Production of the Parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae) in Unicellular Rearing Trays Using the Host *Spodoptera exigua* (Lepidoptera: Noctuidae)[1]

Eric W. Riddick[2]

USDA-ARS, Biological Control and Mass Rearing Research Unit, Mississippi State, MS 39762-5367 USA

J. Entomol. Sci. 39(3): 362-372 (July 2004)

Abstract Factors that might affect parasitism rate and progeny production of *Cotesia marginiventris* (Cresson), a solitary endoparasitoid of lepidopteran larvae, were considered in this study. Hosts were *Spodoptera exigua* (Hübner) larvae, which were feeding gregariously on artificial diet within 270-mL unicellular rearing trays. The following hypotheses were tested: (1) parasitism and superparasitism rates increased as exposure time and parasitoid density increased, and (2) progeny production decreased as parasitoid age increased. Parasitism rate increased significantly as exposure to *S. exigua* larvae increased from 2 to 6 h, but not from 6 to 18 h. Superparasitism rate was not affected significantly by exposure time. Both parasitism and superparasitism rates were greatest at a density of 3 rather than 1 parental parasitoid per tray; no differences were evident between densities of 3 vs 2 or 2 vs 1 parasitoid per tray. Significantly more offspring were produced (with normal sex ratios) when parental females were inserted into rearing trays as adults rather than as pupae (in cocoons). Also, 1- to 2-d-old and 8- to 9-d-old females produced more progeny (with normal sex ratios) than 15- to 16-d-old females. This study suggests that inserting a single, mated 1- to 9-d-old *C. marginiventris* female into a unicellular rearing tray containing an abundance of putative hosts could limit superparasitism without seriously reducing progeny production. Partial automated rearing of *C. marginiventris* is possible.

Key Words Automation, biological control, mass rearing, parasitoids, superparasitism

Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae) is native to the West Indies (Muesbeck 1921) and is currently distributed in North America, from Delaware south to Florida, west to Indiana, Kansas, Texas, Wisconsin, Arizona and California (Marsh 1979); and in Central and South America. It is an important solitary endoparasitoid of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), and other lepidopteran pests in agroecosystems (van den Bosch and Hagen 1966, Ashley et al. 1982, McCutcheon et al. 1990, Ruberson et al. 1994, Novoa and Luna 1996). There has been recent interest in using *C. marginiventris* for biological control of lepidopterans on vegetables grown in greenhouses (Gillespie et al. 1997, Hoffman et al. 1998, Urbaneja et al. 2002).

Inundative releases of *C. marginiventris* might be the best approach for pest sup-

[1]Received 14 August 2003; accepted for publication 23 November 2003. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by USDA for its use.

[2]Address inquiries (email: *ewriddick@ars.usda.gov*).

pression in certain agroecosystems. A prerequisite to inundative releases is the production of large quantities of quality insects (Nordlund 1998). *In-vitro* rearing of this parasitoid to the adult stage has not been achieved (Greany 1986); thus, *in-vivo* rearing is the only option at this time. However, refinement and improvement of *in-vivo* rearing methods are needed.

The state-of-the-art for *in-vivo* rearing of solitary braconids that attack noctuid larvae has involved the use of multicellular (32-cell) rearing trays (55.1 × 14.6 × 1.8 cm, L × W × H) for automated rearing of host larvae on artificial diet; then placement of these trays, without lids, at the base of parasitoid 'sting' cages of various sizes (Powell and Hartley 1987, Tillman et al. 1997, Tillman 2001). Tillman (2001) compared parasitism rate and progeny production of *C. marginiventris* in tall cages (81.5 × 39.5 × 46.0 cm, L × W × H) versus short cages (64.4 × 34.4 × 6.7 cm; L × W × H). Short cages facilitated the greatest parasitism of *S. exigua* larvae and production of *C. marginiventris* progeny, including more female progeny. Thus, restricting the search area of female parasitoids within sting cages greatly increased production efficiency (Tillman 2001).

In this current study, the potential of using 270-mL unicellular trays (~12 × 12 × 2 cm, L × W × H) for efficient *in-vivo* rearing of *C. marginiventris* was explored. The unicellular tray would (1) allow efficient rearing of 50 to 70 *S. exigua* larvae within a single cell, (2) restrict search area of female parasitoids, (3) eliminate the need to transfer *S. exigua* larvae to a 'sting' cage, then transfer parasitized larvae back into diet trays for rearing, and (4) facilitate automated or partial automated rearing of *C. marginiventris.* Automation can be defined as the replacement of human labor with mechanical and/or electrical systems (Harrell and Gantt 1984). Automated rearing may allow cost-effective propagation of large quantities of parasitoids necessary for commercial-scale inundative releases (Nordlund and Greenberg 1994, Nordlund 1998).

Research to determine whether *C. marginiventris* can be produced efficiently from *S. exigua* in unicellular trays has not been reported previously. The suitability of these trays for efficient production of *C. marginiventris* was assessed by (1) dissecting hosts to estimate parasitism and superparasitism rates, relative to exposure time and parental parasitoid density, and (2) rearing hosts to determine progeny production and sex ratio, relative to parental parasitoid age.

Materials and Methods

Insect cultures. The beet armyworm has been reared on an agar soybean flour-wheat germ meridic diet (after King and Hartley 1985) for more than 200 continuous generations at the USDA-ARS, Biological Control and Mass Rearing Research Unit (BCMRRU), Mississippi State, MS. Partial automated rearing of *S. exigua* larvae has been accomplished with form-fill-seal technology, using multicellular (32-cell) rearing trays (Davis et al. 1990, Tillman et al. 1997). The multicellular tray was initially used for rearing larvae of cannibalistic (e.g., *Helicoverpa zea* (Boddie)) and semi-cannibalistic species (e.g., *Heliothis virescens* (F.)) at a density of one larva per cell (3 cm diam) with each cell holding approximately 7 gm of artificial diet (Davis et al. 1990).

Cotesia marginiventris has been reared at BCMRRU for more than 100 continuous generations. The original parasitoids came from a colony maintained at an USDA-ARS facility in Tifton, GA. The culture at BCMRRU has been maintained by exposing

late first to second-instar *S. exigua* larvae (on meridic diet within multicellular trays, without lids) to *C. marginiventris* females for 24 h in a 'sting' box (45.7 × 66.0 × 8.9 cm, W × L × H; 18.9 L clear plastic) with lid. Afterwards, exposed *S. exigua* larvae were removed and transferred, by hand, to new multicellular trays holding fresh diet. Trays were sealed with a mechanical press and placed on metal racks in an environmentally-controlled rearing room (27°C, 60-70% RH, and 16 h photophase). After 2 wk, trays were removed and *C. marginiventris* cocoons (i.e., pupae) were harvested from them.

Unicellular host-rearing trays. Experimental unicellular trays (11.75 × 11.75 × 1.9 cm, L × W × H; 270 mL) were molded from high-impact polystyrene. This tray (and size variations of it) has been used for partial automated rearing of boll weevils, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), at USDA-ARS, Mississippi State, MS, using form-fill-seal technology (Griffin and Malone 1983). Trays with contents were sealed with perforated lids made from double-punched 200-gauge Mylar™ (Oliver Products, Grand Rapids, MI).

Effects of exposure time and parasitoid density on parasitism rate. In two separate experiments, *S. exigua* larvae were dissected to determine the effects of exposure time and parasitoid density on incidence of parasitism and superparasitism. Experimental trays were filled with 200 mL of flash-sterilized meridic diet. Several hours later, 80 *S. exigua* eggs (that had been rinsed free from moth scales and surface-sterilized, using sodium hypochlorite) were added to each tray, with a fine paint brush. Trays containing diet and *S. exigua* eggs were sealed with Mylar™ lids and placed in an environmentally-controlled holding room (27°C, 50 to 55% RH, and 16 h photophase). Four days later, trays contained *S. exigua* late first to second-instar larvae.

A random sample of approximately 250 *C. marginiventris* cocoons, all of the same generation, were harvested from the BCMRRU culture and placed at the base of a white, polypropylene cage (30 × 30 × 30 cm). Each cage was provisioned with honey and maintained in a rearing room (as before). Emerged adults (both sexes) were given continuous access to honey and sterile water. Presumably mated, 1- to 2-d-old *C. marginiventris* females were removed from the polypropylene cage and inserted into experimental trays with potential hosts on 18 January and 1 March 2001 for the exposure time and parasitoid density experiments, respectively. The rearing tray was the experimental unit and a completely randomized design was used with trays replicated six times per exposure time (2, 6 or 18 h) for a total or 18 observations; trays were replicated nine times per parasitoid density (1, 2 or 3 females) for a total of 27 observations. Note that only one female was inserted into replicate trays in the exposure time experiment. In the parasitoid density experiment, all treatment females were exposed to hosts for 18 h. Twenty-four hours after the cessation of experiments, all trays (with contents) were stored in a counter-top freezer (−18 to −20°C), until dissection.

The mean ± SEM number of *S. exigua* larvae per tray on the day that dissections were initiated was 54.0 ± 3.11, 50.0 ± 4.75 and 47.7 ± 4.57 individuals for 2, 6 and 18 h exposure times, respectively, and 60.0 ± 1.67, 53.6 ± 3.36 and 55.8 ± 3.38 individuals for 1, 2 and 3 density treatments, respectively. Twenty *S. exigua* larvae were selected at random from each tray for dissection. A total of 360 larvae was dissected in the exposure time experiment; 540 were dissected in the parasitoid density experiment. The number of *C. marginiventris* first-instar larvae inside each *S. exigua* larva was recorded, and rates of parasitism and superparasitism were determined per

tray per treatment. In this experiment, parasitism rate was the percentage of hosts containing any *C. marginiventris* larvae; whereas, superparasitism rate was the percentage of parasitized hosts containing two or more *C. marginiventris* larvae. Superparasitism has been defined as the tendency of solitary parasitoids to deposit two or more eggs into the same host in two or more encounters. More specifically, this behavior is called self-superparasitism when a single female is involved; it is called conspecific superparasitism when two or more females are involved (van Dijken and Waage 1987).

Effects of parasitoid age on progeny production and sex ratio. In two separate experiments, *S. exigua* larvae were reared to determine the effects of parasitoid age on progeny production and sex ratio. The first rearing experiment compared the effect of inserting five *C. marginiventris* parental pupae versus one adult parental female into unicellular trays. The second rearing experiment compared the effect of inserting a 1- to 2-d, 8- to 9-d or 15- to 16-d-old parental female into unicellular trays. The rearing tray was the experimental unit and a completely randomized design was used with trays replicated 10 times per age group in both experiments. Unicellular trays were filled with 200 mL of flash-sterilized meridic diet. Several hours later, 80 *S. exigua* eggs (treated as before) were added to each tray, with a fine paint brush. Trial date was used as a blocking factor: first experiment; 28 February 2002 (trial 1), 11 April 2002 (trial 2), and 23 May 2002 (trial 3); second experiment: 22 March 2001 (trial 1), and 12 April 2001 (trial 2). For combined trials, there was a total of 60 observations in both experiments.

In the first rearing experiment, five *C. marginiventris* cocoons (from a random sample of approximately 250 cocoons of the same generation harvested from the BCMRRU culture on the same day) were subsequently added, by hand, to one-half of the experimental trays. All other cocoons were placed at the base of a white polypropylene cage (30 × 30 × 30 cm), which was provisioned with honey and held in a rearing room. Trays containing diet and *S. exigua* eggs with or without parental cocoons were sealed and placed in the holding room for 4 d, then trays were transferred to the rearing room. On the same day, one presumably mated, 1- to 2-d-old adult *C. marginiventris* female was inserted into the remaining trays (those not provisioned with parental cocoons). Trays were re-sealed and placed in the rearing room.

In the second rearing experiment, a random sample of approximately 100 to 150 cocoons was harvested from the BCMRRU culture on the same day for three consecutive weeks. Thus, *C. marginiventris* cocoons were from at least two distinct rearing colonies (i.e., isolines) because approximately 2 wk were required to complete a life cycle. Cocoons were placed into separate white, polypropylene cages (30 × 30 × 30 cm), provisioned with honey; one cage for each age group. These cages were maintained in the rearing room. Emerged adults (both sexes) had continuous access to honey and sterile water. Females of all three age groups were separated from male cohorts (after the fourth day that parental cocoons had been placed in cages) so that time with mates could be standardized between treatments. On the day that the 1- to 2-d-old females were removed from their cage, the 8- to 9-d-old and 15- to 16-d-old females were also removed from their cages and placed into trays with putative hosts. A single parasitoid female was inserted into each tray. Trays were re-sealed and placed in the rearing room.

In both experiments, female parasitoids of each treatment group were exposed to host larvae for 24 h, then removed from trays and discarded. Trays were re-sealed and placed back in the rearing room. Twelve days later, trays were opened and all *S.*

exigua larvae (and occasionally pupae) and *C. marginiventris* progeny (i.e., cocoons) were removed and placed in clean, plastic Petri dishes (15 × 100 mm). Cocoons were held in a growth chamber (26.5°C, 60 ± 5% R.H., 16 h photophase) to allow for adult emergence. The number of *C. marginiventris* cocoons and emerged adults and progeny sex ratio (i.e., % females) were determined per tray per treatment.

Statistical analysis. A one-factorial analysis of variance (ANOVA) was used to test for significance of exposure time as well as parasitoid density on the number of *C. marginiventris* larvae found inside *S. exigua* (host) larvae and rates of parasitism and superparasitism. The general linear model (GLM) with a two-factorial ANOVA was used to test for significance of parasitoid age and trial date on progeny production and sex ratio. Absolute data were square-root transformed and percentage data were arcsine-transformed prior to analysis (Zar 1999). Means (and least square means) were considered significantly different at $P \leq 0.05$. When necessary, the Tukey HSD method was used for pairwise mean comparisons after the ANOVA. Statistical analyses were performed with SigmaStat (1994) or Systat (1998) computer software. Untransformed data are presented.

Results

Exposure time and parasitoid density effects on parasitism rate. Exposure time had a significant effect on the number of parasitoid first instars found inside *S. exigua* larvae ($F = 26.8$; df = 2, 357; $P < 0.0001$; Table 1). Less than one first instar

Table 1. Effects of exposure time and parasitoid density on the mean ± SEM number of *C. marginiventris* larvae inside *S. exigua* (host) larvae and percentage of hosts parasitized and superparasitized per tray

Treatment	First instar larvae per host	% Parasitism	% Superparasitism
Experiment I			
Time			
2 h	0.33 ± 0.06 a	25.0 ± 5.77 a	7.5 ± 2.81 a
6 h	0.72 ± 0.075 b	52.5 ± 6.29 b	15.0 ± 4.83 a
18 h	0.925 ± 0.07 c	71.7 ± 6.41 b	15.8 ± 5.07 a
Experiment II			
Density			
1 ♀	1.06 ± 0.09 a	67.8 ± 5.66 a	21.1 ± 4.06 a
2 ♀	2.15 ± 0.18 b	82.2 ± 7.32 ab	45.6 ± 7.97 ab
3 ♀	2.40 ± 0.19 b	89.4 ± 3.94 b	49.4 ± 5.68 b

In experiment I, a single adult parasitoid female was inserted into each replicated experimental tray, then removed per time treatment; in experiment II, treatment females were inserted into replicated experimental trays, then removed after 18 h. Means (±SEM) followed by a different letter in a column are significantly different for each experiment. $P \leq 0.05$ (Tukey's test). n, 360 larvae or 18 trays in experiment I; n, 540 larvae or 27 trays in experiment II.

(on average) was found in hosts at all treatments, but more progeny were found in hosts as exposure time increased from 2 to 6 to 18 h. Some 99.9% of all parasitoid progeny were first instars rather than eggs. Parasitoids had developed beyond the egg stage within 24 h after parental females had been removed from all experimental trays. The percentage of hosts parasitized within 2 h was significantly less than the percentage parasitized within 6 h or 18 h ($F = 10.9$; df = 2, 15; $P = 0.001$); but, there was no difference between the 6 h and 18 h periods. More than half of all *S. exigua* larvae had been parasitized within 6 h of exposure.

The percentage of hosts superparasitized was not affected significantly by exposure time ($F = 1.3$; df = 2, 15; $P = 0.3$), although twice as many hosts had been superparasitized within 18 h and 6 h rather than in 2 h (Table 1). A maximum of three *C. marginiventris* first instars were found inside the same host larva for 2, 6, and 18 h exposure treatments.

Parasitoid density had a significant effect on the number of parasitoid first instars found inside host larvae ($F = 26.1$; df = 2, 537; $P < 0.0001$; Table 1); more parasitoid progeny were found in hosts when 2 or 3 rather than 1 parental female was exposed to hosts for 18 h. Percent parasitism was greater than 60% for all treatments. Parasitism rate was significantly different between 1 vs 3 parental females ($F = 3.9$; df = 2, 24; $P = 0.03$), but not between 1 vs 2 or 2 vs 3 parental females per tray.

Superparasitism rate was significantly different between parasitoid densities of 1 vs 3 parental females ($F = 4.75$; df = 2, 24; $P = 0.02$), but not between 1 vs 2 or between 2 vs 3 parental females. Almost 50% of host larvae contained two or more developing parasitoids at parental densities of 2 or 3 females per tray (Table 1). A maximum of 7, 10 and 15 *C. marginiventris* first instars were found inside the same host larva for 1, 2 and 3 parasitoid density treatments, respectively.

Age effects on progeny production and sex ratio. In the first age experiment, significantly more *C. marginiventris* progeny were produced by parents that had been inserted into rearing trays as adults rather than as pupae (new cocoons: $F = 24.7$; df = 1, 56; $P < 0.0001$; emerged adults: $F = 29.5$; df = 1, 56; $P < 0.0001$, Table 2). The average emergence rate of adult parasitoid progeny from their cocoons was 84.5 and 77% for the parental adult and parental pupae treatments, respectively. A greater percentage of female progeny resulted from the adult treatment than the pupae treatment ($F = 8.7$; df = 1, 56; $P < 0.005$; Table 2). Progeny sex ratio was essentially unbiased when parents were inserted into trays as adults, but strongly male-biased when parents were inserted into trays as pupae (in cocoons). At least 90% of the adult treatment females generated one adult female progeny per tray; whereas, 67% of the pupae treatment females generated one adult female progeny per tray. Trial date had no significant effect on production of cocoons or adults (new cocoons: $F = 1.8$; df = 2, 56; $P = 0.18$; emerged adults: $F = 2.5$; df = 2, 56; $P = 0.09$); but had a marginally significant effect on percentage of female progeny ($F = 3.3$; df = 2, 56; $P = 0.05$).

In the second age experiment, significantly more *C. marginiventris* cocoons and adults were produced by 1- to 2-d-old and 8- to 9-d-old females than by 15- to 16-d-old females (new cocoons: $F = 9.75$; df = 2, 56; $P < 0.0001$; emerged adults: $F = 9.75$; df = 2, 56; $P < 0.0001$, Table 2). The average emergence rate of adult progeny from their cocoons was 85%, 82%, and 74% from 1- to 2-d-old, 8- to 9-d-old, and 15- to 16-d-old parents, respectively. A greater percentage of female progeny resulted from 1- to 2-d-old and 8- to 9-d-old females than from 15- to 16-d-old females ($F = 9.85$; df = 2, 56; $P < 0.0001$). Progeny sex ratio was rather unbiased for the 1- to 2-d-old and 8- to 9-d-old treatment groups, but extremely male-biased for the 15- to

Table 2. Effect of parasitoid age on the least square mean ± SEM number of *C. marginiventris* progeny produced per tray

Treatment	F_1 Cocoons	F_1 Adults	% Females
Experiment I			
Age			
5 Pupae (sex unknown)	10.2 ± 1.2 a	7.8 ± 1.07 a	30.6 ± 4.59 a
1 Adult (1- to 2-d-old ♀)	18.7 ± 1.2 b	16.1 ± 1.07 b	49.0 ± 4.59 a
Experiment II			
Age			
1- to 2-d-old ♀	18.5 ± 1.44 a	15.6 ± 1.32 a	45.9 ± 6.10 a
8- to 9-d-old ♀	18.5 ± 1.44 a	15.2 ± 1.32 a	49.8 ± 6.10 a
15- to 16-d-old ♀	10.2 ± 1.44 b	8.2 ± 1.32 b	13.2 ± 6.10 b

In experiment I, several hours after artificial diet and *S. exigua* eggs were added to replicated trays, five parental parasitoids (as pupae inside cocoons) were added to ½ of these trays. Four days later, when *S. exigua* larvae were first to second instars, a single presumably mated, 1- to 2-d-old parasitoid female was inserted into each of the remaining experimental trays. In experiment II, a single, presumably mated parasitoid female, per age treatment, was inserted into experimental trays containing *S. exigua* first to second instars feeding on artificial diet. Least square means (±SEM) followed by a different letter in a column are significantly different for each experiment, $P \leq 0.05$ (Tukey's test). *n*, 60 trays in experiment I (three trials) and experiment II (two trials).

16-d-old treatment group. The average percentage of treatment females that generated one or more adult female progeny per tray was 70%, 55%, and 40% from 1- to 2-d-old, 8- to 9-d-old, and 15- to 16-d-old parents, respectively. Trial date had no significant effect on production (new cocoons: $F = 1.0$; df = 1, 56; $P = 0.3$; emerged adults: $F = 2.5$; df = 1, 56; $P = 0.1$) or on percentage of female progeny ($F = 3.3$; df = 1, 56; $P = 0.075$).

Discussion

Parasitism and superparasitism. The observation that the number of *C. marginiventris* larvae per host increased as exposure time increased from 2 to 6 to 18 h was expected. However, parasitism rate only increased significantly from 2 to 6 h in this study. Nevertheless, the data suggest a trend of increasing parasitism rate with exposure time (Table 1). The fact that 72% of dissected hosts were parasitized in 18 h suggests that this time period is adequate for a significant proportion of the *S. exigua* larvae to become parasitized within the unicellular tray environment. The host: female parasitoid ratio in each tray was approximately 50: 1. Thus, an average of 36 *S. exigua* larvae would have been parasitized in 18 h. A mated *C. marginiventris* female is capable of parasitizing an average of 91 *Spodoptera litura* (F.) larvae in its lifetime (Jalali et al. 1987). Perhaps, *C. marginiventris* females could be re-used rather than discarded after 18 h inside one tray. Placement of ovipositing females into several unicellular trays (with new hosts) over consecutive days would provide an opportunity for each female to deplete most of their egg load.

The observation that percent superparasitism of hosts did not increase with exposure time was not expected. The fact that 15% of these hosts were superparasitized within 6 h or 18 h of exposure to a female parasitoid suggests that some waste of progeny will occur within unicellular host-rearing trays. Superparasitism behavior of *C. marginiventris* females may have resulted from an inability to distinguish between previously parasitized and unparasitized hosts. This explanation has been suggested for other parasitoids that superparasitized their hosts (van Lenteren and Baker 1975, Hubbard et al. 1999).

The density of searching parasitoids can also have an impact on parasitism rate. Most studies indicate that low parasitoid density, relative to host density, is usually necessary for production of progeny of normal sex ratios and for limiting superparasitism (Wylie 1965, Jones et al. 1999, Sousa and Spence 2000, Wu and Nordlund 2002). For example, at high parasitoid density, the mymarid *Anaphes iole* Girault superparasitized 82% of eggs of the mirid, *Lygus hesperus* Knight, in 24 h; at low parasitoid density, superparasitism was only 10% (Wu and Nordlund 2002). Parasitism rate might be maximized and superparasitism rate minimized when host density exceeds the daily oviposition rate of searching parasitoids (Greenberg et al. 1995).

The finding that both percent parasitism and superparasitism increased with parasitoid density was expected; although, differences were only detected between a density of 1 vs 3 parental female parasitoids per tray. Because parasitism rate did not differ significantly at a density of 1 vs 2 parasitoids, it might be more efficient (i.e., less waste of parasitoid eggs) to insert only one female parasitoid per tray. In order to achieve reasonable parasitism rates for *C. marginiventris* in unicellular host rearing trays it appears that some level of superparasitism of hosts must be tolerated. The question is whether the waste of parasitoid eggs is more critical than the outcome of superparasitism. If superparasitism does not reduce significantly the chances that at least one progeny will complete development and emerge successfully from its host (Riddick 2002), and does not reduce the fertility of female progeny that result from a superparasitized host, then the waste of eggs can be tolerated. In fact, van Alphen and Visser (1990) indicated that self-superparasitism in solitary parasitoids may often result in a waste of time and eggs; however, it can be an adaptive behavior when the presence of two or more eggs per host increases the likelihood that one immature parasitoid will survive.

Progeny production and sex ratio. One or more factors could have been responsible for the observation that production and sex ratio of progeny was less effective when parental parasitoids were inserted into trays as pupae (of unknown sex) rather than as an adult. First, *C. marginiventris* pupae were forced to emerge from cocoons and mate with conspecifics within a very confined space; some females were probably unmated. Second, newly-emerged males may have interfered with mated females (that had been inseminated by a cohort male in the same tray) as they attempted to oviposit into *S. exigua* larvae. Finally, *S. exigua* immatures might not have achieved the preferred developmental stage (that is, late first or early second-instar) by the time that parasitoid females were ready to oviposit.

Despite the lower efficiency, the first age experiment indicates that *C. marginiventris* can be propagated from *S. exigua* larvae within the confines of an ~12 × 12 × 2 cm (L × W × H) unicellular tray when parental pupae (in cocoons) are used. Inserting parasitoid pupae into rearing trays would make complete automation a possibility, because trays could be formed, then filled with artificial diet, *S. exigua* eggs, *C. marginiventris* cocoons; then sealed using a form-fill-seal machine (E.W.R., unpubl.

data). Complete automation reduces handling of insects, reduces labor, is more time efficient, and reduces the potential for entry of microorganisms that could contaminate the diet or infest developing hosts.

In partial automation, artificial diet and *S. exigua* eggs are added to rearing trays and sealed, but parasitoid pupae, which are destined for the rearing trays, are permitted to emerge from cocoons with conspecifics of both sexes in typical emergence cages, with ample room for normal mating behavior. This method is advantageous in the sense that the mating status of parasitoids is more predictable and only a limited number of parentals must be added to trays, because the sex of the adult parasitoid is apparent. Two obvious disadvantages of partial automation are reduced efficiency and increased labor. Also, airborne pathogens have an increased chance of entering trays and infesting the diet and hosts when trays are opened to insert an adult parasitoid. Thus, a technique must be devised to quickly insert adult parasitoids into trays, then reseal the trays with minimal disturbance to the contents.

The observation that progeny production and sex ratio were affected by differences in adult female age was expected. Production of the braconid *Microplitis croceipes* (Cresson) was greatest when 2- to 6-d-old females were used (Tillman 1994). In sting cages, parasitism of *S. exigua* larvae was greatest when *C. marginiventris* females were 0- to 1-d-old rather than 2-d-old (Tillman 2001). Riddick (2001) indicated that older females produced fewer female progeny. King (1987) stated that older parasitoid females tend to oviposit fewer female eggs. One reason for the results in the current study could be the lack of viable sperm in the spermathecae of *C. marginiventris* females. [Females were separated from males when they were 1- to 2-d-old.] Gerling and Rotary (1974) indicated that the walls of the spermathecal reservoir in *Bracon mellitor* Say (Hymenoptera: Braconidae) thickened as females grew older. Release of sperm became more difficult, such that older females produced progeny that were mostly or completely males.

The normal sex ratio of *C. marginiventris* is often variable, ranging from unbiased (Boling and Pitre 1970), slightly male-biased (Kunnalaca and Mueller 1979, Rajapakse et al. 1992) to slightly female-biased (Tillman 2001, Riddick 2002). Some have reported very male-biased sex ratios of progeny (Gillespie et al. 1997). The observation that sex ratio of progeny from 8- to 9-d-old females was essentially unbiased was not expected. It provides evidence that 1-wk-old *C. marginiventris* females are capable of reproducing effectively and can, therefore, be used for production of new progeny from *S. exigua* late first- to second-instar larvae in unicellular trays.

Conclusion. This study suggests that inserting a single, mated 1- to 9-d-old *C. marginiventris* female into a unicellular host-rearing tray containing an abundance of putative hosts could limit superparasitism without seriously reducing progeny production. This research provides evidence to support the use of unicellular trays for partial automated rearing of *C. marginiventris* from non-cannibalistic lepidopteran larvae.

Acknowledgments

I thank Mary Tate for her technical assistance and Bobbie Blair and Clifton Tate for maintaining parasitoid and host colonies, respectively. I thank Frank Davis and Livy Williams, III for their comments on an earlier version of this manuscript. Comments of two anonymous reviewers were very helpful. A portion of this research was performed as a Cooperative Research and Development Agreement (#58-3K95-8-651) between the Agricultural Research Service (USDA) and Biobest USA, Inc. This manuscript was approved for publication as Journal Article No.

J10223 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

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