vittatum*. The biology, population dynamics and behavior of *C. gasseni* needs to be studied on its native host *D. speciosa* in Brazil.

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