Host preference of the egg parasitoids *Telenomus remus* and *Trichogramma pretiosum* in laboratory

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ABSTRACT. Host preference of the egg parasitoids *Telenomus remus* and *Trichogramma pretiosum* in laboratory. This research aimed to evaluate the host preference of the egg parasitoids *Telenomus remus* and *Trichogramma pretiosum*. Trials were carried out in laboratory, under controlled environmental conditions (25 ± 2ºC temperature; 70 ± 10% RH; and 14 h photophase). The parasitoid searching behavior was evaluated based on the distribution (%) of eggs parasitized by each parasitoid, on egg masses of each host species. Results showed the host preference of *T. remus* by *Spodoptera cosmioides* eggs. *T. pretiosum*, reared in *A. gemmatalis* eggs, choose to parasitize always eggs of the host where the parasitoid had been reared. The egg preference was not observed when *T. pretiosum* was reared in *S. frugiperda* eggs. These results show that, in general, host preference of *T. remus* is less influenced by the host where it is developed than *T. pretiosum*. Host preference is an important parameter for biological control programs because more than one pest species may occur in the field, different from those where they were reared in the laboratory.

KEYWORDS. *Anticarsia gemmatalis*; *Anagasta kuehniella*; biological control; *Spodoptera* spp.

RESUMO. Preferência hospedeira dos parasitoides de ovos *Telenomus remus* e *Trichogramma pretiosum* em laboratório. Este trabalho objetivou avaliar a preferência hospedeira dos parasitóides de ovos *Telenomus remus* e *Trichogramma pretiosum*. Os experimentos foram conduzidos em laboratório, sob condições controladas (temperatura de 25 ± 2ºC; umidade relativa de 70 ± 10%; e fotofase de 14 horas). O comportamento de busca dos parasitóides foi avaliado através da distribuição (%) de ovos parasitados por espécie de parasitóide, em massas de ovos de cada hospedeiro na combinação estudada. Os resultados mostraram que *T. remus* preferiu parasitar ovos de *S. cosmioides*. *T. pretiosum*, criados em ovos de *A. gemmatalis*, preferiu parasitar sempre os ovos dos hospedeiros nos quais foram multiplicados. Essa preferência não foi observada quando *T. pretiosum* foi multiplicado em ovos de *S. frugiperda*. Esses resultados mostram que, em geral, a preferência hospedeira de *T. remus* é menos influenciada em relação a *T. pretiosum* pelo hospedeiro em que o parasitóide se desenvolveu. A preferência hospedeira é um importante parâmetro a ser considerado em programas de controle biológico porque em condições de campo mais de uma espécie praga pode estar presente, diferente daquela em que foi criado em laboratório.

PALAVRAS-CHAVE. *Anticarsia gemmatalis*; *Anagasta kuehniella*; controle biológico; *Spodoptera* spp.

Chemical control is the most commonly used technique against insect-pests; however, as a result of the intensive use of insecticides, a series of negative impacts on the environment may occur. The incorrect use of chemicals can, besides turning insect management inefficient, cause outbreaks of secondary pests, selection of resistant insects, and ecological imbalances, for example (Nakano 1986). Thus, the adoption of efficient and low environmental impacting tactics is fundamental for the success of pest control based on the Integrated Pest Management (IPM). One of the most promising tactics is the biological control by releasing egg parasitoids (Parra *et al.* 1987).

Among the egg parasitoids, several species in the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) have been widely used, because they are easily reared on alternative host (Parra 1997; Haji *et al.* 1998). They are efficient parasitoids, mainly on eggs of Lepidoptera (Botelho1997). Releases of *Trichogramma* species for biological control of insect pests have been performed over a hundred years, reaching, nowadays, an area of approximately 16 million hectares, although, some authors refer to 32 million hectares of *Trichogramma* spp. releases (Parra & Zucchi 2004).

Inundatory releases have been used mainly for controlling insect pests in crops such as corn, sugarcane, tomatoes, rice, cotton, sugar beet, apple, prune, vegetables, and forests (Hassan *et al.* 1998; Parra *et al.* 2002). Moreover, studies have demonstrated that *Trichogramma pretiosum* Riley, 1879 (Hymenoptera, Trichogrammatidae) can be used to control *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera, Noctuidae) and *Pseudoplusia includens* Walker, 1857 (Lepidoptera, Noctuidae) on soybean (Bueno *et al.* 2009). However, despite the parasitism potential of *T. pretiosum* and other species of this genus, they are inefficient in parasitizing eggs laid in overlapping layers, as the egg masses of *Spodoptera* *frugiperda* J. E. Smith, 1797 (Lepidoptera, Noctuidae) and *Spodoptera cosmioides* Walker, 1858 (Lepidoptera, Noctuidae). These commonly infesting corn pest species have been recorded injuring soybeans, especially *S. cosmioides* (Bueno *et al.* 2007).

Another egg parasitoid, *Telenomus remus* Nixon, 1937 (Hymenoptera, Scelionidae), has been observed parasitizing eggs of five different species of *Spodoptera*, even on overlapping layer egg masses (Bueno *et al.* 2008). Each female of *T. remus* produces about 270 eggs during its reproductive lifespan (Morales *et al.* 2000). This parasitoid has been released in corn field areas, as part of IPM programs, in Venezuela, where the parasitism rates reached up to 90% (Ferrer 2001), demonstrating the high potential of this control agent of several species of *Spodoptera*.

Despite the great potential in using egg parasitoids as biological control agents, the success or failure of field releases of these insects depends, basically, on the knowledge of the bioecological characteristics of the parasitoids and of their interactions with the target host (Bourchier & Smith 1996). It is important to consider that when the parasitoid is released in the field, it will probably have a larger host choice than in the laboratory. This host diversity may influence the capacity of the parasitoid in finding the target host. Another important point to be considered is the quality of the insect reared under laboratory conditions. In general, an easy-reared alternative host is normally used for mass production. However, the successive rearing on alternative hosts may affect the host preference of the natural enemy, by altering the control efficiency against the target (Cobert 1985). *T. pretiosum* is commonly reared on eggs of the alternative host *Anagasta kuehniella* Zeller, 1879 (Lepidoptera, Pyralidae), which is not a target pest in the field. Thus, if the parasitoid searching behavior changes when moved from the alternative host to the target species, the control efficiency may decrease (Cobert 1985). Therefore, the objective of this research was to evaluate the host preference of two egg parasitoids, *T. remus* and *T. pretiosum*, after being reared on eggs of different hosts.

MATERIAL AND METHODS

The tests were performed in the laboratory, under controlled environmental conditions (25 ± 2ºC temperature; 70 ± 10% RH; and 14 h photophase), in a completely randomized experimental design, with two treatments and 12 replications for each bioassay, aiming to evaluate *T. pretiosum* and *T. remus* host preference for eggs of different hosts. The adults of *T. remus* were reared on eggs of *S. frugiperda* for several generations. The adults of *T. pretiosum* were divided in two groups: in the first, the parasitoids were multiplied – after several generations in eggs of the factitious host *A. kuehniella* – for one generation, in eggs of *S. frugiperda*; in the second group, the multiplication was in eggs of *A. gemmatalis*. For all bioassays, the host eggs were kept under the same environmental conditions.

T. remus multiplication was carried out in a small white cardboard containing 30 *S. frugiperda* egg masses with about 100 eggs each. The 24 h old eggs were glued on the cardboards and introduced into transparent circular plastic containers (20 cm x 10 cm) with *T. remus* adult females. A droplet of honey was used for adult parasitoid feeding. After 24 h parasitism, the cardboard cards were individualized into plastic containers, as previously described, but without the parasitoid. These containers were sealed with a plastic wrap. After about 10 days, the parasitoids emerged and the females were collected to be used in the experiments. The multiplication of *T. pretiosum* followed the same procedure used for *T. remus* rearing, but the hosts were *A. gemmatalis* eggs or *S. frugiperda* egg masses.

The tests on host preference were carried out in arenas with dual-choice chance, as proposed by Thuler *et al.* (2007). Each arena was composed by four transparent polyethylene vials, measuring 4 cm tall x 2 cm in diameter, with four plastic micro-tubes (1.5 ml). These micro-tubes containing a card with approximately 20 eggs of each host and were equidistantly disposed, forming an "X" in the arena. A fifth tube was vertically placed in the center of the arena, where a single female was placed for each replicate. The eggs were exposed to parasitism for 24 h period, and then the females were removed. The eggs were observed daily until the emergence of parasitoids. The number of parasitized eggs was recorded daily.

In the assays performed with *T. remus*, the host comparisons were: 1) *S. frugiperda* x *S. cosmioides*; 2) *S. frugiperda* x *Spodoptera albula* (Walker) (Lepidoptera: Noctuidae), and 3) *S. cosmioides* x *S. albula*. In the bioassays carried out with *T. pretiosum*, the host comparisons for the parasitoids reared on eggs of *S. frugiperda* were: 1) *S. cosmioides* x *A. kuehniella;* 2) *S. frugiperda* x *A. gemmatalis*; 3) *S. frugiperda* x *A. kuehniella*; and 4) *S. frugiperda* x *S. eridania*. Finally, for the parasitoids reared on eggs of *A. gemmatalis*, the comparisons were: 1) *S. frugiperda* x *A. gemmatalis* and 2) *A. gemmatalis* x *A. kuehniella*.

The number of eggs parasitized was evaluated and the parasitism distribution (%) was calculated by dividing the number of parasitized eggs of each species by the total of parasitized eggs of the replication. Results were submitted to exploratory analyses for checking the assumptions of residual normality, the homogeneity of variance of treatments to perform analysis of variance (ANOVA). The means were compared by F test ($P \leq 0,05$) for statistical significance.

RESULTS AND DISCUSSION

Females of *T. remus* evaluated in the choice test for eggs of *Spodoptera* preferred the eggs of *S. cosmioides*. In the comparison between *S. frugiperda* and *S. cosmioides*, 74% of the parasitized eggs were from *S. cosmioides* (Fig. 1A). The preference for parasitizing *S. cosmioides* eggs was also observed when compared with *S. albula* (Fig. 1B). Differently, *T. remus* did not show significant statistical differences in the preference of eggs of *S. frugiperda* and *S. albula* (Fig. 1C).

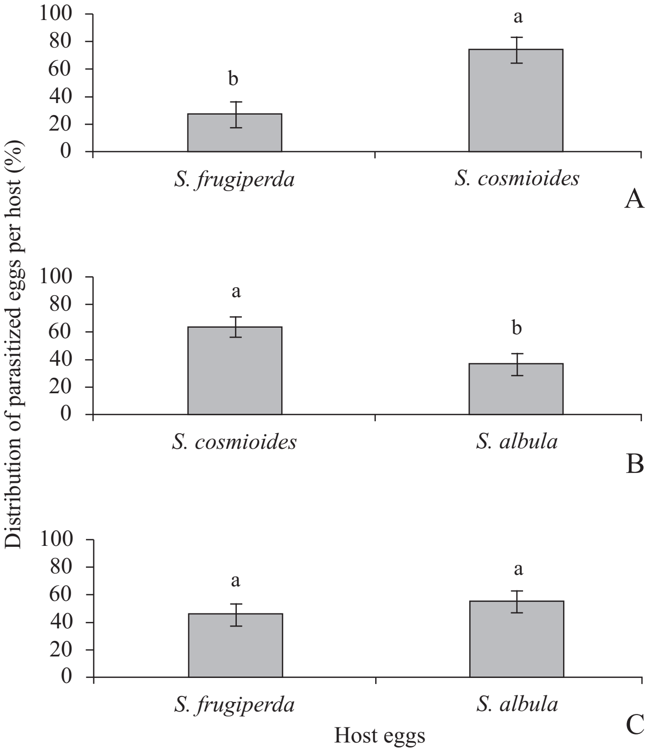

Fig. 1. Distribution (%) of eggs parasitized by *Telenomus remus* (Mean ± SE) on egg masses of different hosts of the genus *Spodoptera* in double choice tests, after that parasitoid was reared in eggs of *Spodoptera frugiperda* for several generations. Means followed by the same letter are not statistically different from each other by F test, at 5% probability.

T. remus females were reared in *S frugiperda* eggs for several generations. Thus, the non-preference of the parasitoid for the eggs of *S. frugiperda* demonstrates that the host acceptance or preference behavior of the parasitoid females cannot be attributed to the pre-imaginal conditioning, previously described by Cobert (1985) and Kaiser *et al.* (1989). This parasitoid preference can not be also attributed to associative learning, or α-conditioning. In this case, females associate new stimuli (acquired) to the innate ones, and are able to adapt themselves to the environment they have lived, as adults or juveniles (Kaiser *et al.* 1989; Vinson 1998; Nurindah *et al.* 1999). These results indicate that *T. remus* can be maintained in laboratory cultures for several generations in eggs of a single host species without reducing the parasitism efficiency for other target pests in the field.

It is important to consider that *S. cosmioides* is becoming a key pest in soybean crops in recent years (Bueno *et al.* 2007). As indicated by the results, *S. cosmioides* is a preferential host for *T. remus*, demonstrating its potential as biocontrol agent against this specie in Soybean-IPM. Further studies, however, are necessary to establish a biological control program using this parasitoid in soybean fields. Moreover, *S. cosmioides* does not display the cannibal habit observed for *S. frugiperda*. Thus, *S. cosmioides* larvae can be reared in laboratory without individualization, a condition that is essential for *S. frugiperda* rearing. Therefore, the mass rearing of *S. cosmioides* may be less laborious and less expensive as compared to *S. frugiperda*, and thus more economically feasible. This is a favorable characteristic for using *S. cosmioides* for massal rearing of *T. remus* in the laboratory because costs can be reduced.

Additionally, *T. remus* is described in the literature as an excellent egg parasitoid for the *Spodoptera* complex and perfectly feasible in integrated management programs for *S. frugiperda* (Figueiredo *et al.* 1999). It is important to consider that not only *S. cosmioides*, but also other species of the *Spodoptera* complex have increased their importance on crops such as soybean, corn and cotton. Another noticeable issue is that *T. remus* can effectively parasitize the inner layers of the egg masses (Cruz & Figueiredo 1994), whereas the parasitoids from the *Trichogramma* genus are able to parasitize only the outer layers (Beserra *et al.* 2002). Therefore, *T. remus* can be considered as an efficient biocontrol agent of *S. frugiperda* eggs (Johnson 1984), as well as of the other species of *Spodoptera* that lay eggs in layered clusters, such as *S. cosmioides*.

When one generation of *T. pretiosum* is reared in eggs of *S. frugiperda*, females preferred *S. frugiperda* eggs (100% of the parasitized eggs) instead *S. eridania* (Fig. 2A). However, in the dual-choice parasitism test, there were no statistically significant differences between treatments using eggs of *S. frugiperda* and *A. gemmatalis* as well as eggs of *S. frugiperda* and *A. kuehniella* (Figs. 2B, 2C). Probably, this means that even if the parasitoid was reared for one generation in eggs of *S. frugiperda*, this was not the preferential host for parasitism. *T. pretiosum* also did not show any preference between eggs of *S. cosmioides* and *A. kuehniella* (Fig. 2D).

Females of *T. pretiosum*, reared for one generation in eggs of *A. gemmatalis*, preferred the eggs of *A. gemmatalis* instead of *S. frugiperda* and *A. kuehniella* eggs for parasitism (Figs. 3A, 3B). Differently, results obtained with parasitoids reared in eggs of *S. frugiperda* suggest that the host in which the insect was reared is actually important. Nevertheless, host selection cannot be explained simply in relation to the host in which the parasitoid was multiplied. The host preference of females of the genus *Trichogramma* presents a complex relation with the nutritional quality of the hosts chosen for parasitism and is also related to the host in which the parasitoid was multiplied, as previously observed by Molina *et al.* (2005) and Volpe *et al.* (2006).

Characteristics of the host egg include differences in the surfaces, size and structure of the chorion, and changes in color during embryonic development, as well as in size and egg volume. All these peculiarities, which are specific to each host egg, can influence not only the time necessary for *Trichogramma* spp. exploratory behavior, but also the development of the parasitoid in the specific host (Cônsoli *et al.* 1999). Therefore, differences of the host in which the parasitoid developed also influence the preference of the female parasitoid in choosing eggs of the same host used for its development.

The parasitism potential of *T. remus* and *T. pretiosum* could be confirmed in our study and their use in biological control

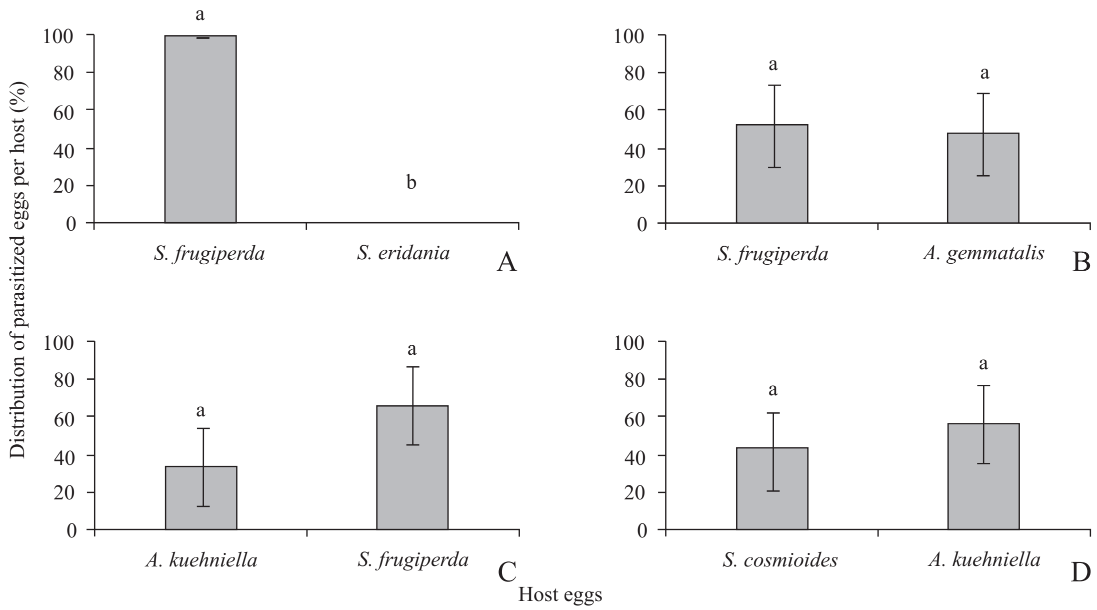

Fig. 2. Distribution (%) of eggs parasitize by *Trichogramma pretiosum* (Mean ± SE) on egg masses of different hosts, in double choice tests, after that parasitoid was reared for one generation in eggs of *Spodoptera frugiperda*. Means followed by the same letter are not statistically different from each other by F test, at 5% probability.

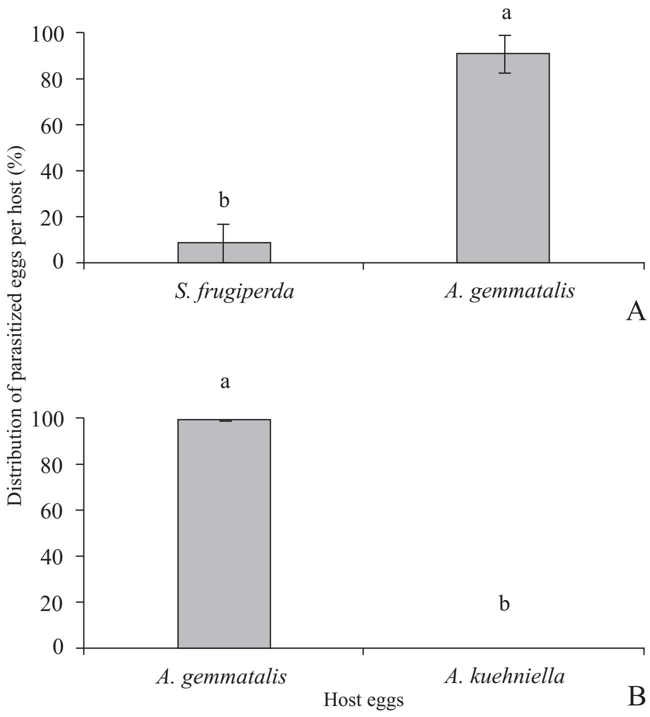

Fig. 3. Distribution (%) of eggs parasitized by *Trichogramma pretiosum* (Mean ± SE) on egg masses of different hosts, in double choice tests, after that parasitoide was reared for one generation in eggs of *Anticarsia gemmatalis*. Means followed by the same letter are not statistically different from each other by F test, at 5% probability.

programs can be recommended. Further field studies related to biology and parasitism capacity of *T. remus* and *T. pretiosum* in eggs of different species of the genus *Spodoptera* and other pests are needed.

CONCLUSIONS

The parasitoid *Telenomus remus* prefers to parasitize eggs of *Spodoptera cosmioides* instead of eggs of *S. frugiperda* in laboratory conditions. *Trichogramma pretiosum*, reared in *A. gemmatalis* eggs, prefers to parasitize eggs of the host where the parasitoid had been reared. Egg preference does not occur when *T. pretiosum* is reared in *S. frugiperda* eggs. In general, host preference of *T. remus* is less influenced by the host where it has developed than *T. pretiosum*. For both parasitoids, host preference is an important parameter to be studied since it might influence parasitism mainly in the field where several pest species may occur.

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