Effect of host egg age on preference, development and arrestment of \textit{Telenomus remus} (Hymenoptera: Scelionidae)

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\textbf{Abstract.} Age of host eggs can be a limiting factor for egg parasitoids. It is expected that old eggs are less preferred by egg parasitoids, which can discriminate between eggs of different ages by using chemical cues. The objective of this study was to determine the preference, development and arrestment of \textit{Telenomus remus} Nixon (Hymenoptera: Scelionidae) parasitizing \textit{Spodoptera frugiperda} (J.E. Smith) (Lepidoptera: Noctuidae) eggs of different ages. This egg parasitoid preferred to parasitize 1- and 2-day-old eggs rather than 3-day-old eggs in choice and no-choice assays. However, although the percentage emergence of parasitoids is significantly lower from 2- and 3-day-old eggs, the sex ratio and developmental time are unaffected. Parasitoids spent longer searching substrates impregnated with extracts of 1- and 2-day old eggs than 3-day-old eggs. Our results reveal that \textit{T. remus} is able to distinguish the most suitable (1-day-old) from the least suitable (3-day-old) host eggs, but unable to recognize the unsuitability of intermediate aged eggs. Egg arrestants may be responsible for the preference of \textit{T. remus} for ovipositing in 1- and 2-day old eggs.

\section*{INTRODUCTION}

Host selection by parasitoids consists of the following behavioural steps: host habitat location, host location, host recognition and host acceptance (Vinson, 1976; 1984). For this reason, parasitoids mostly exploit chemical and/or physical cues associated with the host, plant, or interaction between plant and host (Borges et al., 1999; Hilker & Meiners, 2002).

Egg parasitoids have to be efficient at locating hosts as the duration of the egg stage is generally short. Moreover, old eggs are less suitable as hosts as they adversely affect several parasitoid biological parameters, such as percentage parasitism, developmental time (Da Rocha et al., 2006), adult emergence (Bruce et al., 2009), body size and sex ratio (Ruberson & Kring, 1993). These effects may be due to changes in the chemical composition as nutrients are gradually consumed by the host embryo and/or changes in the physical characteristics of the chorion, which becomes more rigid as the egg ages (Barret & Schmidt, 1991; Bai et al., 1992). Preference for hosts of a particular age may enhance the fitness of egg parasitoids (Pyke et al., 1977; Strand & Vinson, 1983; Schmidt & Smith, 1985; Vinson et al., 1998). Thus parasitoids might make use of the physical characters and/or chemicals in and on the surface of eggs, which change with age, as cues indicating the suitability of hosts.

Once egg parasitoids encounter host sites, they are arrested, i.e., they change their behaviour from random walking to an intense directed examination of host-derived chemicals (Kennedy, 1978). This behaviour is well documented for egg parasitoids (Beevers et al., 1981; Gazit et al., 1996), although it is unknown whether the arrestants change with host age and thus affect parasitoid behaviour. \textit{Telenomus remus} Nixon (Hymenoptera: Scelionidae) is a specialist parasitoid of the eggs of the genus \textit{Spodoptera} (Schwartz & Gerling, 1974). As \textit{T. remus} is able to parasitize the eggs in the basal layers of \textit{Spodoptera} egg masses, even when they are covered by moth scales, the percentage parasitism by this parasitoid is usually higher than that by \textit{Trichogramma} parasitoids (Cave, 2000). Furthermore, \textit{T. remus} can effectively control fall armyworm in corn fields (Narváez & Zocco, 1996). Thus information on this parasitoid’s preference and the suitability of fall armyworm eggs of different ages is of great importance for mass rearing purposes.

The objective of this study was to determine if \textit{T. remus} can discriminate between eggs of \textit{Spodoptera frugiperda} (J.E. Smith) (Lepidoptera: Noctuidae) of different ages and if egg kairomones play a role in determining their preference for eggs of a particular age.

\section*{MATERIAL AND METHODS}

\textbf{Insects}

Larvae of \textit{S. frugiperda} were collected from maize crops and fed on an artificial diet (Greene et al., 1976) until pupation. Adults were then transferred to cylindrical rearing cages (10 cm in diameter and 21 cm high) for mating and oviposition. They were fed on a water solution of 10% honey (v/v). Cages were covered by paper in which the moths laid egg masses. Papers containing eggs were collected and replaced daily.

For rearing the egg parasitoid \textit{T. remus} egg masses of \textit{S. frugiperda} were attached to a white paper strip (2.5 cm wide and 5 cm long) and placed inside glass tubes (8.5 cm high and 2.5 cm in diameter) containing gravid wasps. Parasitoid adults were fed...
on honey drops placed on the wall of the glass tube. For all bio-
assays, only 3 and 4 day old honey-fed gravid and naive para-
sitoids were used. The parasitoid wasps came from cultures
maintained at EMBRAPA Milho e Sorgo (Sete Lagoas, MG,
Brazil). Occasionally, we introduced individuals collected from
corn fields in Piracicaba, SP, Brazil.

Both the host and the egg parasitoid were reared in an accli-
matized room at 25 ± 2°C, 60 ± 10% R.H. and a photoperiod of
12L : 12D.

No-choice bioassay

The percentages of 1, 2 and 3 day old eggs of *S. frugiperda*
parasitized by *T. remus* females and the development of their
larvae in these eggs were evaluated using a no-choice bioassay.

Eggs were carefully separated using a fine brush. Fifty eggs
of each age were fixed with nontoxic water soluble glue to a
square of paper (1 cm²) each of which was placed in a glass tube
(8.5 cm high and 2.5 cm in diameter). Each glass tube contained
one *T. remus* female and one paper square with eggs. In total,
there were 30 tubes, 10 replicates of each egg age. All tubes
were kept in a climatic chamber at 28 ± 0.5°C, 70 ± 10% R.H.
and a photoperiod of 12L : 12D. After 2 h (time enough for *T.
remus* to parasitize 50 eggs), *T. remus* females were removed
and the glass tubes kept in the climatic chamber until adult
emergence. Larvae that hatched from un-parasitized eggs were
removed and counted twice a day.

Day of emergence, number of male and female parasitoids
that emerged and the number of dark eggs that did not give rise
to parasitoid adults or larvae were recorded. These values were
used to estimate percentages of parasitism and emergence, the
period of time required for development from egg to adult and
the sex ratio (number of females / total number of individuals).

Choice bioassay

The percentages of 1, 2 and 3 day old eggs of *S. frugiperda*
parasitized by *T. remus* females and the development of their
larvae in these eggs were evaluated using a bioassay in which
they were able to choose between eggs of different ages.

For this bioassay 3 squares of paper each 1 cm² and con-
taining 50 1, 2 or 3 day-old eggs were randomly fixed with non-
toxic water soluble glue to a paper strip. Each set of paper
squares were placed inside a glass tube (8.5 cm high and 2.5 cm
diameter) containing one parasitoid female. One *T. remus*
parasitizes, on average, 20–25 eggs/h (C.D. Bezerra da Silva,
data not published). In order to assess the wasf preference in
choice assays, we allowed *T. remus* to parasitize the eggs for 4
h. In total, there were 15 glass tubes that were kept in a climatic
chamber under similar conditions to those used in the no-choice
bioassays.

After 4h the paper squares with 1, 2 and 3 day old eggs for
each replicate were separated and placed in glass tubes. Larvae
that hatched from the un-parasitized eggs were removed and
counted twice a day.

Arrestment bioassay

Arrestment was measured based on the time females of *T.
remus* spent (residence time) on a piece of filter paper impreg-
nated with an hexane extract of eggs (Gazit et al., 1996). Assays
were conducted in an acclimatized (25 ± 2°C, and 60 ± 10%
R.H) room, which was illuminated with red light. Ten minutes
before the assay, squares of filter paper (1 cm²) were impreg-
nated with 20 µl of egg extract, and placed in a Petri dish (14.2
cm diameter) previously cleaned with alcohol (v/v 90%) and
acetone (v/v 99%). Then one *T. remus* female was released at
the center of the filter paper and the timing started. Each obser-
vation lasted up to 5 min and the time spent by the parasitoid
female on the filter paper was recorded (Gazit et al., 1996). If
the female left the filter paper during the 5-min period and did
not return within 20 s the observation ended. However, if a
female returned to the filter paper within 20 s, the observation
continued. As a control, filter papers impregnated only with
hexane were used. Each treatment consisted of 20 replicates and
insects were tested only once.

Egg extracts

Moth scales were removed from the eggs before the extrac-
tions. In addition egg masses that were well covered with moth
scales were not used. However, not all moth scales were always
removed.

*S. frugiperda* eggs were carefully separated to avoid
rupture, weighed and placed in clean glass vials. Sufficient sol-
vent (hexane) was added to give a concentration of 60 mg/ml.
After 3 h, the solvent in the vial containing the eggs was trans-
ferrred to another new vial, which was kept at −20°C until
required. Before testing, egg extracts were evaporated in N₂ gas
to reduce volume of solvent to 200 µl resulting in a concentra-
tion of 150 mg/ml.

Treatments

As moth females age, they lose scales and produce less ovu-
duct secretion. Both represent important egg kairomones
(Beeners et al., 1981; Norlund et al., 1987). So, preliminary
tests were performed to verify if moth age affected the attrac-
tiveness of extracts of 1, 2 and 3-day old eggs. To do this
extracts of 1-day-old eggs that were laid by 4, 6, 7, 8 and
9-day-old moths were tested.

Since the eggs deposited by females of 4-to-8-days-old did
not differ significantly in their attractiveness for *T. remus* (see
results of Fig. 5), the extracts were of eggs laid by 4-to-8-
day-old females. The results of these treatments were compared
with the solvent (hexane) control using the method described
above.

Statistical analysis

All data was tested for parametric assumptions (normal distri-
bution and equal variance) using a Kolmogorov-Smirnov test.

Based on that test the data on percentage parasitism and sex
ratio from no-choice assays were analyzed using a Kruskal-
Wallis test and means compared by Dunn’s method (P ≤ 0.05).
While data on emergence, percentage parasitism in choice
assays and residence time (arrestment assays) were analyzed
using One-Way ANOVA and means compared using a Duncan
test (P ≤ 0.05).

RESULTS

In the no-choice bioassay *T. remus* equally parasitized 1-
and 2-day-old eggs. However, the percentage para-
sitism of 3-day-old eggs was significantly lower (Fig. 1;
Kruskal-Wallis; *H* = 17.11; *P* < 0.01; Dunn *P* < 0.05).
Similarly, in the choice bioassay, the parasitism of 3-
day-old eggs was significantly lower than that of 1- and
2-day-old eggs (Fig. 2, ANOVA *F* = 3.581 *P* = 0.037; 
Duncan *P* < 0.05).

Mean percentage parasitoid emergence significantly
decreased with egg age. The highest emergence was
recorded from 1-day-old eggs and the lowest from
3-day-old eggs. Percentage parasitoid emergence from
2-day-old eggs was intermediate and differed from that of 1-
and 3-day-old eggs (Fig. 3; ANOVA *F* = 49.935; *P* <
0.001; Duncan *P* < 0.05). In contrast, the sex ratio was
unaffected by the age of the eggs (Fig. 4; Kruskal-Wallis
H = 0.324; P = 0.850) and the parasitoid developmental time was 10 days in all treatments.

In the arrestment tests (measured in terms of parasitoid residence time), females usually remained longer on filter papers treated with egg extracts: walking in circles, or stopping and vibrating their antennae. Sometimes, the parasitoid left the filter paper but returned within a few seconds. The extracts of eggs laid by 4-to-8-day-old moths elicited the same arrestment response from *T. remus* females. Parasitoid residence time was longer on filter papers impregnated with egg extracts derived from these eggs compared to the extract of eggs laid by 9-day-old moths and the hexane control (Fig. 5; Kruskal-Wallis H = 48.87; P < 0.001; Dunn P < 0.05). Therefore, the extracts of eggs of different ages were obtained using eggs collected from 4-to-8-day-old *S. frugiperda*.

In the egg age test, *T. remus* spent longer on filter papers impregnated with egg extracts than the hexane control (Fig. 6; ANOVA F = 10.337; P < 0.001) and significantly more time on those impregnated with the extract of 1-day-old eggs than of 3-day-old eggs (Duncan P < 0.05).

DISCUSSION AND CONCLUSIONS

Host quality for egg parasitoids basically consists of two parameters: species and host age (Liu et al., 1998). The latter can be restrictive because host embryo development depletes the nutrients stored in the egg. Therefore, old eggs are usually low-quality hosts. Moreover, a parasitoid larva is not capable of digesting the cuticle of its host and therefore cannot consume nutrients from embryos in an advanced stage of development (Strand et al., 1986).

Thus, the selection of young eggs is advantageous because of their better nutritional quality for the development of parasitoid offspring. Egg parasitoids usually prefer young or intermediate aged eggs for parasitism (Reznik & Umarova, 1990; Monje et al., 1999; Moreno et al., 2009), including members of the Scelionidae (Romeis et al., 2000; Da Rocha et al., 2006).

Most studies indicate that old eggs are poor quality hosts for egg parasitoids (Souza & Spence, 2001; Tunçbilek & Ayvaz, 2003). However, there are reports that old eggs do not have any deleterious effect on preference or offspring performance (Pak et al., 1986; Jacob et al., 2006).
This study revealed that the parasitoid T. remus prefers to parasitize young hosts (1- and 2-day old eggs). The old hosts (3-day-old eggs) were not only less preferred than young hosts for parasitism, but also less acceptable as the parasitoid parasitized fewer old eggs in no-choice assays (Figs 2 and 3). Percentage of adults emerging from 2- and 3-day old eggs was less than from 1-day old eggs (Fig. 4). These results suggest that T. remus females are able to discriminate between the most (1-day-old eggs) and the least suitable (3-day-old eggs) hosts, but are unable to recognize the unsuitability of intermediate aged eggs. These findings are not consistent with the predictions of optimal foraging theory, which predicts that parasitoids prefer hosts that provide the best conditions for offspring performance (Pyke et al., 1977), since T. remus does not discriminate 2-day-old eggs, which are not as good as 1-day-old hosts in terms of offspring performance. However, T. remus avoids parasitizing 3-day-old eggs, which were the least suitable of the different ages of eggs tested. In this case, T. remus behaviour agrees with optimal foraging theory.

On the other hand, egg aging did not affect the sex ratio of T. remus (Fig. 5) or its developmental time. Although a reduction in the proportion of males emerging from old eggs is reported (Sousa & Spence, 2001), this effect is not recorded for other species of Scelionidae, such as Gryon gallardoi (Brethes) (Da Rocha et al., 2006) and Telenomus isis (Hymenoptera: Scelionidae) (Bruce et al., 2009).

Suitable egg age depends on host species as embryo development may vary among species (Pak et al., 1986; Monje et al., 1999; Chabi-Olaye et al., 2001). This may account for the differences between the results obtained in this and other studies in which T. remus parasitized the eggs of other species of Spodoptera (Gerling & Schwartz, 1974; Dass & Parshad, 1983).

The parasitoid T. remus was arrested by hexane extracts of S. frugiperda eggs (Fig. 6). Although all the hexane egg extracts invoked arrestment behaviour, T. remus spent longer on filter paper impregnated with extracts of 1- and 2-day old than with 3-day-old eggs (Fig. 6). This arrestment response was equal to the preference of T. remus for 1- and 2-day-old eggs. Therefore, it is possible that the egg kairomones associated with 3-day-old eggs were altered or reduced.

Gazit et al. (1996) report a residence time for T. remus on extracts derived from 1-day-old S. frugiperda eggs of 195 ± 19 s (mean ± SE). We obtained similar averages for extracts of 1- and 2-day-old eggs, which were 204 ± 24 s and 195 ± 22 s, respectively. The residence time was significantly lower, 125 ± 24 s, on extracts of 3-day-old eggs.

Qualitative or quantitative changes in the chemical composition of the surface of 3-day-old S. frugiperda eggs may be the cue used by T. remus to avoid ovipositing in old hosts. That is, the lower percentage parasitism of 3-day-old eggs by T. remus could be partly due to the detection of chemicals on the surface of eggs.

Chemicals associated with eggs are well known to induce arrestment behaviour in Scelionidae parasitoids (Strand & Vinson, 1983; Bin et al., 1993). They may play a role in host recognition and act as short-range attractants (Norlund et al., 1987; Boo & Yang, 2000).

Hexane extracts may not contain the host recognition kairomone from S. frugiperda eggs as they consist of heavy proteins that are associated with moth scale compounds, which are arrestants (Strand & Vinson, 1983; Gazit et al., 1996). A preliminary bioassay of the arrestment recorded for 1-day-old eggs laid by 9-day-old moths did not induce arrestment in T. remus (Fig. 5). We suppose that the lack of arrestment in this case is due to the reduction in the number of scales on old moths. Hence, 3-day-old egg extracts may contain fewer moth scale residues and are therefore less effective at inducing arrestment behaviour in T. remus.

Nevertheless, not only contact chemical cues are important for egg parasitoids in recognizing suitable hosts, but also physical characteristics, such as the hardening of the chorion, which hampers the penetration of a parasitoid’s ovipositor (Leibee et al., 1979; Irvin & Hoddle, 2005).
The ability to parasitize eggs of a wide range of ages is one attribute that might confer a higher potential for controlling pests in the field. In the case of T. remus, results suggest that location of S. frugiperda eggs can be critical for eggs older than 2 days, and may also limit percentage of parasitism and emergence.

Furthermore, the results of this study may help in improving the mass rearing of T. remus. We show that in order to maintain a high productivity in mass rearing it is important to offer 1-day-old S. frugiperda eggs to T. remus.

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