Insectary Production of *Biosteres tryoni* (Cameron) (Hymenoptera: Braconidae), a Larval Parasitoid of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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ABSTRACT

The results of this study contribute to the development of mass rearing procedures for *Biosteres tryoni* (Cameron) by providing a method for producing adequate numbers for releases, and by determining the potential for the use of this species in suppression programs against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann).

Progeny production per cage of 200 females was 1,483.6 adult parasitoids per generation when oviposition exposure period was 6 hours per day. A yield of 94.4% of total progeny was obtained during a parasitoid age interval of 5 to 20 days. Progeny production and ratio of females declined significantly during the age interval of 21 to 30 days. Host larvae exposure periods of 4, 6, and 8 hours per day appeared to be optimum for parasitoid production even though parasitoids achieved their highest rate of reproduction per unit time after 2 hours exposure for oviposition.

KEY WORDS: *Ceratitis capitata*, *Biosteres tryoni*, Parasitoid production, Oviposition rate, Oviposition exposure.

Factors contributing to the lack of success with fruit fly parasitoids in places other than Hawaii include improper rearing procedures in colonization programs and low numbers of viable, mated females received in shipments for releases (Clausen 1956, 1978). Therefore, improvements in mass culturing and handling of fruit fly parasitoids are needed to achieve a maximum degree of efficiency in their production.

Biosteres tryoni (Cameron) is considered a candidate parasitoid for augmentation programs against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Hawaii (Wong et al. 1984). Information on the basic biology of this species has not been adequate to answer the many questions pertaining to criteria for its mass rearing. The objectives of this study were to examine the population growth rate in the laboratory and the rates of progeny production during different oviposition exposure periods.

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MATERIALS AND METHODS

Progeny Production in Laboratory. Five replicates, each containing 200 females and 200 males per cage of newly emerged *B. tryoni*, were used. Parasitoid cohorts were collected from the field (Kula, Maui) and were continuously propagated in the laboratory since 1981. Parasitoids were allowed to oviposit daily, starting from the fifth post eclosion day. Previous results (M.M.R., unpublished data) showed that ovipositing females lived for less than one month. Therefore, this test continued for 30 days after emergence.

Mature late third instar *C. capitata* larvae (7-day-old) reared by the methods of Tanaka et al. (1969, 1970) were exposed to parasitoids for six hours daily. Larvae placed in modified petri dishes (9 cm diameter, < 0.5 cm deep) containing larval media and covered with lids made of fine-mesh nylon organdy were then exposed to parasitization, a method similar to that of Nishida (1956). These oviposition units (average 285 host larvae per unit) were placed into the parasitoid cages (26 cm^3 covered on three sides with fine-mesh saran filter cloth). Parasitoid cages were provided with undiluted cream honey smeared on top screen as source of food for parasitoid cohorts. Water was also provided. After oviposition, the petri dishes were taken out of the cages and the larvae were placed in plastic cups until pupation. Host pupae were held at 26 ± 1°C and 60 ± 10% R.H. for one month after removal from parasitoid cages to allow for complete emergence of parasitoids. The numbers of parasitoids and *C. capitata* that emerged were counted. Numbers of dead female parasitoids (cohorts) were recorded once a day. Females attracted to the oviposition units were referred to as competitors and these were counted every two hours.

Expected parasitism was determined by examining the unemerged host pupae. The presence of dead immature stages of parasitoids in the puparia was positive evidence of parasitism. Samples of unemerged pupae were taken randomly for examination. Sample sizes of 10, 25, 50, and 100% were taken when the total uneclosed pupae were > 200, 100 to 200, 50 to 99, and < 50, respectively. Expected parasitization (potential parasitism) was the mean of dead pupae with parasitoid cadavers plus pupae that produced living parasitoids.

Effect of Host Exposure Period on Progeny Production. Test cages, each with 100 pairs of newly emerged *B. tryoni*, were used in this study. Early third instar *C. capitata* larvae were placed in ovipositional units (ca. 500 larvae/unit) and introduced into the test cages. Host larvae were exposed to parasitoids for periods of 2, 4, 6, 8, 10, 12, and 24 hours. The laboratory was illuminated throughout the exposure periods. A separate test cage was used for each exposure period, and the number of female parasitoids attracted to the oviposition units was counted every hour. Each test was replicated three times using 5-day-old parasitoids.

When the prescribed exposure period was over, the oviposition unit was removed from each cage and the host larvae were reared until pupation and

adult emergence. Thirty days after pupation, the unemerged pupae were dissected to determine the number of unemerged parasitoids.

Data were subjected to an analysis of variance and means were separated by Duncan's multiple range test at $P = 0.05$ level (SAS Institute 1985) of: 1) the percentage of female progeny produced by different ages of female parasitoids and 2) progeny production for different oviposition periods.

RESULTS

Progeny Production. Fig. 1 presents the results of progeny production (per cage of 200 females) of *B. tryoni* when allowed to oviposit into late third instar larvae of *C. capitata*. The data have been grouped to show the economy of progeny production during 5-day age intervals. The highest

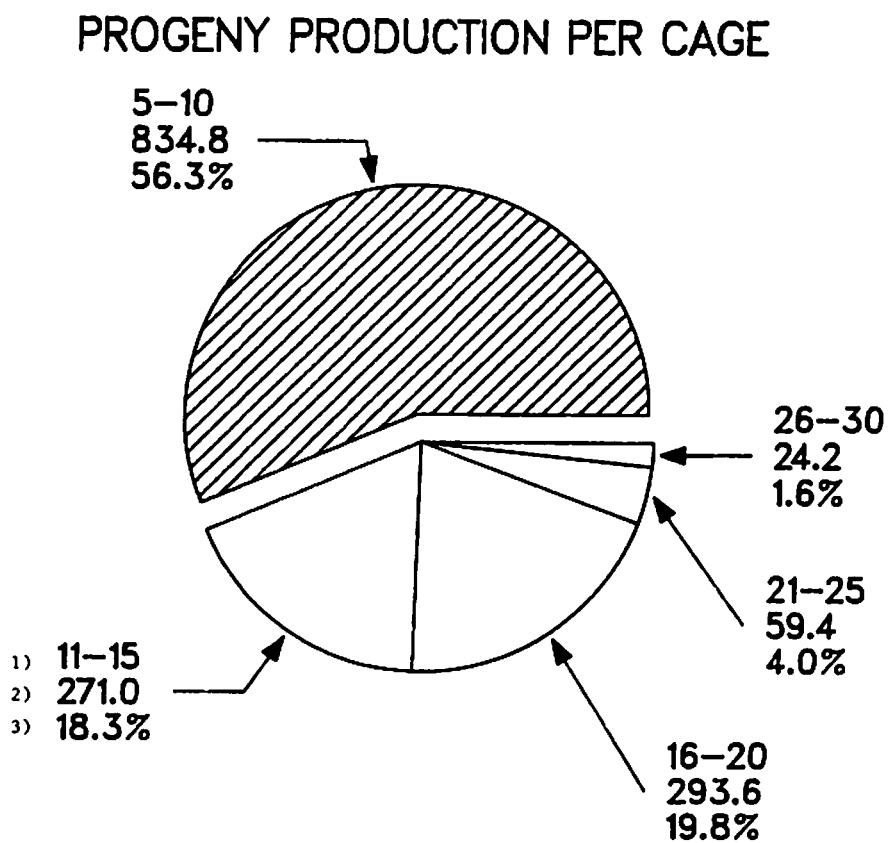

FIGURE 1. Sequence of progeny production by *B. tryoni* when reared on late third instar of *C. capitata* larvae. Production data during five days were pooled. Every data segment represents (1) age interval in days, (2) mean number of offspring, and (3) percent progeny production.

progeny production was in the age interval of 5 to 10 days. During this interval, progeny production averaged 834.8 ± 55.6 parasitoids per cage, which represented 56.3% of the total yield per cage. Production dropped off (18.3 and 19.8% of the total yield) during the age intervals of 11 to 15 and 16 to 20 days, respectively, after which the yield declined significantly ($P < 0.0001$) to 4.0 and 1.6% in the last age intervals (21 to 25 and 26 to 30 days). Duncan's multiple range test showed that progeny production was grouped into three significantly different ($P < 0.0001$) age intervals: a period of maximum production in the age interval of 5 to 10 days, a period of intermediate production in the age interval of 11 to 20 days, and a minimum production period in the age interval of 21 to 30 days.

Of the initial female parasitoids in the cage, 97.1% contributed to the total progeny production until they died. As shown in Fig. 2, very few females were still alive when the test was terminated after 30 days. The early competitors reached their optimum density on oviposition units in the first two hours after the introduction of host larvae. The maximum number of females ovipositing on a given day occurred on the tenth day when 29.4 ± 1.3% of the initial cohorts were attracted to oviposition units.

Data on daily realized and expected progeny production per cage of *B. tryoni* are presented in Fig. 3. Maximum production, found on the sixth and seventh days, coincided with maximum ovarian maturation (M.M.R., unpublished data).

Overall mean production per cage during the reproductive period of 5 to 30 days of age, was 1,483.6 adult parasitoids produced by 200 females. Of this number, 74.6% were produced during the age interval 5 to 15 days and 94.4% during age 5 to 20 days. Only 5.6% of the total parasitoids were produced in the last ten days of the test. This average yield per cage was about 7.4 times the number of the initial cohorts. Unfortunately, this production rate represented only 38.5% of the average expected parasitism per cage, which was 3,852.6, based on the assumption that at least one parasitoid is lost in every uneclosed pupa containing parasitoid cadavers. However, in many instances, as many as 7 or more head capsules of first instar parasitoid larvae were dissected from a single host puparium.

The mean percentage of female progeny was highest in the age interval of 5 to 10 days, reaching 49.9 ± 4.3% females. During this period, as many as 80% females were obtained in the eighth day, with a mean of 65.8 ± 10.7%. During the last 10 days of this test, the percentages of female offspring declined significantly ($P < 0.0001$) to a mean of only 10.0% ± 5.0%.

Effect of Host Exposure Periods on Progeny Production. As soon as oviposition units were introduced into the cages, females attacked host larvae persistently and aggressively. Ovipositional activity reached a peak at six hours of exposure and then declined. However, the percentages of ovipositing females showed no significant differences ($P > 0.3909$) between exposure periods (2 to 24 h).

The maximum observed and expected progeny production were in the "24-hour treatments" and the minimum values were in the "2-hour treat-

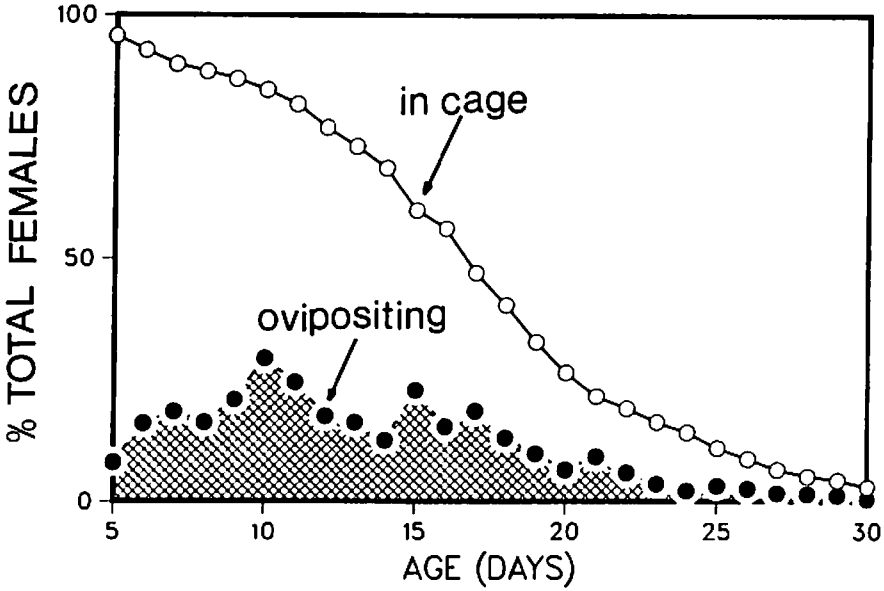

FIGURE 2. Percentage of total females in cage and percentage of females on oviposition units when exposed to late third instar larvae of *C. capitata*. Each data point is a mean of 5 replicates.

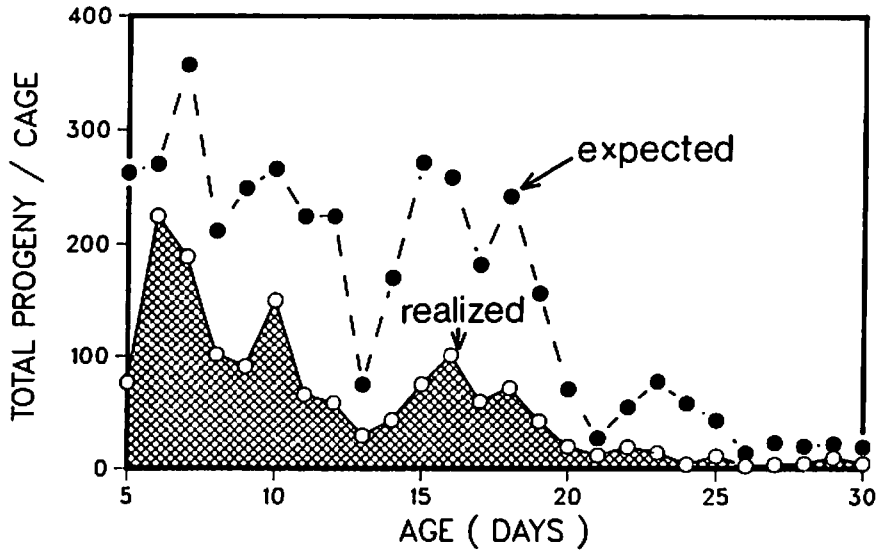

FIGURE 3. Daily ovipositional rates per 200 females per cage of *B. tryoni* exposed to late third instar larvae of *C. capitata*.

TABLE 1. Characteristics of progeny production by *Biosteres tryoni* (Cameron) under several oviposition exposure periods.

Exposure hours	Progeny production		% Ovipositing[b]	Reproductive fitness[c]		% Uneclosed pupae
	Observed[a] ($\bar{X}$ ± SEM)	Expected ($\bar{X}$ ± SEM)	($\bar{X}$ ± SEM)	Observed ($\bar{X}$ ± SEM)	Expected ($\bar{X}$ ± SEM)	($\bar{X}$ ± SEM)
2	66.3 ± 2.9d	76.0 ± 1.5d	11.3 ± 0.4a	2.9 ± 0.02a	3.4 ± 0.2a	2.4 ± 0.2c
4	140.0 ± 37.8dc	154.0 ± 38.7dc	15.1 ± 3.4a	2.3 ± 0.1ab	2.5 ± 0.1b	2.8 ± 0.03c
6	118.3 ± 21.1dc	138.3 ± 25.4dc	19.2 ± 4.1a	1.2 ± 0.3dc	1.4 ± 0.4c	4.1 ± 0.7cb
8	128.3 ± 27.7dc	158.7 ± 34.4dc	17.3 ± 1.9a	0.9 ± 0.2d	1.2 ± 0.3c	4.2 ± 0.6cb
10	174.3 ± 28.0c	201.3 ± 28.4c	16.4 ± 1.7a	1.1 ± 0.1dc	1.2 ± 0.1c	4.9 ± 0.5b
12	258.0 ± 20.5b	296.3 ± 13.7b	13.6 ± 2.5a	1.7 ± 0.4bc	2.0 ± 0.4bc	5.4 ± 0.5b
24	366.0 ± 25.5a	455.0 ± 34.4a	13.6 ± 1.4a	1.2 ± 0.2dc	1.4 ± 0.2c	13.1 ± 1.1a

[a]Means within columns followed by the same letter are not significantly different (P>0.05; Duncan's multiple range test [SAS Institute 1985]).
[b]Mean number of females seen ovipositing/total number of females in cage.
[c]Progeny production per ovipositing female per hour.

ments". Differences in the yields of the 4, 6, and 8-hour treatments were not statistically significant (Table 1).

A clearer measure of progeny production would be provided if evaluation were based upon the ability of a female to provide viable progeny per unit of time. This measure is referred to as the reproductive fitness (Price 1975) of a female. Females oviposited intensively during the first two hours, reaching a peak rate of 2.9 ± 0.02 per hour during this period. However, oviposition declined as exposure period increased. From six hours onward, a reduction of more than 50% of the maximum reproductive fitness was observed.

Analysis of variance of the fitness values of 6, 8, 10, 12, and 24-hour treatments showed three slightly different groups (Table 1). The percentages of unemerged pupae increased steadily as the length of exposed period increased.

DISCUSSION

There are many factors affecting the daily parasitization rates of *B. tryoni*, such as the host density available for oviposition, the host larval stages, the number of ovipositing females and their ages, the oviposition exposure periods, and the percentages of unproductive (unemerged) parasitized pupae. All these factors make it difficult to predict a constant reliable value of parasitization.

Rates of oviposition are greatly influenced by the time available for the parasitoids to encounter the hosts. However, at long oviposition exposure periods a great loss of parasitoid eggs is expected in undiscriminating species (i.e., those such as *B. tryoni* which oviposit in previously parasitized hosts), especially when the host density is limited. Therefore, in a solitary parasitoid such as *B. tryoni*, timing of oviposition periods is important to minimize the rates of superparasitism, and thereby the loss of parasitoid eggs. Short oviposition exposure periods may lower rates of superparasitism, but many of the hosts will escape parasitism, thus affecting rearing costs.

The high progeny production of the 24-hour exposure treatment should not be taken as the optimum yield of the female. The parasitoid female acts according to a physiological age determined by the amount of nutrient (metabolites transferred to the adults from larval and adult feeding) in its body for egg production and as a source of energy. Therefore, the faster it uses this nutrient, the sooner it dies. Greany et al. (1976) showed that *Biosteres longicaudatus* Ashmead females when provided with hosts died much sooner than those deprived of hosts. Therefore, the high progeny yield of the 24-hour exposure treatment was at the expense of some longevity of the females. Moreover, the number of unemerged pupae in this treatment was the highest, indicating a high loss in the parasitoid's yield due to superparasitism. In the 2-hour exposure treatment, a limited portion of the available host larvae was encountered by the females. So, the 4, 6, and 8-hour exposure treatments appear to be the most practical and should be

selected according to the amount of time available to the breeder to maintain stock cages, and also according to the number of females on the oviposition units, which is strongly correlated with the age of the ovipositing females.

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