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Factors Affecting Parasitization of *Spodoptera exigua* (Lepidoptera: Noctuidae) and Sex Ratio of the Parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae)[1]

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Abstract Biological factors hypothesized to affect parasitization by *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), an endoparasitoid of larvae of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), as well as its sex ratio, were examined in the laboratory. Highest parasitization occurred when (1) adult female parasitoids were closely associated with hosts, (2) adult female parasitoids were 1 day old, (3) a host:female parasitoid ratio of between 10:1 and 30:1 was maintained, (4) second-instar beet armyworms were used as hosts, and (5) adult female parasitoids were exposed to hosts for 24 h. Percentage of female progeny was higher when females were closely associated with second instars.

Key Words *Cotesia marginiventris, Spodoptera exigua,* sex ratio, parasitization

Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae) is an important solitary larval endoparasitoid of many lepidopteran hosts in a range of agricultural crops worldwide. *Cotesia marginiventris* is often the most prevalent parasitoid of the soybean looper, *Pseudoplusia includens* (Walker), and the corn earworm, *Helicoverpa zea* (Boddie), in soybeans (McCutcheon et al. 1990). It also is a predominant parasitoid of the western yellow-striped armyworm, *Spodoptera praefica* (Grote), in alfalfa (Miller 1977), the beet armyworm, *Spodoptera exigua* (Hübner), in cotton (Ruberson et al. 1994), and the green cloverworm, *Plathypena scabra* (F.), in soybeans (Kunnalaca and Mueller 1979). Other economically important hosts include *Anticarsia gemmatalis* (Hübner) (McCutcheon et al. 1990) and *Heliothis virescens* (F.), *Hymenia perspectalis* (Hübner), *Leucania inconspicua* Herrich-Schäffer, *Spodoptera frugiperda* (Smith), *Spodoptera ornithogalli* (Guenée), and *Trichoplusia ni* (Hübner) (Marsh 1979). All of these hosts are in the family Noctuidae, except for *H. perspectalis,* which is in the family Pyralidae.

Conservation and augmentation of *C. marginiventris* could be successful biological control strategies for control of *S. exigua.* The beet armyworm has in recent years been a serious pest in cotton in the Southeast (Wier and Boethel 1995, Mascarenhas et al. 1999). Natural control of the beet armyworm by a large and diverse complex of natural enemies can suppress populations of this pest below economically damaging levels (Ruberson et al. 1994). Parasitism rates in populations of beet armyworm larvae collected in Georgia were 46.8% in 1992 and 40.2% in 1993 (Ruberson et al.

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1994). *Cotesia marginiventris* was the predominant parasitoid for both years. Also, *C. marginiventris* is a very promising candidate in an augmentation program for control of beet armyworms feeding on vegetables in greenhouses. The ability of this parasitoid to control this pest in greenhouses is currently being evaluated by Koppert Biological Systems in the Netherlands (R. Timmer, pers. commun.). Understanding biological factors that affect parasitism by *C. marginiventris* is important in both conservation and augmentation of this parasitoid. In the field, a female parasitoid may have an "optimum window of opportunity" to parasitize hosts. During this critical period it would be important to avoid the use of harsh insecticides to protect the parasitoid population in the field. The first goal in releasing *C. marginiventris* is to develop methods to maximize parasitization and production of healthy female progeny in the laboratory. Also, developing release methodologies that consider the effects of biological factors on parasitization and sex ratio can increase the likelihood of the success of an augmentation program for this natural enemy. This study was conducted to evaluate the effect of proximity of female parasitoids to hosts, day female parasitoids are exposed to hosts, cage size, parental age of inexperienced females, host:female parasitoid density, host instar, and host exposure time on parasitization of hosts and sex ratio of *C. marginiventris* in the laboratory.

Materials and Methods

Colony culture. A *C. marginiventris* colony was established from a healthy colony in Tifton, GA. The colony was maintained with a constant supply of dilute honey water (50%) at 60 ± 10% RH, and a 12:12 h (L:D) photoperiod. Parasitoid rearing was conducted at 27°C because developmental time is shorter, adult longevity is longer, and sex ratio is higher for *C. marginiventris* reared at 27°C than at 24°C (Riggin et al. 1992). The *C. marginiventris* colony was reared in a 64.4 × 34.4 × 6.7 cm cage (Tillman and Scott 1997) on larvae of *S. exigua.* For colony culture and all experiments, the host was fed an agar soybean flour-wheat germ diet (King and Hartley 1985) and reared using a mechanized rearing system described by Tillman et al. (1997).

Proximity of female parasitoids to hosts. A short and tall cage with 4.9 and 44.2 cm, respectively, between hosts and the top of the cage were the two treatments used to determine if confined proximity of the adult females to hosts affected parasitization and percentage of female progeny. This study was conducted because previous efforts at producing a colony in large cages failed because the percentage of female progeny was low. The internal dimensions of the short cage and the tall cage were 64.41 × 34.4 w × 6.7 h cm and 81.51 × 39.5 w × 46.0 h cm, respectively. Second instars of *S. exigua* were placed singly in rearing cells provisioned with artificial diet in a 32-celled rearing tray 1.8 cm deep (Tillman et al. 1997). Four of these rearing trays (128 host larvae) were placed on the bottom of the cage. Before the test, 2 to 6-h-old females were paired individually with a single male in a Petri dish (100 × 15 mm) to mate. Mating was observed, and then 10 females/cage were placed in each treatment cage and held in a rearing room maintained at ≈27°C, 70 to 80% RH, and a photoperiod of 12:12 h (L:D). Light from a 400-watt metal halide lamp mounted on the ceiling spread evenly over the treatment cages. After 24 h exposure to parasitoids, host larvae were placed singly in rearing cells provisioned with artificial diet in a 32-celled rearing tray that was then covered with 200-gauge Mylar (Oliver Products, Grand Rapids, MI). These host larvae were held in an environmental chamber main-

tained at 27°C, 60 ± 10% RH, and a 12:12 h (L:D) photoperiod and checked every day until adult parasitoid emergence. If a host appeared to be dying or did not pupate, it was dissected to determine the presence of an immature parasitoid. This procedure was replicated 8 times. Mean number parasitized, percentage of female progeny, percentage immature parasitoid mortality, and percentage of cocoons from which adult parasitoids emerged was compared between the two treatments using *t*-tests.

Daily apparent fecundity and sex ratio. A test was conducted to determine daily apparent fecundity (total number of progeny) and sex ratio (percentage of female progeny) for the lifetime of 18 individual female wasps. Before the test, 2 to 6-h-old females individually were paired with a single male in a Petri dish (100 × 15 mm) to mate. After mating was observed, the female was placed in an ovipositional cage (35.61 × 25.4 w × 12.7 h cm with a 26.71 × 15.9 w cm screen on top) containing 64 second instars of *S. exigua* (2 trays of artificial diet each with 32 hosts, one tray on a bottom wire rack and the second tray on a top wire rack). This ovipositional cage was placed in an environmental chamber maintained at 27°C, 70 to 80% RH, and a photoperiod of 12:12 h (L:D). Female parasitoids were exposed to fresh second instars of the host every day from adult eclosion until the death of the female. Host larvae that had been exposed to a parasitoid for 24 h were removed and placed singly in rearing cells provisioned with artificial diet in a 32-celled rearing tray that was then covered with 200-gauge Mylar. These host larvae were held in an environmental chamber maintained as above and checked every day until adult parasitoid emergence. If a host appeared to be dying or did not pupate, it was dissected to determine the presence of an immature parasitoid. PROC NLIN (SAS Institute 1998) was used to determine if there was a relationship between day that the females were exposed to hosts and parasitization or percentage of female progeny. Mean number of progeny, percentage parasitization and female progeny, and longevity for these females were calculated using PROC MEANS (SAS Institute 1998).

Ovipositional cage type. Three types of cages were tested: (1) small cage (64.41 × 34.4 w × 6.7 h cm) which was clear on the top and dark on the sides and bottom and held 256 hosts (128 hosts on the bottom of the cage and 128 hosts on a top wire rack 2.54 cm above the first tray), (2) large cage (66.01 × 44.5 w × 25.7 h cm) which was clear so that light could penetrate through the sides and bottom of the cage and held 512 hosts (4 layers of 128 hosts per layer with 2.54 cm between each layer), and (3) large cage (same size and host number as above) which was painted so that light could not pass through the sides and bottom of the cage. A larger cage size was desired for rearing since the same number of insects could possibly be produced quicker than with the smaller cage. It was hypothesized that preventing light from penetrating the large cage would increase production of *C. marginiventris* because the adult females would spend more time parasitizing hosts than walking on the sides of the cage trying to escape towards the light. Early second instars of *S. exigua* were used as hosts. After hosts were placed in the cages, 1-d-old *C. marginiventris* females, with an equal number of males, were aspirated inside the cage at a host:female parasitoid ratio of 15:1. The large ovipositional cage was used to try to develop a mass-rearing program for this insect. In a mass-rearing project, sexing the individuals for all the cages would be too time consuming, and thus, both sexes would be placed in ovipositional cages. Therefore, an equal number of *C. marginiventris* males was aspirated into an oviposition cage in this and subsequent tests. Mating between individual males and females was not observed in this test and subsequent tests, but it was assumed that mating occurred when female progeny were produced. Each

ovipositional cage was held in an environmental chamber maintained at 27°C, 70 to 80% RH, and a L12:D12 photoperiod. After 24 h exposure to parasitoids, host larvae were removed from the cage and placed singly in rearing cells provisioned with artificial diet in a 32-celled rearing tray that was then covered with 200-gauge Mylar and held in an environmental chamber maintained as above. Starting on day 8, these host larvae were checked every 24 h until immature parasitoids pupated. Hardened cocoons were collected and held at the same environmental conditions as above until adult emergence. Thus, for this test and subsequent tests, percentage parasitization was based on percentage of hosts from which cocoons were obtained, and sex ratio was ascertained from the number of males and females that emerged from these cocoons for each replicate. Treatments were replicated 8 times. Percentage parasitization and sex ratio data were converted by arcsin transformation and analyzed using PROC ANOVA (SAS Institute 1998). Mean number of progeny produced per female was calculated using PROC MEANS (SAS Institute 1998).

Female age at first oviposition. Three age classes were tested: (1) newly-emerged females, (2) 1-d-old females with no ovipositional experience, and (3) 2-d-old females with no ovipositional experience. A large, clear cage (same size and host arrangement as in previous test) that held 512 second-instars of *S. exigua* was used as a treatment replicate. After hosts were placed in a cage, *C. marginiventris* females at the appropriate age were aspirated inside the cage at a host:female parasitoid ratio of 15:1. Each cage was held in an environmental chamber maintained at 27°C, 70 to 80% RH, and a 12:12 h (L:D) photoperiod. After 24 h exposure to parasitoids, host larvae were removed from the cage. These host larvae and subsequent cocoons were handled in the same manner as in the previous experiment. The test was replicated 8 times. Percentage parasitization and sex ratio data were converted by arcsin transformation and analyzed using PROC ANOVA (SAS Institute 1998). When significant differences were obtained, means were separated by Fisher protected least significant difference (LSD).

Host:female parasitoid density. This experiment varied the host:female parasitoid densities, using ratios of 5:1, 10:1, 15:1, 20:1, 25:1, and 30:1. This test was conducted to determine the optimal number of adult parasitoid females needed for the greatest production of female progeny. The 30:1 host:parasitoid ratio was used as the highest host:parasitoid ratio since preliminary findings showed that females oviposited a mean of around 30 eggs during peak egg production. A large cage (same size and host arrangement as in previous test) that held 512 second instars of *S. exigua* was used as a treatment replicate. After hosts were placed in the cage, 1-d-old *C. marginiventris* females were aspirated inside the cage at the appropriate host:female parasitoid ratio. Each ovipositional cage was held in an environmental chamber maintained at 27°C, 70 to 80% RH, and a 12:12 h (L:D) photoperiod. After 24 h exposure to parasitoids, host larvae were removed from the cage. These host larvae and subsequent cocoons were handled in the same manner as in the previous experiment. The test was replicated 12 times. Data analyses were the same as in the previous experiment.

Host instar. Three classes of host instar were tested: (1) late first instars, (2) second instars, and (3) third instars. Host instar was determined by observing egg mass hatch and the presence of exuviae. A large cage (same size and host arrangement as in previous test) that held 512 *S. exigua* larvae was used as a treatment replicate. After the appropriate host instar was placed in the cage, 1-d-old *C. marginiventris* females were aspirated inside the cage at the 15:1 ratio of host:female

parasitoid ratio. Each ovipositional cage was held in an environmental chamber maintained at 27°C, 70 to 80% RH, and a 12:12 h (L:D) photoperiod. After 24 h exposure to parasitoids, host larvae were removed from the cage. These host larvae and subsequent cocoons were handled in the same manner as in the previous experiment. The test was replicated 12 times. Data analyses were the same as in the previous experiment.

Host exposure period. Host exposure periods of 4, 7, and 24 h were tested. A large cage (same size and host arrangement as in previous test) that held 512 second instars of *S. exigua* was used as a treatment replicate. After hosts were placed in the cage, 1-d-old *C. marginiventris* females were aspirated inside the cage at the 15:1 ratio of host:female parasitoid ratio. Each ovipositional cage was held in an environmental chamber maintained at 27°C, 70 to 80% RH. The 4 and 7 h treatments were always maintained at constant light whereas the 24 h exposure period was maintained at a photoperiod of 12:12 h (L:D). After exposure to parasitoids, host larvae were removed from the cage. These host larvae and subsequent cocoons were handled in the same manner as in the previous experiment. The test was replicated 12 times. Data analyses were the same as the previous experiment.

Results

Proximity of female parasitoids to hosts. Parasitization and sex ratio of progeny were affected by constrained proximity of female parasitoids to their hosts. A significantly higher number of progeny was produced when *C. marginiventris* females were more closely associated with hosts in short cages than in tall cages ($t = 2.99$, df = 14, $P = 0.0097$) (Table 1). Percentage of female progeny also was significantly higher when parent females were closely associated with hosts in short cages than with hosts in tall cages ($t = 4.57$, df = 14, $P = 0.0004$). Proximity of female parasitoids to hosts did not affect percentage immature mortality ($t = 0.4372$, df = 14, $P = 0.6686$) or percentage of adults that emerged from parasitoid cocoons ($t = 0.9137$, df = 14, $P = 0.3564$).

Daily apparent fecundity and sex ratio. For individual *C. marginiventris* females, the number of progeny produced was related to the day that the females were exposed to hosts ($F = 91.8$, df = 4, 7, $P < 0.001$) (Fig. 1A). Peak production of progeny

Table 1. Effect of constrained proximity of female *C. marginiventris* to second instars of *S. exigua* on mean number of hosts parasitized, percentage of female progeny, percentage mortality of immature parasitoids, and percentage parasitoid adult emergence

Cage type*	Mean ± SEM no. hosts parasitized**	% ± SEM Female progeny**	% ± SEM Mortality of immature parasitoids**	% ± SEM Parasitoid adult emergence**
Short cage	95.0 ± 4.8 a	65.1 ± 3.8 a	6.2 ± 4.3 a	82.2 ± 2.5 a
Tall cage	71.6 ± 6.2 b	42.3 ± 3.2 b	7.1 ± 1.5 a	85.1 ± 1.9 a

* Short cage had 4.9 cm between hosts and top of cage. Tall cage had 44.2 cm between hosts and top of cage. For each cage, 128 hosts were exposed to 10 parasitoid females for 24 h.

** Means in columns followed by the same lower case letter are not significantly different (*t*-test, $P > 0.01$).

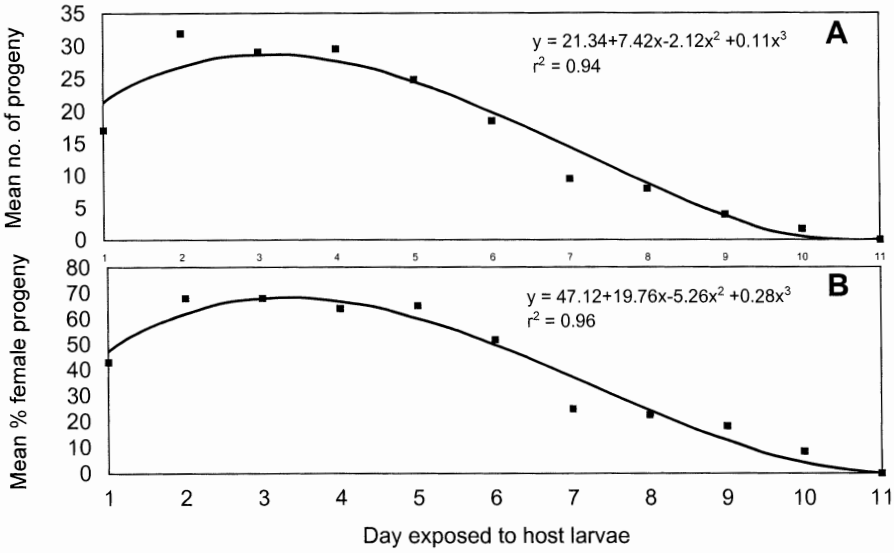

Fig. 1. Relationship of ovipositional day to number of progeny (A) and percentage of female progeny (B) for *C. marginiventris* females. Each point represents a mean value. Each female parasitoid was allowed to oviposit for 24 h in a cage with 64 hosts.

occurred from day two through five (Table 2). The mean (±SEM) number of progeny produced and percentage parasitism for those three days was 30.19 (±1.54) and 47.17% (±1.39), respectively. The sex ratio of the progeny also was influenced by the day that the females were exposed to hosts (F = 153.23, df = 4, 7, P < 0.001) (Fig. 1B). Peak production of female progeny was from day two through five (Table 2). Mean (±SEM) longevity for these females was 6.83 (±1.92) days with a range of 6 to 10 days.

Ovipositional cage type. Ovipositional cage type did not influence parasitization or sex ratio. Percentage parasitization for *C. marginiventris* was not significantly different between small cages (mean ± SEM = 51.63 ± 2.19), large, painted cages (mean ± SEM = 51.25 ± 1.94), and large, clear cages (mean ± SEM = 51.75 ± 2.66) (F = 0.02, df = 2, P = 0.9788). Sex ratio of progeny for *C. marginiventris* did not differ significantly between small cages (mean ± SEM = 62.22 ± 3.23), large, painted cages (mean ± SEM = 62.4 ± 2.96), and large, clear cages (mean ± SEM = 57.75 ± 4.22) (F = 0.26, df = 2, P = 0.774). The mean (±SEM) number of progeny per female for these cages was 7.89 (±0.17).

Female age at first oviposition. Parasitization was affected by parental age of inexperienced females (F = 10.56, df = 2, P = 0.0008) (Table 3). Percentage parasitization was higher for newly-emerged and 1-d-old *C. marginiventris* females than for 2-d-old females (Fisher protected LSD, P < 0.01). Sex ratio of progeny was not affected by parental age of these females (F = 0.89, df = 2, P = 0.4283).

Host:female parasitoid density. Parasitization was influenced by the ratio of

Table 2. Mean (±SEM), minimum, and maximum number of progeny and percentage female progeny per female over the lifetime of 18 *C. marginiventris* females*

	Number of progeny			% Female progeny		
Day	Mean (±SEM)	Minimum	Maximum	Mean (±SEM)	Minimum	Maximum
1	17.1 ± 1.9	3	32	31.4 ± 4.6	0	60.0
2	31.9 ± 2.1	19	47	50.3 ± 4.5	21.7	85.2
3	29.1 ± 2.0	16	47	45.5 ± 4.6	20.0	83.3
4	29.6 ± 1.8	18	51	43.2 ± 4.2	21.9	76.9
5	24.8 ± 1.6	17	42	45.8 ± 4.0	20.8	82.4
6	18.5 ± 2.4	3	37	33.8 ± 4.8	0	68.4
7	8.9 ± 2.7	0	38	18.1 ± 5.8	0	62.5
8	7.9 ± 2.6	0	29	13.5 ± 4.5	0	52.9
9	3.9 ± 1.6	0	19	14.2 ± 5.9	0	71.4
10	1.0 ± 0.5	0	9	6.0 ± 3.4	0	44.4

* Each female parasitoid was allowed to oviposit for 24 h in a cage with 64 hosts.

female parasitoids to hosts (F = 4.62, df = 5, P = 0.0012) (Table 3). Percentage parasitization was lower for the host:female parasitoid ratio 5:1 than for all the other host ratios (Fisher protected LSD, $P < 0.01$). Sex ratio of progeny was not significantly different for each host:female parasitoid ratio tested (F = 0.04, df = 5, P = 0.9989).

Host instar. Host instar influenced both parasitization (F = 64.71, df = 2, P = 0.0001) and sex ratio of progeny (F = 8.03, df = 2, P = 0.0028) (Table 3). Percentage parasitism was significantly higher for second instars than for the other two host instar classes (Fisher protected LSD, $P < 0.01$). The percentage female progeny was higher for second and third instars than for first instars (Fisher protected LSD, $P < 0.01$).

Host exposure period. Parasitization was affected by host exposure time (F = 40.8, df = 2, P = 0.0001) (Table 3). Percentage parasitization was higher with a 24 h host exposure period than with the other two exposure periods tested (Fisher protected LSD, $P < 0.01$). Sex ratio of progeny was not influenced by host exposure period (F = 0.99, df = 2, P = 0.3976).

Discussion

In laboratory rearing of *C. marginiventris*, close association with hosts assured higher parasitization of hosts and subsequent production of female progeny than could be attained when female parasitoids were given room in the rearing cage to fly away from hosts. Even in the large cages that held layers of host trays, parasitization and sex ratio were good. Constrained proximity to hosts, and not cage size, was a limiting factor in rearing this parasitoid in large cages.

Differential mortality of immature parasitoids or adult emergence was not responsible for these differences in parasitization and sex ratio in the "proximity" test be-

Table 3. Effect of female age, host:female parasitoid density, host instar, and host exposure period on percentage parasitization of *S. exigua* and percentage of female progeny for *C. marginiventris**

Test	Treatment	% (±SEM) Parasitization**	% (±SEM) Female progeny**
Female age	newly-emerged	48.2 ± 3.8 a	55.1 ± 2.6 a
	1-day-old	47.5 ± 2.4 a	61.1 ± 4.9 a
	2-day-old	31.5 ± 2.6 b	55.6 ± 2.3 a
Host:female parasitoid ratio	5:1	37.0 ± 2.5 a	60.0 ± 4.6 a
	10:1	49.0 ± 1.9 b	59.4 ± 3.5 a
	15:1	49.5 ± 1.8 b	61.1 ± 3.3 a
	20:1	46.8 ± 1.5 b	59.3 ± 2.8 a
	25:1	47.9 ± 1.6 b	59.9 ± 2.8 a
	30:1	49.2 ± 1.4 b	59.9 ± 2.8 a
Host instar	first	21.4 ± 1.7 a	46.8 ± 3.15 a
	second	48.1 ± 1.7 b	62.3 ± 3.2 b
	third	29.4 ± 1.7 c	61.0 ± 2.9 b
Host exposure period	24 h	48.6 ± 1.8 a	60.1 ± 1.9 a
	7 h	32.1 ± 1.4 b	57.1 ± 1.8 a
	4 h	27.1 ± 1.7 b	57.0 ± 1.6 a

* Unless otherwise stated in table, 512 second-instar hosts were exposed to 1-d-old parasitoid females at a 15:1 host:parasitoid ratio for 24 h.

** Means in columns followed by the same lower case letter are not significantly different (Fisher protected LSD, $P > 0.01$).

cause there were no differences in these biological factors between treatments. Instead, the differences in sex ratio were due to differential oviposition of fertilized and unfertilized eggs. Charnov (1979) stated that the uniqueness of haplodiploidy is that the mother can easily control the sex of her offspring. Like most Hymenoptera, *C. marginiventris* exhibits sex determination where females are produced from fertilized eggs and males from unfertilized ones. Flanders (1956) hypothesized that activation of sperm may be affected by environmental stimuli. Unfortunately, the environmental stimuli that may have affected sex ratio of *C. marginiventris* in this test are unknown, but this would be worthy of further investigation.

It is well known that plant and host cues attract female parasitoids to host plants and hosts. Single and dual choice tests in a flight tunnel revealed that plants damaged by beet armyworm larvae are the main source of the volatiles that attract females of the parasitoid *C. marginiventris* to the microhabitat of their host (Turlings et al. 1991). Frass and host larvae, the other two components of a complete plant-host complex, were significantly less attractive than the damaged plants. The plant component is necessarily eliminated from laboratory rearing. Parasitism by *C. marginiventris* fe-

males may have been lower in cages in which the parasitoids were free to fly because the main cues which attract the females to hosts, plant volatiles, were lacking, and females at the top of the high cages may not have been strongly attracted to hosts at the bottom of the cage. Forcing the female parasitoids to be close to the hosts may circumvent the necessity of the plant to attract the female parasitoids to hosts. Another possible scenario could be that cages in which hosts and female parasitoids are closely confined simulates the situation in the field where hosts on the underside of cotton leaves are in close proximity to leaves in the lower canopy of the plant.

The amount of light entering the cages in the "proximity" test probably did not directly affect parasitization and sex ratio of this parasitoid. First, the light was evenly spread over the cages in the "proximity" test. Secondly, parasitization and sex ratio did not vary between the large, painted cages and the large, clear cages, so differences in amount of light in large cages did not directly affect parasitization and sex ratio. However, it is possible that females in the "tall" cages with lots of space between the hosts and top of the cage were attracted to the light at the top more than to hosts far away at the bottom of the cage and, therefore, were more inclined to sitting on the top of the cage than in parasitizing hosts.

Because penetration of light into the sides of the cage and cage size did not affect parasitization and production of female progeny, the large, clear cage probably should be used for parasitoid production. Using clear cages avoids wasting time painting cages and also increases the efficiency of production because insects can be produced more quickly than with the smaller cage. Unfortunately, the number of progeny per female per day was lower in the tests with the large cages than in the fecundity tests. This difference, however, was mainly due to the fact that mortality of immature parasitoids was taken into consideration in the latter test, but not in the former tests.

Higher parasitization occurred on the second and third day of oviposition than on the first day. In tests to determine apparent fecundity of *C. marginiventris* from the green cloverworm, observations indicated that females were more active on the second and third days than on the first and fourth days (Kunnalaca and Mueller, 1979). Data from our study corroborate the observations of Kunnalaca and Mueller (1979). Lower parasitization on the first day of oviposition was probably due two factors, a preovipositional period and lack of experience of parasitoids to hosts. Some of the newly-emerged females produced very few progeny while other newly-emerged females produced ten times more progeny than other females. Thus, it appears that *C. marginiventris* females have a variable preovipositional period. Experience with hosts probably was another factor accounting for the higher production of progeny the second day of oviposition because experience with hosts and/or frass can significantly enhance the response of *C. marginiventris* to hosts (Dmoch et al. 1985). To maximize a mass rearing program, new females could be exposed to a small number of larvae to attain ovipositional experience. This first group of progeny from these females could be used for maintaining a stock culture. Cocoons produced from these females for the second and third day of oviposition could be appropriated for augmentative releases.

Longevity of *C. marginiventris* females was similar to longevity reported for this parasitoid by Kunnalaca and Mueller (1979). In this study, the mean range for longevity of females ranged from 4.91 to 8.75 days at 27°C while the mean range for longevity of females in the other study was 7.5 to 17.3 days at 25°C and 4.5 to 9.3 days at 30°C.

Inexperienced *C. marginiventris* females receiving their first ovipositional experience after the 1-d-old age appear to be unable to meet their fecundity potential at the host:parasitoid ratios in this study. Also, peak apparent fecundity occurs the first few days of the life cycle of the female parasitoid. Thus, newly-emerged or 1-d-old females should be protected by using less toxic insecticides (Tillman and Scott 1997), and females no older than 1-d-old in age should be used to optimize an augmentation program.

The lower parasitization which resulted at the 5:1 host:parasitoid ratio in comparison to the other ratios tested may have resulted from adult female competition in the cage. Also, because male insects can interrupt oviposition by females (Martens and Rehfeldt 1989), *C. marginiventris* males may have been interfering with oviposition by females in this treatment. It already has been suggested that newly-emerged females should obtain a day of experience parasitizing hosts before using the females in a large production program. At the same time females are being experienced with hosts and their ovipositional systems are reaching their full potential, females should be allowed to mate with males. Then, inclusion of males in the large rearing boxes with experienced females the next day could be dropped possibly increasing production of progeny.

Regardless of the age and experience of the females, early second instars of *S. exigua* should be used in an augmentation and rearing program to maximize both parasitoid production and sex ratio of progeny. Using early first instars may result in lower parasitoid production and percentage of females. Even though sex ratio of progeny was the same for second and third instars, percentage parasitization was higher for second instars than for third instars. Thus, the second instar was the optimum host instar for parasitoid rearing. The low parasitism obtained when using first instars may be due to a greater acceptance of this parasitoid for second instars of *S. exigua* over first instars for oviposition. In a 2-yr field study, percentage parasitism by *C. marginiventris* from *S. exigua* larvae collected from cotton was higher for second instars than for first instars for 1 yr and the same for the two instars for the second year (Ruberson et al. 1994). The parasitoid, thus, showed a greater acceptance for second instars of *S. exigua* than for first instars for at least 1 yr. Percentage parasitism was the same for second and third instars for both years of the field study conducted by Ruberson et al. (1994). The parasitism rate observed in third instars was probably carryover from the second instar (J. Ruberson, pers. commun.). Thus, our results support those of Ruberson et al. (1994). The small size of first instars may have influenced sex ratio of progeny of *C. marginiventris.* Host size is known to affect sex ratio for other parasitoid species (Brunson 1937, Van den Assem 1971, Jones 1982, Tillman and Cate 1993) resulting in more males emerging from small hosts and more females emerging from large hosts. Overall, early second instars would probably be best for an augmentation program for the reasons given above and also because the hosts would still be small and congregated in the spot where they hatched from the egg batch.

In conclusion, designing a program around the environmental factors that enhance parasitization and sex ratio of *C. marginiventris* can optimize conservation and augmentation biological control strategies for this parasitoid. Thus, assuring that 1-d-old mated female parasitoids experienced in parasitizing hosts are in close proximity to second instars of *S. exigua* can greatly increase the success of the biological control strategy selected for control of this pest.

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