Laboratory Biological Parameters of Trichogramma fuentesi (Hymenoptera: Trichogrammatidae), an Egg Parasitoid of Cactoblastis cactorum (Lepidoptera: Pyralidae)

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LABORATORY BIOLOGICAL PARAMETERS OF *TRICHOGRAMMA FUENTESI* (HYMENOPTERA: TRICHOGRAMMATIDAE), AN EGG PARASITOID OF *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Trichogramma fuentesi Torre was identified attacking *Cactoblastis cactorum* (Berg), a serious pest of *Opuntia* spp. in North America, raising the possibility of using this egg parasitoid as an inundative biological control agent. Studies were conducted to assess the biological parameters of this parasitoid under laboratory conditions. Nutritive quality influence of the rearing supplement on the parasitoid's longevity, mating, and age was evaluated based on the level of parasitism. The presence and type of food source had a positive impact on female longevity, and female parasitoids given a diet composed of pure honey lived the longest; an average of 11 d. Mated females parasitized a greater number of *C. cactorum* host eggs than did unmated females. Percent parasitism significantly decreased with female age. Two- to 3-day old female parasitoids had the highest level of parasitism. Two-day old host eggs were the optimal host egg age for parasitization by *T. fuentesi.* In the context of implementing an inundative biological control program against *C. cactorum*, two-day old mated and honey fed *Trichogramma* females should be released to increase level of control.

Key Words: *Trichogramma fuentesi*, *Cactoblastis cactorum*, cactus moth, augmentative biological control, host age, biological parameters

RESUMEN

Identificamos al parasitoide *Trichogramma fuentesi* Torre atacando a huevecillos de *Cactoblastis cactorum* (Berg), una plaga importante de cactáceas del género *Opuntia* en Norte América. Este descubrimiento nos llevo a considerar la posibilidad de utilizar este parasitoide como agente de control biológico para *C. cactorum* a través de liberaciones inundativas. Realizamos estudios para examinar los parámetros biológicos de *T. fuentesi* bajo condiciones de laboratorio. Entre los parámetros examinados estuvieron el efecto de un suplemento alimenticio sobre la longevidad del parasitoide, asi como el efecto de la cópula y la edad de los parasitoides sobre el nivel de parasitismo de los mismos. La presencia y el tipo de alimento tuvo un impacto positivo sobre la longevidad de las hembras; las hembras que recibieron un suplemento de miel pura vivieron el mayor tiempo - un promedio de 11 dias. Las hembras previamente apareadas parasitaron un número mayor de huevecillos de *C. cactorum* que las hembras no apareadas. El porcentaje de parasitismo se redujo a medida que la edad de los parasitoides se incrementó. Esta reducción fue estadisticamente significativa. Las hembras de dos a tres dias de edad tuvieron el nivel mas alto de parasitismo. Los huevecillos de dos dias de edad son óptimos para ser parasitados por *T. fuentesi*. En el contexto de la implementación de un programa inundativo de control biológico para *C. cactorum*, se deben liberar parasitoides hembras de dos dias de edad, previamente apareadas y alimentadas con miel pura, lo que aumentará el nivel de contol por *T. fuentesi*.

In the late 1980s, a phycitine moth native to South America, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), was found in south Florida (Dodd 1940; Dickel 1991; Zimmermann et al. 2001). The moth has been considered an important ecological and economic threat to *Opuntia* spp. cactus particularly in the Southwestern U.S. and in Mexico. Current management techniques involve pest population monitoring with sex pheromone baited traps, field sanitation through removal of infested plants, and area wide control through inundative releases of sterile *C. cactorum* adult males (Hight et al. 2005; Bloem et al. 2007). In addition to these management efforts, biological control by egg parasitoids in the genus *Trichogramma* has been suggested (Bennett & Habeck 1995; Pemberton & Cordo 2001; Logarzo et al. 2008; Paraiso et al. 2011). *Trichogramma* spp. are facultative gregarious egg parasitoids that have been used as inundative biological control agents against a wide range of agricultural pests including corn borers, sugarcane borers, and cotton bollworm (Li 1994; van Lenteren 2000; Godin & Boivin 2000). *Trichogramma* wasps are particularly efficient against lepidopteran pests that lay their eggs in clusters such as the European pine shoot borer (*Rhyacionia buoliana* Denis & Schiffermüller: Tortricidae), and the European corn borer (*Ostrinia nubilalis* Hübner: Pyralidae) (Dahlsten & Mills 1999; Kogan et al. 1999).

Previous surveys for natural enemies in North Florida led to the discovery of *T. pretiosum* (Riley) and *T. fuentesi* Torre attacking *C. cactorum* (Paraiso et al. 2011). Inundative releases of several *Trichogramma* spp., such as *T. exiguum* Pinto & Platner against heliothine pests of cotton (Suh et al. 2000), or against the Nantucket pine tip moth (*Rhyacionia frustrana* (Comstock): Tortricidae) in Virginia pine (*Pinus virginiana* Mill.) (Philip et al. 2005), have failed to provide an adequate level of pest suppression. Therefore, prior to field release it is important to undertake detailed studies of biological and ecological characteristics of prospective agents (van Lenteren et al. 2003; Dannon et al. 2010). Some of the important characteristics that need to be studied to optimize rearing conditions and release strategy of *T. fuentesi* against *C. cactorum* include: sex ratio, longevity, and influence of parasitoid and host age on parasitism and fecundity.

Trichogramma wasps require a source of carbohydrate to maintain basic physiological activities (Romeis et al. 2005). Generally, raisins and pure or diluted honey are used in experimental studies as a source of food to maintain *Trichogramma* adult rearing cultures (Morrison 1985). The presence and nutritional quality of a supplemental source of food have been reported to increase the longevity of *Trichogramma* spp. (Laetimia et al. 1995; Oliveira et al. 2003). In addition, a source of carbohydrate can, in some cases, influence fecundity and egg resorption in parasitoids (Heimpel et al. 1997). Female parasitoid age is another biological characteristic that can affect the success of an inundative biological control agent (Amalin et al. 2005). Studies have shown that trichogrammatids under or over a certain age are not able to parasitize their hosts (Rajapakse 1992; Amalin et al. 2005). Host age also impacts the level of parasitism since trichogrammatid parasitoids favor young host eggs (Sequeira & Mackauer 1988; Amalin et al. 2005), and younger hosts generate parasitoid offspring that are characterized by greater fitness (Sequeira & Mackauer 1994).

In the present study, we examined the influence of 3 types of supplemental food sources on the longevity of *T. fuentesi* in the laboratory. In addition, optimum age for oviposition by female *T. fuentesi* was assessed in order to furnish information for optimizing inundative releases against *C. cactorum*. This paper provides information on the influence of supplemental food sources, parasitoid female age, mating and host age on parasitism rates, number of parasitoid progeny, number of emerged parasitoid per parasitized eggs, and sex ratio.

Materials and Methods

Rearing Procedures and General Methods

Experiments were conducted at the USDA-Agricultural Research Service and the Florida A&M University Center for Biological Control laboratory in Tallahassee, Florida. *Trichogramma fuentesi* females used in the study came from a rearing colony originating from field collected material. Species identity was confirmed by DNA ITS 2 sequences performed by Dr. R. Stouthamer (Department of Entomology, University of California, Riverside, California). *Cactoblastis cactorum* eggs derived from a colony maintained on artificial diet were used as hosts during parasitoid rearing and for the experiments. To culture *T. fuentesi*, host eggsticks were glued on note card strips (4 × 2 cm) with non-toxic carpenter's glue (Elmer's® glue, Elmer's Products Inc., Columbus, Ohio). The note card strips were placed into plastic petri dishes (9 × 2 cm) lined with filter paper (Ahlstrom®, Mt. Holly Springs, Pennsylvania). A fresh raisin was glued on a 1 × 1 cm note card in the center of each petri dish to provide a supplemental source of food to emerging wasps. Petri dishes were sealed with parafilm® (Pechiney-Plastic Packaging, Menasha, Wisconsin) and arranged on plastic trays lined with moist wipes to increase relative humidity to 60-80% RH. The cultures were maintained in a growth chamber at 28 ± 1 °C, 16:8 h L:D. Additional growth chambers maintained at 25 ± 1 °C, 16:8 h L:D and 60-80% RH were used for incubation of experimental units in all experiments. At 25 ± 1 °C, *C. cactorum* eggs require an average of 26 d to hatch (McLean et al. 2006).

Effect of Presence and Type of Diet on Female Parasitoid Longevity

We tested the effect of no supplemental source of food, presence of undiluted honey, or presence of raisins on the longevity of female parasitoids. Individual, newly emerged (0-24 h-old) *T. fuentesi* females were collected from the rearing colony and placed in a plastic petri dish (3.5 × 1 cm) lined with filter paper (Ahlstrom®, Mt. Holly Springs, Pennsylvania). A source of food consisting of either a drop of honey or a raisin was added to the center of each container. A set of control petri dishes did not have any supplemental source of food. Petri dishes were sealed with parafilm and arranged on a plastic tray lined with moist paper wipes to maintain the relative humidity. The containers were incubated and checked daily until all females died. Each treatment was replicated 20 times.

Influence of Female Parasitoid Age and Mating Status on Parasitism

The effect of female parasitoid age on percentage of host eggs parasitized was tested for wasps ranging from 2-6 d old. One-d old unmated female parasitoids were isolated from the rearing colony and individually transferred to petri dishes (3.5 × 1 cm) lined with filter paper, as described above. In addition, newly emerged (0-24 h) females and males were collected from the rearing colony and stored in a petri dish for 24 h to allow mating. Mated and unmated females were transferred individually into petri dishes (3.5 × 1 cm) lined with filter paper. The experimental arena did not have a source of food for the wasps. Adult female parasitoids, without food, lived an average of 4 d (data from above Experiment). Eggsticks were replaced daily until the females were 5 d old. Petri dishes were sealed with parafilm and arranged on a plastic tray lined with moist paper wipes and incubated at 25 ± 1 °C, 16:8 h L:D and 60-80% RH. Individual females were exposed to 60, 2-d old, *C. cactorum* eggs. The experiment was replicated 20 times. The number of parasitized eggs was determined on a daily basis and the rates of parasitism for these mated females were compared against unmated.

Influence of Host Age on Percent Parasitism

Cactoblastis cactorum females lay an average of 70-90 eggs/eggstick (Zimmermann & Pérez-Sandi 2006). Eggs hatch in approximately 3 wk (Zimmermann et al. 2001). A no-choice experimental design was used to assess the influence of 10 different *C. cactorum* host age groups (1, 6, 7, 9, 11, 12, 13, 14, 15, and 20 d old). Three day old, randomly chosen, honey-fed, mated female parasitoids were isolated from the rearing colony and each placed in an individual petri dish (3.5 × 1 cm) lined with filter paper. Sixty host eggs belonging to one of the different age groups were placed in the center of each petri dish. Eggsticks were removed after 48 h and individually transferred into plastic cups (30 mL) for 10 d to allow all parasitoids to emerge. Each treatment was replicated 20 times and the percent parasitism, total number of emerged parasitoids, number of emerged parasitoids per parasitized eggs, and sex ratio were determined.

Statistical Analysis

The effect of presence and type of diet on female parasitoid longevity and influence of host age on number of egg parasitized by *T. fuentesi* were analyzed by analysis of variance (PROC ANOVA & PROC GLM). In addition, the effect of host egg age on level of parasitism, number of progeny, number of successfully emerged eggs, and percentage of females produced was analyzed by polynomial regression. A logistic regression was used to analyze the effect of *T. fuentesi* age and whether the female wasp was mated or unmated on the level of parasitism. A one way analysis of variance (PROC LOGISTIC) with Firth correction was applied to the proportion of eggs parasitized by *T. fuentesi*. Comparison of the number of eggs parasitized versus mated and unmated *T. fuentesi* females at each age was evaluated using Tukey least mean comparison test. A Bonferroni approach was used to adjust the alpha value for the pairwise comparisons. The SAS Statistical Software Version 9.2 (SAS Institute, Cary, North Carolina) was used to perform the statistical analyses.

Results

Effect of Presence and Type of Diet on Female Parasitoid Longevity

The provision of a food supplement had a significant influence on the survival of female parasitoids as indicated for each food source. Without a supplemental food source, females survived the shortest period; an average of 4 ± 0.58 d (F = 23.14, df = 1, 12, $P < 0.001$, $R^2 = 0.85$). Females supplied with a raisin or honey supplement lived for 8 ± 0.07 d (F = 595.03, *df* = 1, 12, $P < 0.001$, $R^2 = 0.99$) and 11 ± 0.79 d (F = 670.73, df = 1, 12, $P < 0.001$, $R^2 = 0.99$), respectively. Type of food (raisin or honey) significantly affected the longevity of female parasitoids (F = 43.20, df = 2, 57, $P < 0.001$, $R^2 = 0.60$). The longest survival time was 16 d for a female fed honey.

Influence of Female Parasitoid Age and Mating Status on Percent Parasitism

The number of parasitized eggs varied significantly with female parasitoid age (F = 20.86, df = 4, 190, $P < 0.0001$). The number of parasitized eggs was the highest for 2 and 3 d old female parasitoids and declined rapidly thereafter (Table 1). In addition, successful parasitism increased when females were mated (F = 21.14, df = 1, 190, $P < 0.0001$). Unmated females had none to a low level of parasitism after the third day. In contrast, parasitized eggs were still recovered after the fifth day with mated females (Table 1).

TABLE 1. INFLUENCE OF AGE AND MATING STATUS OF AN EGG PARASITOID, *TRICHOGRAMMA FUENTESI*, ON NUMBER OF PARASITIZED *CACTOBLASTIS CACTORUM* HOST EGGS.

	Mean # of Eggs Parasitized (± *S.E.*)*	
Age of Female *T. fuentesi* (Days) $n = 20$	Mated	Unmated
2	5.4 ± 0.97 a A	4.0 ± 1.06 a A
3	5.9 ± 1.39 a A	1.8 ± 0.78 b B
4	2.8 ± 0.75 b A	0.0 ± 0.05 c B
5	0.9 ± 0.65 c A	0.0 ± 0.00 c B
6	0.0 ± 0.00 d A	0.0 ± 0.00 c A

*Means with different lower case letter in columns or capital letter in rows are significantly different according to Tukey's Least Mean Comparison test at $P \leq 0.002$.

Influence of Host Age on Percent Parasitism

Numbers of eggs parasitized and emerged parasitoids per eggstick declined with age of host eggs ($F = 19.53$, df = 3, 196, $P < 0.0001$; and $F = 16.53$, df = 3, 196, $P < 0.0001$, respectively) (Table 2). The relationship between host egg age and the number of eggs parasitized was best represented by a cubic polynomial equation ($y = 4.81 - 1.02x + 0.074x^2 - 0.0018x^3$, $R^2 = 0.23$). *Trichogramma fuentesi* displayed a preference for 1 d old host eggs, and the highest level of parasitism and number of progeny produced was recorded for 1 d old host eggs. Female parasitoids did not produce any progeny in host eggs older than 14 d (Table 2). A cubic polynomial equation also described the relationship between egg age and the number of parasitoids that emerged ($y = 16.17 - 3.56x + 0.27x^2 - 0.0065x^3$, $R^2 = 0.20$). However, host egg age did not have a significant influence on the sex ratio or on the total number of parasitoids emerging per parasitized egg (Table 2). Although polynomial regression revealed significant relationships between host egg age and the number of parasitized eggs and the number of parasitoids per eggstick, only about 20% of the variation in the data was explained by our models. The high significance lends credibility to the reality of the influence of host egg age, but additional factors are important in explaining the variation in our data.

DISCUSSION

This study provides information on the biological parameters of *T. fuentesi*, a potential inundative biological control agent for *C. cactorum*. Although there is considerable amount of information on the biology of some *Trichogramma* spp., little is known on this particular species. *Trichogramma fuentesi* has been recorded in the neotropics including Argentina, Colombia, Mexico, Peru, and Venezuela and in several U.S. states (Alabama, California, Florida, Louisiana, New Jersey, South Carolina, and Texas) (Fry 1989; Pinto 1999). Its primary hosts are mainly noctuid species (Fry 1989; Wilson & Durant 1991; Pintureau et al. 1999; Querino & Zucchi 2003). *Trichogramma* wasps require sugar as a source of energy to sustain major physiological processes (Romeis et al. 2005). In nature, Trichogrammatids may obtain sugar from floral nectar, extrafloral nectar, honeydew, and plant sap (Wackers 2005). Food deprived parasitoids will first search

TABLE 2. INFLUENCE OF HOST AGE (*CACTOBLASTIS CACTORUM* EGGS) ON THE NUMBERS OF EGGS PARASITIZED, EMERGED PARASITOIDS, EMERGED PARASITOIDS PER PARASITIZED EGG, AND SEX RATIO.

	Mean # (± *S.E.*)*			
Age of Host (Days)	Eggs Parasitized	Emerged Parasitoids/Eggstick	Emerged Parasitoids/Parasitized Egg	% Females
1	4.0 ± 1.28 a	13.2 ± 4.70 a	3.4 ± 0.80	60 ± 14
6	0.5 ± 0.25 b	2.0 ± 0.89 b	5.5 ± 1.34	98 ± 2
7	0.6 ± 0.43 b	1.0 ± 0.55 b	2.0 ± 0.58	50 ± 29
9	0.8 ± 0.34 b	2.8 ± 1.43 b	3.3 ± 0.75	50 ± 0
11	0.3 ± 0.21 b	1.4 ± 0.82 b	5.8 ± 1.17	81 ± 9
12	0.3 ± 0.15 b	1.6 ± 0.87 b	6.5 ± 2.73	71 ± 5
13	0.2 ± 0.20 b	0.6 ± 0.65 b	3.2 ± 0.00	85 ± 0
14	0	0	—	—
15	0	0	—	—
20	0	0	—	—

*Means within a column with different letters are significant according to Tukey's Least Mean Comparison test at $P \leq 0.05$. Since no significant relationship was found between age of host egg and number of parasitoids per parasitized egg or percent females, means were not separated.

for food resources before they search for hosts (Hegazi et al. 2000). In addition, sugar feeding influences overall individual flight, foraging behavior (Forsse et al. 1992; Pompanon et al. 1999; Romeis et al. 2005), and longevity (Oliviera et al. 2003). Hegazi et al. (2000) observed that honey-deprived *T. cacoeciae* Marchall only attacked one host patch whereas fed females were more likely to move to a second host egg patch. Females without supplemental sources of food in our study never lived more than 5 d while fed females lived an average of 11 d on honey and 8 d on raisin. Honey contains proteins, enzymes, fructose, glucose, and amino acids (Vela et al. 2007). The main sugars of raisins are fructose and glucose with minimal amounts of sucrose. They are a good source of vitamins, minerals, and phytochemicals (Williamson & Carughi 2010). Although a comparison of nutritional quality was not done between the different types of food to document their influence on insect physiology, the difference in longevity may be explained by the greater accessibility of carbohydrates in honey compared with raisin.

Various studies have shown that the age of parasitoids can affect the level of parasitism (Hentz 1998; Honda & Kainoh 1998). This information is important in deciding at what age parasitoids should be released in the field to obtain a significant level of parasitism (Amalin et al. 2005). The highest level of parasitism was observed in 2 d old unmated parasitoids and 2-3 d old mated females (Table 1). The level of parasitism decreased after the third d until the death of the parasitoids. Mating status (mated or unmated) may affect oviposition behavior of parasitoids (Hardy et al. 2007). In our study, the percent parasitism did not significantly increase for mated females except for 3 d old females (Table 1). The 2 d old mated and unmated females parasitized the same number of eggs (Table 1). Therefore, inundative releases of 2 d old mated *T. fuentesi* females should be used against *C. cactorum* eggs to increase control levels.

A range of ages of *C. cactorum* eggs coexist in the field during the flight period. *Trichogramma* spp. generally prefers younger host eggs (Godin & Boivin 2000; Takada et al. 2000). The number of Trichogrammatid progeny decreases significantly with older host eggs (Miura & Kobayashi 1998; Takada et al. 2000) due to depletion of essential nutrients (Ruberson & Kring 1993) or host embryo cephalic capsule sclerotization (Guang & Oloo 1990). In our study, the highest level of parasitism and number of parasitoid progeny was observed on 1 d old host eggs (Table 2). The number of eggs parasitized decreased as the host eggs age increased. *Trichogramma fuentesi* females did not parasitize *C. cactorum* eggs older than 13 d old (Fig. 1). Although sex allocation was not influenced by host age, female-biaised progeny were observed in all experimental treatments (Table 2).

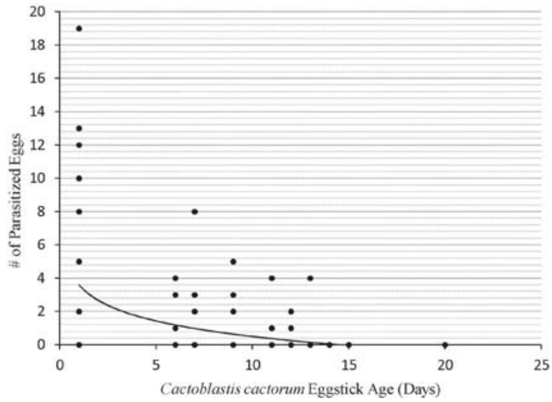

Fig. 1. Regression analysis showing the influence of *Cactoblastis cactorum* host egg age (1-20 days) on level of parasitism by *Trichogramma fuentesi*.

Female *Trichogramma* preferentially lay a higher percentage of females in younger host eggs due to their implication with higher fitness.

Our results showed that honey must be provided in mass reasing programs to establish a sustainable population of *T. fuentesi* in the laboratory. Two to 3 d old mated *T. fuentesi* females should be released in the field to obtain a significant level of parasitism. *Trichogramma fuentesi* displayed a preference for younger host eggs. Therefore, the inundative releases of this egg parasitoid should be timed to coincide with the beginning of *C. cactorum* oviposition period and/or frequent releases made to attack newly layed *C. cactorum* eggs.

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