BIOLOGICAL CONTROL

Effects of Egg Age of *Spartocera dentiventris* (Berg) (Hemiptera: Coreidae) on Parasitism by *Gryon gallardoi* (Brethes) (Hymenoptera: Scelionidae)

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Efeito da Idade dos Ovos de *Spartocera dentiventris* (Berg) (Hemiptera: Coreidae) no Parasitismo por *Gryon gallardoi* (Brethes) (Hymenoptera: Scelionidae)

RESUMO - Visando avaliar o efeito da idade dos ovos de *Spartocera dentiventris* (Berg) no parasitismo por *Gryon gallardoi* (Brethes), foram expostos grupos de 12 ovos de 2, 3, 4, 5, 6, 7, 8, e 12 dias de idade para uma fêmea de *G. gallardoi* em um tubo de ensaio, sendo esta observada por 2h. Após, os ovos foram individualizados e incubados (25 ± 1°C; fotoperíodo de 12h) até a emergência dos adultos. Para a avaliação da idade preferencial, grupos de 12 ovos de 1, 3, 5, 7 e 12 dias de idade foram expostos, simultaneamente, para uma fêmea do parasitóide por 2h. Posteriormente, os grupos foram individualizados e incubados até ser confirmado o parasitismo. Em todas as idades testadas verificou-se parasitismo em 88,5 ± 2,64% dos ovos oferecidos, com diminuição no percentual de emergência com o aumento da idade ($P < 0,01$). O tempo de desenvolvimento aumentou com a idade dos ovos ($P < 0,01$), sendo que os machos se desenvolveram mais rapidamente. A razão sexual, em todas as idades testadas foi desviada para fêmeas (0,49:1) e a proporção de machos não variou com a idade do hospedeiro ($P > 0,05$). Quando os grupos de ovos foram expostos simultaneamente, a fêmea preferiu os ovos de um e três dias de idade. Os resultados indicaram preferência de *G. gallardoi* por ovos mais jovens de *S. dentiventris* podendo a idade exercer efeito negativo no tempo de desenvolvimento e na emergência da prole.

PALAVRAS-CHAVE: Insecta, parasitóide de ovos, interação hospedeiro-parasitóide

ABSTRACT **-** To evaluate the effect of egg age of *Spartocera dentiventris* (Berg) on the parasitism by *Gryon gallardoi* (Brethes), groups of 12 eggs of 2, 3, 4, 5, 6, 7, 8, and 12 days old were exposed to one female of *G. gallardoi* on a test tube, with the wasp observed for 2h subsequently. Later, the eggs were individualised and incubated (25 ± 1°C; 12h photoperiod) until adult emergence. To identify the preferred age, groups of 12 eggs of 1, 3, 5, 7 and 12 days of age were simultaneously exposed to a parasitoid female on an arena for 2h. These groups were also individualised and incubated until parasitism was confirmed. On all ages tested parasitism was at about 88.5 ± 2.64%, with a reduction in adult parasitoid emergence with age increasing ($P < 0.01$). The development time increased with increasing egg age ($P < 0.01$), with males developing faster. Parasitoid sex ratio for all ages tested was female-biased (0.49:1), with the proportion of males invariant with host age ($P > 0.05$). When the egg groups were exposed simultaneously, the female preferred eggs one and three days old. The results indicate a preference of *G. gallardoi* for younger eggs of *S. dentiventris*, with host egg age exerting a negative effect on development time and offspring emergence of the parasitoid.

KEY WORDS: Insecta, egg parasitoid, host-parasitoid interaction

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Host evaluation by eggs parasitoids has a substantial effect on the efficiency of the latter in biological control programmes (Pak 1986, De Bach & Rosen 1991, Ruberson & Kring 1993, Heng-Moss *et al*. 2001). With increases in age, insect egg chemical composition changes rapidly, passing from a fluid medium to complex tissues. This can in turn change host acceptance and suitability for parasitoid offspring development, exerting negative effects on parasitism as pre-imaginal mortality and overall offspring fitness (Vinson & Iwantsch 1980, Pak 1986, Vinson 1998, Brodeur & Boivin 2004), besides modifications in sex ratio (King 2002). Because of this, many parasitoids prefer certain stages of development of the host. Knowledge of these preferences is fundamental for mass rearing, since the use of hosts at inadequate ages could lead to the production of low-quality parasitoids and, consequently, less efficient biological control (Ruberson & Kring 1993). In field releases, when the preferred host stage is absent from the area, parasitoids could disperse instead of becoming established (De Bach & Rosen 1991, Heng-Moss *et al.* 2001).

The influence of host age on parasitism success has been studied in the last decades, especially in Scelionidae, a family that includes a large number of species of Heteropteran egg parasitoids (Hernández & Diaz 1996, Romeis *et al.* 2000, Souza & Spence 2001). Among these *Gryon gallardoi* (Brethes) has been referred as the main natural enemy of *Spartocera dentiventris* (Berg), the tobacco-grey-bug, in Rio Grande do Sul, Brazil, being responsible for a considerable mortality of the egg stage of this bug (Santos *et al.* 2001). Studies with scelionids demonstrated that parasitoids are more successful in development when reared from younger hosts (Strand 1986, Navasero & Oatman 1989, Hernández & Diaz 1996, Souza & Spence 2001).

This work aimed at evaluating, under laboratory conditions, the effect of egg age of *S. dentiventris* on the parasitism by *G. gallardoi*, recording adult emergence, development time, proportion of males per group of eggs and order of oviposition, and host age preferred.

Material and Methods

The insects used in the experiments were obtained from an experimental tobacco culture, adjacent to forest areas of the Serra de Santana, in Porto Alegre (30°01'S, 51°13'W), state of Rio Grande do Sul, Brazil. From the moment *S. dentiventris* eggs were recorded on the plants some of the females were confined individually in cages to obtain eggs of known ages. Other females were kept free so their eggs could be parasitized by *G. gallardoi*. Naturally parasitized eggs in the field, characterised by a dark grey colour (Santos *et al.* 2001), were brought to the lab to start a parasitoid rearing on the eggs obtained from the caged bug females. All experiments were run under controlled conditions (25 ± 1ºC, 12h photoperiod). Emerged adults of *G. gallardoi* were kept in plastic cages and fed a 10% honey solution. Only 2-5 day-old adults, mated and with previous oviposition experiences, were used in the experiments. Soon after emergence, females were presented to males and later kept with eggs of *S. dentiventris* for 24h, so they could acquire experience in oviposition.

To evaluate the influence of host egg age on emergence success, development time and offspring sex ratio, groups of 12 unparasitized eggs of *S. dentiventris* with 2, 3, 4, 5, 6, 7, 8 and 12 days of age, were individually exposed in a test tube (1 x 6 cm) to one parasitoid female for 2h, with wasp behaviour observed with a stereomicroscope. As to permit egg identification, maps of the egg groups were drawn. Only eggs marked by the female after retreating the ovipositor were considered parasitized. Ten females (replications) were used for each egg age. Later, parasitized eggs were transferred individually to eppendorff tubes (15 ml) and kept in a climatically controlled environment (25 ± 1ºC, 12h photoperiod), being observed daily until adult emergence. Only adults successfully emerged were used to evaluate sex ratio.

The preferred host egg age was obtained by exposing each female simultaneously to five groups of 12 unparasitized eggs, of 1, 3, 5, 7 and 12 days of age disposed in a circle inside a round plastic arena (9 cm diameter and 10 cm height). Each female was observed until it demonstrated an interest for one group of eggs and begun to oviposit; removal of the female from the arena was standardised at 2h after this first oviposition. Each egg group was individualized in petri dishes until parasitism was confirmed by egg colour change. Twenty replications were employed, with egg age disposition on the bottom of the arena randomly assigned.

Parasitism percentages and adult emergence were evaluated individually for each egg age, arcsine transformed (Sokal & Rolhf 1981) and analysed with linear regressions. Offspring sex ratio was compared with a heterogeneity *G*-test and age effect on this sex ratio by regression. The order of oviposition in which males and females emerged, within each group of eggs, was compared with a replicate *G*-test. Data referring to offspring development time produced on the different egg ages, for each sex, were compared under analysis of covariance (ANCOVA). Parasitism proportions for each egg age when eggs were offered simultaneously (preference experiment) were arcsine transformed and analysed with a Kruskal-Wallis test.

Results and Discussion

On average, 88.5 ± 2.64 % of the eggs were parasitized per egg group, when the groups of eggs were offered individually. Parasitism was recorded for eggs of all ages, with no variation with age increasing ($F = 1.63$; $r^2 = 1.03\%$; $P = 0.20$). This lack of preference is probably related to the experimental design, which did not allow any other choice to the female. The percentage of offspring emergence of the parasitized eggs decreased considerably when host egg age increased ($F = 9.37$; $P < 0.01$) (Fig. 1).

Parasitism on all egg ages of *S. dentiventris* and higher percentages of adult *G. gallardoi* emergence on younger eggs are expected. According to Strand (1986) and Vinson (1998), hymenopteran parasitoids can parasitize and develop on all developmental stages of their hosts, being able to adapt to a range of host conditions. However, some stages, especially the younger ones, seem to be more appropriate to offspring development success, as demonstrated by a series of studies with scelionids, like *Telenomus heliothidis* Ashmed (Strand 1986); *Telenomus solitus* Johnson (Navasero & Oatman 1989), *Telenomus remus* Nixon (Hernández & Diaz 1996) and *Tiphodytes gerriphagus* Marchal (Souza & Spence 2001).

According to Strand (1986), a low emergence rate in

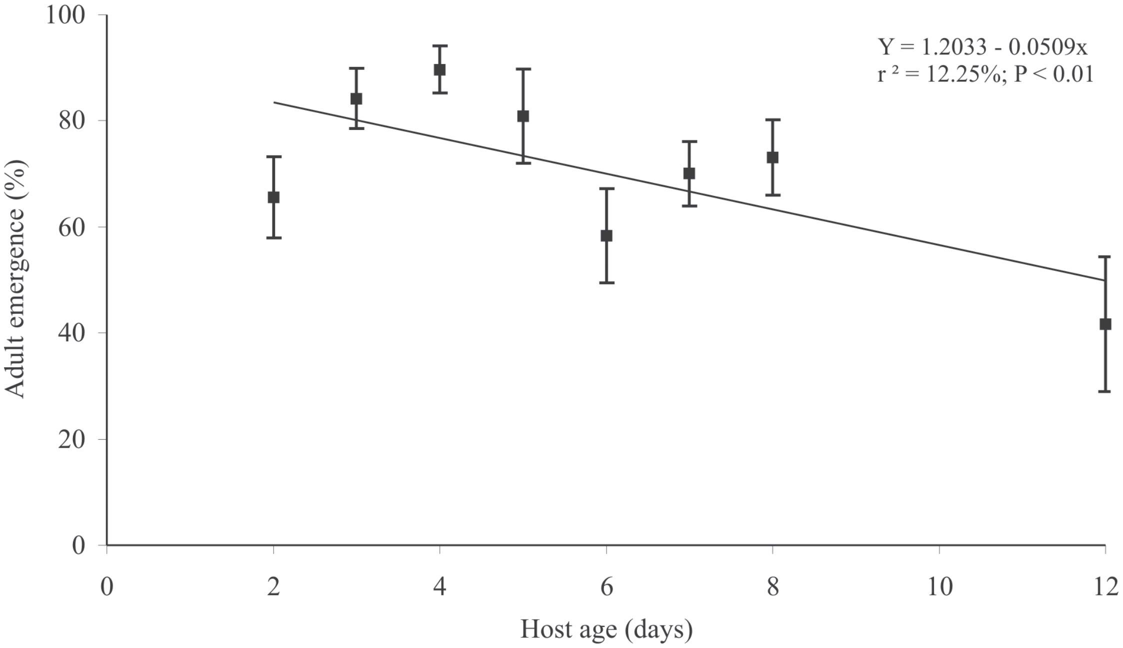

Fig. 1. Emergence percentages (mean ± s.e.) for *G. gallardoi* offspring from eggs of *S. dentiventris* at different ages.

parasitoids reared from older eggs can be attributed to the embryonary development of the host, which in more developed stages could prevent larval feeding by the parasitoid, incapable of digesting the cuticle. This was demonstrated by Nechols *et al.* (1988) for "old" eggs of *Anasa tristis* (De Geer) (Hemiptera: Coreidae) parasitized by *Gryon nixoni* (Masner) (Hymenoptera: Scelionidae), with dissected eggs showing that most of the parasitoid larvae did not reach the second instar.

Nevertheless, many studies have demonstrated, especially for scelionids, that females are able to "transform" host composition so as to facilitate parasitoid larval digestion through the injection of certain substances during oviposition ("poisoning") (Vinson & Iwantsch 1980, Nechols *et al.* 1988, Vinson 1998, Beckage & Gelman 2004) or by substances produced by teratocytes (Volkoff & Colazza 1992). Barret & Schimidt (1991), on the other hand, state that this modified content in older hosts can still be inferior in energetic terms because many nutrients, as free aminoacids, were already incorporated to embryonary tissue during host development, thus justifying the low percentage of *G. gallardoi* emergence recorded for older eggs of *S. dentiventris.*

The low nutritional quality attributed to older host eggs can also slow down offspring development rate and this may be interpreted as an adaptive strategy to maximize the period of availability of the food source (Godfray 1994). Barret & Schimidt (1991) conclude that the older the host, the higher the amount of free aminoacids incorporated to the host embryo tissues, with less nutritive material available to digestion by the parasitoid larva. In the present study, increases in host age negatively influenced *G. gallardoi* development time ($F = 51286.6$; $P < 0.001$) (Fig. 2), with males developing significantly faster than females ($F = 17205.3$; $P < 0.001$). The interaction between age and sex was not significant ($F = 3.06$; $P > 0.05$), showing that males and females were equally affected by host age.

Sex ratio was biased towards females for all host egg ages tested (heterogeneity *G*-test, $P > 0.05$). Female-biased sex ratios are common for many hymenopteran parasitoids species, including Scelionidae (Navasero & Oatman 1989, Noda & Hirose 1989, Hernández & Diaz 1996, Souza & Spence 2001), with these proportions explained in terms of local mate competition (LMC) (Hamilton 1967, Waage 1982). According to these authors, in parasitoids attacking gregarious hosts, as eggs in groups, mating generally occurs among siblings, with mother females adjusting offspring sex ratio so as to produce only a number of males minimally sufficient to guarantee mating for all offspring females. Thus, the proportion of males in the offspring is connected to the characteristic size of the host egg groups (Waage 1982, Charnov & Skinner 1985).

Here, the proportion of males did not vary with increases in egg age ($F= 0.3042$; $P > 0.05$), staying on average at 0.49:1. Vinson (1997) states that there has been little interest on host age effects on parasitoid sex ratio, and so far little or no effect of host age on Scelionidae parasitoids (Navasero & Oatman 1989, Noda & Hirose 1989). However, a few authors as Hernández & Diaz (1996) verified a higher proportion of males of *T. remus* in older eggs of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). Similarly, Souza & Spence (2001) demonstrated a higher male proportion in *T. gerriphagus* for eggs of *Limnoporus dissortis* (Drake & Harris) (Hemiptera: Gerridae) with more than four days of age. In both cases, as eggs of different ages were simultaneously offered, the increase in the proportion of males was attributed to a low proportion of older eggs parasitized.

Regarding order of oviposition, the distribution of

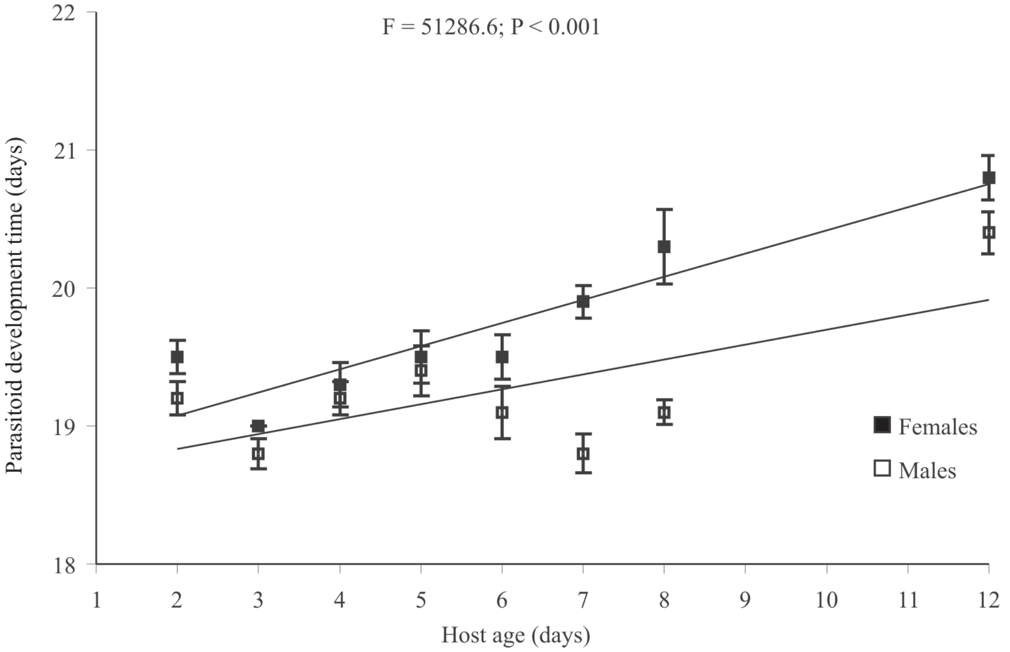

Fig. 2. Comparison between development time (days) (mean ± s.e.) of males and females of *G. gallardoi* with egg age of *S. dentiventris*.

offspring sex within egg groups was heterogeneous on all ages tested ($G_h = 34.941$; $P < 0.001$), with males tending to appear from the first oviposition on an egg group, with higher frequency on the second oviposition ($G_h = 13.437$; $P < 0{,}001$), whilst females were obtained more commonly on the 11th and 12th ovipositions ($G_h = 4.983$ and 6.160; $P < 0.05$, respectively) (Fig. 3). There was no discernible variation in the distribution of offspring sex with host age ($F = 0.383$; $P > 0.05$). Similar results were found by Noda & Hirose (1989) for *Gryon japonicum* (Ashmead)

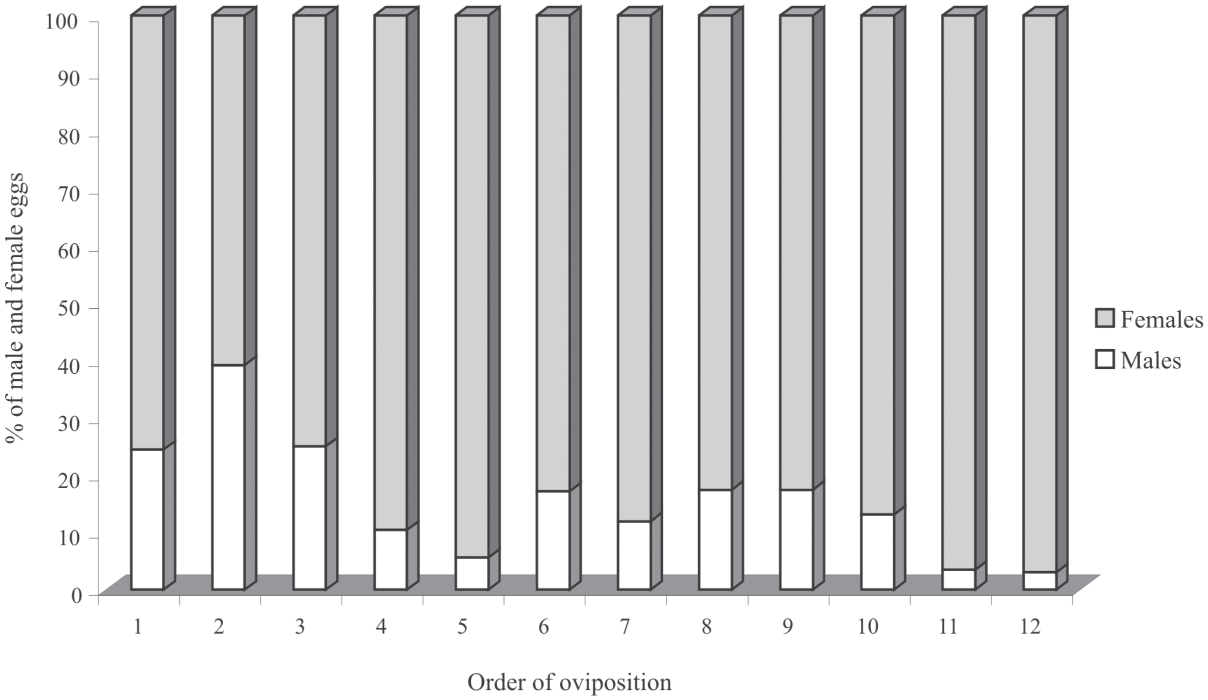

Fig. 3. Percentage of male eggs oviposited by *G. gallardoi* females in relation to the order of oviposition on egg groups of *S. dentiventris* for all egg ages.

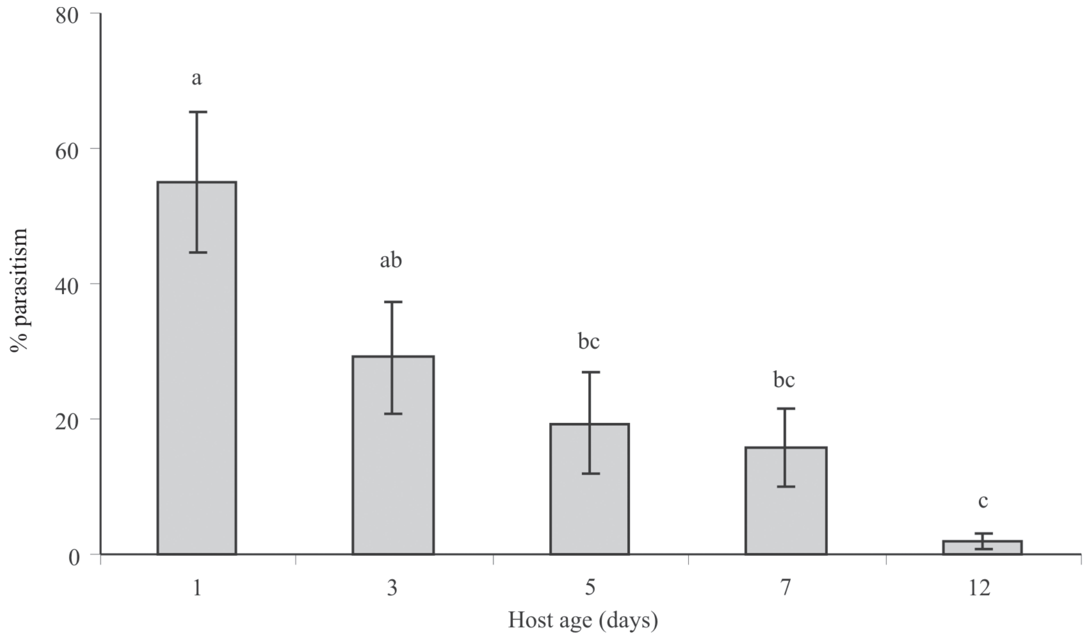

Fig. 4. Parasitism percentages (mean ± s.e.) for eggs of *S. dentiventris* at different ages offered simultaneously to *G. gallardoi* females. Means followed by the same letters do not differ significantly by the Kruskal-Wallis.

(Hymenoptera: Scelionidae), with no difference in order of oviposition among different egg ages of *Riptortus clavatus* (Thunberg) (Hemiptera: Coreidae), with oviposition of males occurring more frequently on the second egg oviposited. In that case, the distribution of male eggs in the host egg group is important to assure mating with all females. Generally, in Scelionidae and other solitary egg parasitoids, females oviposit male eggs on the first ovipositions (Waage 1982), however this can vary according to host egg group size and presence of conspecifics (Godfray 1994).

When egg groups of different ages were offered simultaneously, *G. gallardoi* females preferred younger ones (1-3 days old) (H = 13.841; P < 0.01) (Fig. 4). These lab results for *G. gallardoi* corroborate those found under field condition by Santos *et al.* (2001), where higher parasitism rates were found on eggs *S. dentiventris* up to four days of age. Similar results were found by Morril & Almazon (1990), who recorded higher parasitism rates for younger eggs of *Leptocorisa oratorius* (Fabricius) (Hemiptera: Alydidae) attacked by *G. nixoni*, with older eggs not parasitized at all. Romeis *et al*. (2000) also demonstrated that *Gryon clavigrallae* Mineo (Hymenoptera: Scelionidae) attack younger eggs of *Clavigralla* spp. (Hemiptera: Coreidae) more frequently.

Preference for younger eggs is known for many species of Scelionidae. According to Vinson (1998), parasitoids can locate hosts of all ages, but tend to reject older ones, because of changes in chemical and/or physical stimuli for recognising and accepting hosts which can occur with increases in host age. Despite the clear effects of host age on parasitism evidenced by this and other experiments discussed here, it is valid to question whether the parasitoid female, when exposed to eggs of various ages simultaneously, rejects older eggs because they are more difficult to recognise or because she can assess the nutritional status of the host. Vinson (1997) ponders that there is great plasticity on all levels of the behavioural process of selecting hosts and assessing their suitability. This plasticity is not only related to genetic factors but also to previous experience and learning by the female parasitoid. Thus, more information is necessary in order to reach a more comprehensive description of the host selection process and the factors involved.

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