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Effects of the host and parasitoid densities on the quality production of *Trichogramma chilonis* on lepidopterous (*Sitotroga cereallela* and *Corcyra cephalonica*) eggs

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Abstract
The present study was conducted for efficient and quality production of the stingless wasp, *Trichogramma chilonis* Ishii with respect to rearing host and parasitoid densities of the angoumois grain moth, *Sitotroga cereallela* (Olivier) and the rice meal moth, *Corcyra cephalonica* (Stainton) on its biology. For host density, percentage parasitism and adult longevity was the highest at 20 eggs whereas emergence was the highest from 10 eggs of *S. cereallela* while of *C. cephalonica* percentage parasitism and emergence was the highest at 10 eggs. For parasitoid density, parasitism was the highest at 5 pairs of *T. chilonis*. Percentage of emergence and longevity remained similar among all the 5 treatments. It is concluded that *S. cereallela* eggs more suitable as compare to *C. cephalonica* eggs for mass and quality rearing of parasitoid, *T. chilonis*.

Keywords adult longevity; *Corcyra cephalonica*; developmental period; host age; parasitism; *Sitotroga cerealella*; *Trichogramma chilonis*.

1 Introduction

Conservation as a biological control method includes crop management practices that protect and encourage natural enemies and increase their impact on pests. The selective use of natural enemies, which are planting by strip on crops in and around the fields, necessarily food and habitat are provided to them, have been exercised. The parasitoid stingless wasp, *Trichogramma chilonis* (Ishii) has been conserved along with other natural enemies in Pakistan (Irshad, 2008).

Several factors affect the mass rearing and quality production of *T. chilonis*, i.e., temperature, relative humidity, photoperiod, host and parasitoid eggs densities and host egg quality. For rearing of *T. chilonis* on a commercial scale, it is necessary to use a factitious rearing host, such as the Mediterranean flour moth, *Ephestia kuehniella* (Zell), the angoumois grain moth, *Sitotroga cereallela* (Olivier) and the rice meal moth, *Corcyra cephalonica* (Stainton) rather than the natural or target host. The choice of factitious host is often dictated by the ease of rearing and not necessarily by any factors related to the likely success of the wasps being produced. Factitious hosts are selected on the simplicity of their mass production, mechanization of rearing processes and cost of production compared with that of using the target pest (Greenberg et al., 1998). Environmental factors and host quality can effect developmental period, longevity, parasitism, adult emergence from parasitized eggs and sex ratio (Corrigan and Laing, 1994).

Trichogramma chilonis is an important egg parasitoid used for the control of sugar cane borers (Ashraf and Fatima, 1993). *Sitotroga cerealella* originally proposed by Flanders (1930), is one of the most commonly used as fictitious host for rearing *Trichogramma sp*. It is known that host and parasitoid eggs densities are one of the most important factors determining host acceptance in insect parasitoids (Vinson, 1985).

The periodic release of the natural enemies does not occur naturally in sufficient numbers to keep the pest below damaging levels. Augmentation can be carried out by inundated releases or inoculate releases. The inundated is achieved by flooding the crop with multiple releases of insectary reared natural enemies. The released parasitoids/predators control pests present at the time, but there is little expectation that later generation of these natural enemies will persist at sufficient levels to provide control. This approach requires a large number of natural enemies at the precise time when pest eggs are present and crop weather conditions are conductive to the release (Singh et al., 2002).

In a *T. chilonis* production facility, the host eggs are collected at specified time intervals. The host and parasitoid eggs densities involves in *T. chilonis* production in at least two ways. Firstly, the oviposition preference of the parasitoid females (Pak, 1986) and secondly, as an indicator of the resource quality available for the developing parasitoid larvae thus affecting the physiology of host parasitoid interaction (Vinson and Iwantsch, 1980). Guang and Oloo (1990) and Schmidt et al. (1999) found that *T. chilonis* significantly decreased its parasitization when the eggs were older than 48 h at the time of encounter. Farid et al. (2001) found that *T. chilonis* preferred young eggs when offered along with older eggs simultaneously. Old eggs were not parasitized in the presence of younger eggs. Under no choice test, the parasitoids offered only one age group host at a time, parasitism, adult emergence, and adult longevity did not differ among different age groups of host. Only difference of host age is that the female ratio of emerging *T. chilonis* dropped below 1%. The present research was conducted to check the effect of host and parasitoid densities on biology, i.e., parasitism, adult emergence, adult longevity and sex ratio of *T. chilonis* feeding on *S. cereallela* and *C. cephalonica* eggs under laboratory conditions for efficient and quality production of *T. chilonis*.

2 Material and Methods

2.1 Insects rearing

For the present research, the cultures of *S. cerealella* and *C. cephalonica* were maintained under laboratory conditionon on the natural diet, i.e., wheat grains. The rearing temperature was maintained at 28±1 ºC, 16 h light: 8 h dark photoperiod and 50±60% relative humidity in the Biological Control Laboratory (BCL), Insect Pest Management program (IPM), Institute of Plant and Environmental Protection (IPEP), National Agriculture Research Centre (NARC), Islamabad, Pakistan during July-December, 2010 (Henderson, 1993).

2.2 Angoumois grain moth, *Sitotroga cerealella*

For rearing, eggs of *S. cerealella* were released in trays (36×30×5 cm) having 5 kg wheat grains in upper part of mass rearing chamber made up of tin sheets, consisted of two parts, its upper part was rectangular (37×37×50 cm) while lower was tapered downward with an opening (3×3 cm). Adults collecting box was attached with it. Upon hatching larvae were fed on wheat grains, then pupated and adults were emerged within 25–30 days and dropped directly into adults collecting box. When it contained sufficient number of adults, was daily replaced with empty one. Adults were placed on starch in plastic plates, they laid eggs, were collected by sieving starch with 80 no of mesh sieve. The eggs were used for further experimental purpose (Fig. 1).

Fig. 1 Rearing of Angoumois grain moth, *Sitotroga cereallela* (Olivier); **step 1**: eggs of *S. cereallela* were released in trays (36×30×5 cm) filled with 5 kg wheat grains, tag showing dates on which eggs were added in the wheat grains: a; eggs of *S. cereallela* in trays: b; **step 2**: five mass rearing chambers made up of tin sheets, in which trays filled with 5 kg wheat grains, eggs of *S. cereallela* were placed in the upper parts of chambers which have two parts: the upper one (37×37×50 cm) was rectangular while lower one tapers downwards with an opening (3×3 cm). An adult collection box was attached to the lower side; the adults were dropped directly into oviposition jars, attached to the lower end of the chamber, were replaced daily with new ones: c; **step 3:** oviposition jars were placed on starch present in plastic plates, adults laid eggs in starch; the eggs were collected and used for further experiments: d.

2.3 Rice meal moth, *Corcyra cephalonica*

Under the same laboratory conditions, for maintenance of culture of *C. cephalonica*, 150-200 eggs were mixed daily up to 25 days in the jars containing 1½ kg of rice grain in transparent glass jars (22×55 cm), then the jars were covered with muslin cloth. The larvae fed on rice grain and moulted then pupated. Adults were emerged after 60–75 days. The adult female laid eggs inside of the muslin cloth and then eggs were collected with brush and used for further experimental purpose (Fig. 2).

a b

Fig. 2 Rearing of Rice meal moth, *Corcyra cephalonica* (Stainton): **step 1:** *C. cephalonica* was reared on rice grains (1½ kg) in transparent glass jars (height: 55 cm; dm: 22 cm), eggs of *C. cephalonica* were mixed daily in the jars covered with muslin cloth, larvae fed on rice grain then pupated and adults were emerged after 60–75 days and laid eggs on the outer side of the muslin cloth: a; **step 2**: eggs were collected with brush from the muslin cloth and used for further experiments: b.

2.4 Parasitoid: stingless wasp, *Trichogramma chilonis*

The culture of *T. chilonis* was maintained under the same laboratory conditions on eggs of *S. cerealella and C. cephalonica* were glued on hard paper (8×3 cm²) and put in glass jars (55×12 cm) having *T. chilonis* for parasitization. Adults *T. chilonis* usually emerged after 8-9 days and were provide with a new fresh cards for culture maintenance continuously.

2.5 Identification of sex of parasitoid

Parasitized eggs were kept in transparent gelatin capsule for adult emergence. Then under a high power stereoscope, parasitoid, *T. chilonis* male with long antennae and whorl of hairs and small segments, while female with smaller antennae with tiny hairs and large terminal segments have been identified (Fig. 3).

2.6 Effect of host densities on the biological parameters of *Trichogramma chilonis*

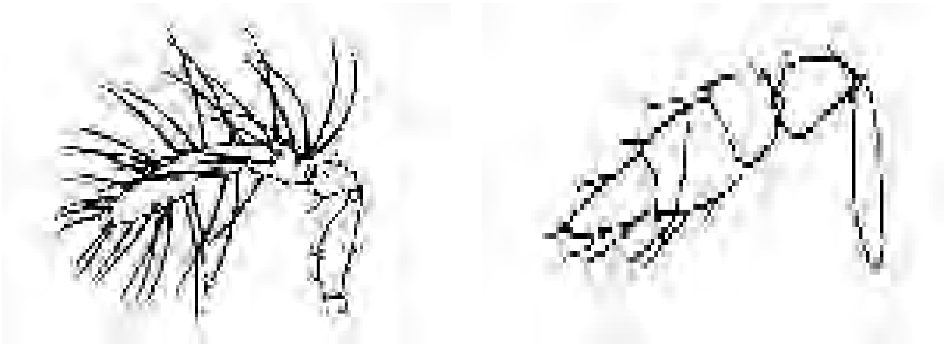
Eggs of both host *S. cerealella* and *C. cephalonica* were glued on the card (8×3 cm) at densities of 10, 20, 30 and 40. There were 10 replications for each insect host eggs. The cards were placed in transparent glass jars (height: 12 cm; dm: 3.5 cm). One day old *T. chilonis* pair was released in each jar, were covered with plastic sheet at the top, tightened with rubber band. After 24 h the *T. chilonis* adults were removed and kept the card at the same temperature and humidity as used for raring of insects. After 4-5 days, the number of parasitized eggs, developmental period, number of adult *T. chilonis* emergence from the parasitized eggs and adult longevity were recorded.

2.7 Effect of parasitoid densities on the biological parameters of *Trichogramma chilonis*

Fresh eggs of 2 h old of both host *S. cereallela* and *C. cephalonica* at density 100 were glued on each card of the same size as mentioned above and kept in jars each with 10 replications. After sexing 1, 2, 3, 4 and 5 pairs of *T. chilonis* were kept as mentioned above paragraph. After 24 h exposure *T. chilonis* were removed and after 4-5 days the data on percentage (%) parasitism [percentage (%) eggs that turn black] and on 11-12 days % adult emergence from parasitized eggs, developmental period and adult longevity were recorded.

2.8 Data analysis

Data were analyzed by one-way analysis of variance (ANOVA) (Concepts, 1989) at $P<0.05$ using LSD test.



a b

Fig. 3 Antennae of male (a) and female (b) of parasitoids Stingless wasp, *Trichogramma chilonis* Ishii

3 Results

3.1 Effect of host density on the biological parameters of *Trichogramma chilonis*

The results indicate that at different densities of eggs of *S. cerealella*, the rate of parasitism of *T. chilonis* was significantly different ($P<0.05$) at last three egg densities (20, 30 and 40) but it was not significantly different ($P<0.05$) at two densities, i.e., 10 and 30. However, it was the highest (85.0±2.44) at 20 egg and the lowest (77.5±1.33) at maximum (40) egg densities. The adults’ emergence was significantly different ($P<0.05$) at all tested densities, it was the highest (96.2±0.82) at minimum (10) and the lowest (87.4±3.51) at maximum (40) egg densities. However, developmental period and adult longevity were not significantly different ($P<0.05$) at all tested densities (Table 1).

Table 1 Effect of host angoumois grain moth, *Sitotroga cereallela* (Olivier) eggs densities on the biological parameters of prasitoids stingless wasp, *Trichogramma chilonis* Ishii

Treatments (number of eggs)	n	Parasitism (M±SD)*	Adult emergence (M±SD)*	Developmenta period (d) (M±SD)*	Adult longevity (d) (M±SD)*
10	10	$80.0±0.66^{b}$	$96.2±0.82^{a}$	$9.0±0.48^{a}$	$3.0±0.32^{a}$
20	10	$85.0±2.44^{a}$	$92.3±2.66^{b}$	$9.1±0.4^{a}$	$3.2±0.23^{a}$
30	10	$80.0±3.97^{b}$	$89.5±2.99^{c}$	$9.0±0.48^{a}$	$3.1±0.25^{a}$
40	10	$77.5±1.33^{c}$	$87.4±3.51^{d}$	$9.0±0.48^{a}$	$3.1±0.25^{a}$

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at $P<0.05$. Means within columns followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days

When host eggs densities of *C. cephalonica* were tested, it was observed that the rate of parasitism of *T. chilonis* was significantly different (*P*<0.05) at 10 and 30 as well as 20 and 40, however, the adults' emergence was significantly different (*P*<0.05) at last three (20, 30 and 40) but not significantly different (*P*<0.05) at 10 and 20 egg densities. However, they were the highest (69.2±1.37; 62.3±1.15) at minimum egg (10) and the lowest (53.7±2.71; 41.3±3.10) at maximum egg (40) densities, respectively. The developmental period was not significantly different (*P*<0.05) at all tested densities. However, the adult longevity was significantly different (*P*<0.05) at 10 and 20 as well as 30 and 40 egg densities, respectively, it was the highest (3.2±0.23) at 30 egg and the lowest (3.0±0.32) at 20 and 40 egg densities (Table 2).

Table 2 Effect of host rice meal moth, *Corcyra cephalonica* (Stainton) eggs densities on the biological parameters of prasitoids stingless wasp, *Trichogramma chilonis* Ishii

Treatments (number of eggs)	n	Parasitism (M±SD)*	Adult emergence (M±SD)*	Developmental period (d) (M±SD)*	Adult longevity (d) (M±SD)*
10	10	69.2±1.37[a]	62.3±1.15[a]	8.3±1.17[a]	3.1±0.25[a]
20	10	68.5±3.49[a]	61.3±3.92[a]	8.0±0.64[a]	3.0±0.32[b]
30	10	54.3±2.00[b]	49.6±3.51[b]	8.1±0.54[a]	3.2±0.23[a]
40	10	53.7±2.71[b]	41.3±3.10[c]	8.3±1.36[a]	3.0±0.32[b]

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at *P*<0.05. Means within colum followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days

Table 3 Effect of parasitoid densities on the biological parameters of prasitoids stingless wasp, *Trichogramma chilonis* Ishii on angoumois grain moth, *Sitotroga cereallela* (Olivier)

Treatments [Pair(s)]	n	Parasitism (M±SD)*	Adult emergence (M±SD)*	Developmental period (d) (M±SD)*	Adult longevity (d) (M±SD)*
1 pair	10	31.2±1.22[e]	93.4±1.10[a]	9.0±0.48[a]	3.8±0.59[a]
2 pairs	10	55.3±4.08[d]	88.0±4.96[b]	9.1±0.4[a]	3.0±0.32[b]
3 pairs	10	63.1±2.99[c]	91.1±5.25[a]	9.2±0.57[a]	3.2±0.23[b]
4 pairs	10	79.6±4.14[b]	83.4±6.02[d]	9.0±0.48[a]	3.1±0.25[b]
5 pairs	10	88.3±4.24[a]	94.2±3.91[a]	9.1±0.4[a]	3.0±0.32[b]

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at *P*<0.05. Means within colum followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days

3.2 Effect of parasitoid density on the biological parameters of *Trichogramma chilonis*

When parasitoid densities of *S. cereallela* were tested, it was observed that the rate of parasitism of *T. chilonis* was significantly different (*P*<0.05) at all (1-5 pairs) densities but it was the highest (88.3±4.24) at 5 pairs and the lowest (31.2±1.22) at 1 pair densities. However, the adults' emergence was significantly different (*P*<0.05) at 1, 2 and 4 pairs densities but not significantly different (*P*<0.05) at 1, 3 and 5 pairs densities. Moreover, it was the highest (94.2±3.91) at 5 pairs and the lowest (83.4±6.02) at 4 pairs densities. The developmental period was not significantly different (*P*<0.05) at all tested (1-5 pairs) densities. However, it was the highest (9.2±0.57) at 3 pairs and the lowest (9.0±0.48) at 1 and 4 pairs densities. However, the adult longevity was significantly different (*P*<0.05) at 1 and 2 pairs but not significantly different (*P*<0.05) 2-5 pairs densities, respectively; it was the highest (3.8±0.59) at 1 pair and the lowest (3.0±0.32) at 2 and 5 pairs densities (Table 3).

When parasitoid densities of *C. cephalonica* were tested, it was observed that the rate of parasitism of *T. chilonis* was significantly different (*P*<0.05) at all (1-5 pairs) densities but it was the highest (74.2±4.39) at 5 pairs and the lowest (21.3±2.90) at 1 pair densities. However, the adults' emergence was significantly different (*P*<0.05) at 1, 2 and 3 pairs densities but not significantly different (*P*<0.05) at 2, 4 and 5 pairs densities. Moreover, it was the highest (74.6±1.66) at 1 pair and the lowest (43.3±7.48) at 3 pairs densities. The developmental period was not significantly different (*P*<0.05) at all tested (1-5 pairs) densities. However, it was the highest (8.3±1.09) at 2 and 4 pairs and the lowest (8.0±0.64) at 3 pairs densities. However, the adult longevity was significantly different (*P*<0.05) at 1, 2 and 3 pairs but not significantly different (*P*<0.05) 3-5 pairs densities, respectively; it was the highest (3.8±0.43) at 1 pair and the lowest (3.0±0.32) at 4 pairs densities (Table 4).

Table 4 Effect of parasitoid densities on the biological parameters of stingless wasp, *Trichogramma chilonis* Ishii on rice meal moth, *Corcyra cephalonica* (Stainton) eggs

Treatments [Pair(s)]	n	Parasitism (M±SD)*	Adult emergence (M±SD)*	Developmental period (d) (M±SD)*	Adult longevity (d) (M±SD)*
1 pair	10	21.3±2.90^{e}	74.6±1.66^{a}	8.1±0.54^{a}	3.8±0.43^{a}
2 pairs	10	47.1±5.40^{d}	61.3±2.37^{b}	8.3±1.09^{a}	3.2±0.23^{b}
3 pairs	10	60.2±2.44^{c}	43.3±7.48^{c}	8.0±0.64^{a}	3.1±0.25b^{c}
4 pairs	10	71.3±1.70^{b}	62.1±4.76^{b}	8.3±1.10^{a}	3.0±0.32^{c}
5 pairs	10	74.2±4.39^{a}	61.3±4.79^{b}	8.2±0.99^{a}	3.1±0.25b^{c}

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at *P*<0.05. Means within colum followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days

4 Discussion

Smith (1996) recognizing the potential of *Trichogramma* species as biological control agents, entomologists in the early 1900s began to mass rear *Trichogramma* for insect control. Although a small commercial production of *Trichogramma* eventually developed in the U.S., insect control research and commercial efforts focused on the development of chemical pesticides following the discovery of DDT. Goodenough and Witz (1985) reported that success of biological control with *Trichogramma* species depends on basic studies on host, temperature, plant architecture and phenology, searching area, wind and chemicals such as insecticides which could affect searching behavior of these organisms. It has not been widely used in Pakistan, therefore, *Trichogramma* has been used for the present research.

Eduardo et al. (2004) reported that most biological control programs using Trichogrammatidae against lepidopterans have made use of Nearctic or Palearctic species of *Trichogramma*, and there is little information concerning the effectiveness of Neotropical *Trichogramma* spp., despite increasing interest in using egg parasitoids in many South American countries i.e., Argentina, Brazil, Chile, Colombia, Peru and Uruguay. Therefore, the present research was conducted to determine the effect of host insect eggs densities, i.e., 10, 20, 30 and 40 eggs of both insects for parasitism by using *T. chilonis*. The result indicates that maximum rate of parasitism (85.4%) in 20 eggs density on *S. cereallela* eggs, which were significantly different from the rest of the host density ratio while minimum (77.5%) parasitism by *T. chilonis* was in 40 eggs densities. At 20 and 30 eggs density rate of parasitism were non-significant to each other but significantly different from the rest of the treatments. The study further indicates that *Corcyra* eggs densities for rate of parasitism by *T. chilonis* indicates maximum rate of parasitism were (69.2%) in 10 eggs density, while minimum was (53.7%) in 40 eggs density. The results further indicate that 10, 20 ratios were non-significant to each other but significantly different from 30, 40 eggs density for rate of parasitism by *T. chilonis* (Tables 1-4).

Previous worker, Khan et al. (2004) reported the highest parasitism (48.25%) at 20 eggs/single pair which is in agreement with our present study. For comparison of both insect eggs for parasitism and adult *T. chilonis* emergence *S. cereallela* was best as compared to *C. cephalonica* eggs in all treatments. The developmental period from eggs to adult emergence were almost non-significant to each other on different host eggs density but *S. cereallela* eggs were significantly different in all treatments than *C. cephalonica* eggs for rate of parasitism by *T. chilonis.*

Lu (1992) observed that increase in parasitoid density reduced the number of parasitized host eggs per female. Sex ratio was unaffected by changes in host density. Vorgas and Nishida (1982) observed that the parasitized species and the relative density of hosts affected the number of parasitized eggs. The percentage of parasitized eggs was independent of host density at low densities of parasitoids and was inversely dependent at high densities. The present study was conducted on the effect of parasitoid densities, i.e., 1-5 pairs of *T. chilonis* using *S. cereallela* and *C. cephalonica* eggs. The result indicates that maximum rate of parasitism (88.3%) from 5 pairs of *T. chilonis* when feeding on *S. cereallela* eggs, which were significantly different from the rest of the parasitoid density ratios while minimum (31.2%) parasitism by 1 pair of *T. chilonis*. All treatments are significantly different from each other. The study further indicates that parasitoid densities for *C. cephalonica* eggs, parasitism by *T. chilonis* indicates maximum rate of parasitism was (86.3%) from 5 pairs of *T. chilonis* density, while minimum was (21.3%) in 1 pair density. The result further indicates that all treatments are significantly different from each other for rate of parasitism by *T. chilonis.* For comparison of both insect eggs for parasitism and adult *T. chilonis* emergence *S. cereallela* was best as compared to *C. cephalonica* eggs in all treatments. At the presently, the similar results were obtained as reported Vorgas and Nishida and Lu.

Thorpe and Dively (1985) reported that rates of parasitism were significantly higher at the highest wasp density on all arenas and the effect of host density was not consistent among the arena. Khan et al., (2004) showed the effect of host and parasitoid density on parasitism efficiency of *T. chilonis* (Ishii) using eggs of *S. cerealella* as a host. Four different host densities viz., 5, 10, 20 and 40 eggs were provided to single pair of *T. chilonis*. Percent parasitism was highest (48.25%) at 20 eggs/single pair, whereas percent emergence was highest (88.89%) from 5 eggs but all treatments were statistically similar. Female ratio was also highest at 5 eggs/female. The longevity was highest at 20 eggs/pair. The developmental period from eggs to adult emergence were almost non-significant to each other on different parasitoid density but *S. cereallela* eggs were significantly different in all treatments than *Corcyra* eggs for rate of parasitism by *T. chilonis.* The present study further indicates adult *T. chilonis* emergence from parasitized eggs of different parasitoid densities maximum adult *Trichogramma* emergence were (94.2%) in 5 pairs using eggs of *S. cereallela* while minimum emergence were (83.4%) in 4 pairs. While on *C. cephalonica* eggs rate of adult emergence were (74.4%) from 1 pair while minimum (43.4%) from 3 pairs. The result for both insects' eggs indicates significantly different for different parasitoid densities. Highest parasitism was obtained from 5 pairs of *T. chilonis* on *S. cereallela* eggs. *S. cereallela* eggs were suitable as compare to *C. cephalonica* eggs for rate of parasitism by *T. chilonis* and adult *T. chilonis* from parasitized eggs under the same conditions. Previous worker Henderson (1993) reported somewhat similar results where he found that a single pair of parasitoid parasitized about 20 eggs. The number of eggs parasitized significantly increased with egg density, tending to stabilize at densities above

30 eggs/ parasitoid which is also in agreement with the present results. Further research should be needed to clarify this mechanism.

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