SCIENTIFIC NOTE

Egg Laying and Development of Different Strains of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) in Artificial Eggs

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Oviposição e Desenvolvimento de Diferentes Linhagens de *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) em Ovos Artificiais

RESUMO – O objetivo deste trabalho foi avaliar a capacidade de diferentes linhagens de *Trichogramma pretiosum* Riley em parasitar e se desenvolver em ovos artificiais. Ovos artificiais foram produzidos utilizando-se um sistema térmico para a deformação de películas de polietileno de alta densidade, obtendo-se 64 depressões semi-esféricas (ovos artificiais) por película plástica. Os ovos foram preenchidos com dieta artificial composta por holotecidos pupais de *Diatraea saccharalis* (Fabr.), gema de ovo, soro fetal bovino, hidrolisado de lactoalbumina e antibióticos. Cada ovo artificial foi oferecido a seis fêmeas recém-emergidas de *T. pretiosum*, durante 24 h. A aceitação dos ovos artificiais (medida pelo número de ovos do parasitóide/ovo artificial) foi bastante distinta entre as diferentes linhagens observadas. A sobrevivência dos estágios imaturos em meio artificial também foi variável entre as linhagens. As implicações dos resultados para o desenvolvimento de técnicas de criação *in vitro* para diferentes espécies e/ou linhagens são discutidas.

PALAVRAS-CHAVE: Insecta, controle biológico, técnicas de criação, aceitação do hospedeiro, exigências nutricionais, criação *in vitro*.

The *in vitro* rearing of parasitoids has been developed for many parasitoid species, but successful results have been obtained mainly for the egg parasitoids in the genus *Trichogramma*. Research on *in vitro* rearing of these natural enemies has been conducted since the 70's, and many countries have been trying to mass-produce them by using this new technology. However, the success of in mass producing and releasing *in vitro*-reared *Trichogramma* has been achieved only by the Chinese (Cônsoli & Parra 1997a; Grenier 1997).

The development of an artificial diet is always done by using one population of a species, and this diet is expect to be suitable for rearing other species or strains. However, species from a single genus may have differences in their nutritional requirements, even for that polyphagous species exploring the same kind

of resource, such as the egg parasitoids *Trichogramma* (Cônsoli & Parra 1996, 1997 b). Yet, strains that were used to develop on artificial diets may become unable to be *in vitro* reared, whilst other strains can still do (Pintureau & Grenier 1992).

The selection of strains of *Trichogramma* is one of the major steps to successfully achieve the biocontrol of a specific host species (Hassan 1994). However, strains may have differences in their fitness to control insect pests associated with different agroecosystems. In such cases, it would be necessary to produce many species or strains of *Trichogramma*, in order to use the most adequate species/strain in each pest: agroecosystem association. In this work we aimed to evaluate the ability of different strains of *Trichogramma pretiosum* Riley in parasitize and develop on artificial host eggs by using a standard diet.

Strains of *T. pretiosum* were reared on UV-treated eggs of the factitious host, *Anagasta kuehniella* (Zeller), under controlled conditions (temperature: $25 \pm 1^{\circ}C$; $60 \pm 10\%$ RH; photophase: 14 h). The factitious host was bred on alternating diets composed of yeast (3%) + wheat flour (97%) or corn (40%) + wheat flour (60%) (Parra 1997). *T. pretiosum* strains employed in our study where continuously reared and maintained on our strain collection, being identified as strain L1 (host: *Helicoverpa zea* (Boddie); crop: tomato; origin: Alegre - ES, Brazil; sex ratio: 1), strain L2 (host: *H. zea*; crop: sweet corn; origin: Jaguariúna - SP, Brazil; sex ratio: 1), strain L4 (host: *H. zea*; crop: tomato; origin: Jerônimo Monteiro - ES, Brazil; sex ratio: 0.95) and strain L8 (host: *H. zea*; crop: sweet corn; local: Piracicaba - SP, Brazil; sex ratio: 0.66). Both strains L1 and L2 were thelytokous.

To produce the artificial eggs, a high-density polyethylene membrane (7-8 mm thick) was laid over the central surface of a hot iron plate that had spherical holes. Below this central area, a chamber was connected to a vacuum pump. After the iron plate was heated, the vacuum pump was turned on and the nega-

tive pressure formed inside the chamber located under the central area pulled the plastic membrane into the spherical holes. After few seconds the vacuum pump was turned off and 64 semi-spheres were formed in the plastic membrane.

These membranes were filled with an artificial diet previously reported for the *in vitro* rearing of *T. pretiosum* and *Trichogramma galloi* Zucchi, made with pupal holotissues of *Diatraea saccharalis* (Fabr.) (65%), egg yolk (18%), bovine fetal serum (8.5%), lactalbumin hydrolysate (8.5%) and preservatives (amphotericin B + streptomycin sulphate - 3%). Pupal holotissues were collected from two to five days-old *D. saccharalis* pupae produced on an artificial diet based on wheat germ and yeast (Macedo *et al.* 1983). Pupae were heat-treated (60-$62^{\circ}C$ - 10min) to inactivate the phenoloxidases. Afterward, they were surface sterilized with 0.2% hypochloride solution (2 min), transferred for a laminar flow chamber and squeezed into a disposable syringe. The holotissues were collected in a sterile vial, centrifuged and the solid phase was discarded. All steps for diet manipulation were conducted in a laminar flow chamber (Cônsoli & Parra 1997a).

The artificial eggs, filled with the artificial diet, were offered to newly-emerged *T. pretiosum* females in a proportion of six females:artificial egg, during 24 h. We used five replicates and each artificial egg membrane (with 64 artificial eggs) was considered as a replication. The ability of the strains of *T. pretiosum* to parasitize and develop in an artificial host egg was assessed by evaluating the acceptance of the artificial host egg and the parasitoid immature survival. Egg acceptance was measured by counting the number of *T. pretiosum* eggs laid/artificial egg. After the 24 h oviposition period, females were removed and 16 artificial eggs/artificial egg card were sampled and the number of *T. pretiosum* eggs laid was assessed using a compound microscope. Immature survival was measured by counting the number of mature larvae that reached the pupal stage and the number of adults eclosed/artificial egg. All the experi-

mental procedure was conducted under controlled conditions (temperature: $25 \pm 1°C$; $60 \pm 10\%$ RH; photophase: 14 h).

Egg laying in artificial host eggs by *T. pretiosum* strains was very distinct, with strain L4 laying the highest number of eggs/artificial egg and strain L1 the lowest number (Figure 1). The lower rate of parasitism by strain L1 could be explained by the low egg accept-

survivorship among strains when parasitizing the same host is related to the genetic variability among populations, as previously described for *T. brassicae* and *T. voegelei* (Chassain & Boulétreau 1991; Mimouni 1991). Also, the success of the preimaginal development seems to be host dependent and it is variable among strains of a single species (Gomes & Parra 1998).
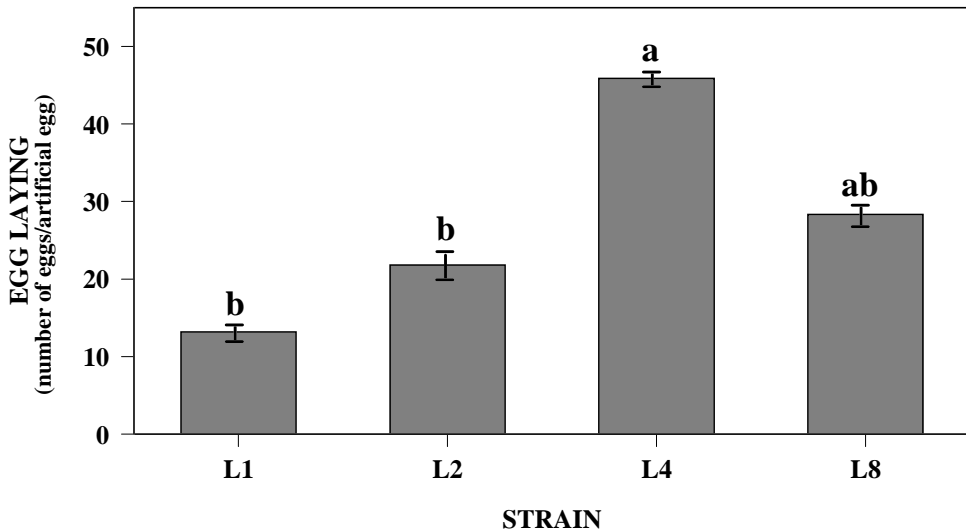

Figure 1. Egg laying (number of eggs/artificial egg) of different strains of *T. pretiosum* in artificial host eggs (temperature: $25 \pm 1°C$; $60 \pm 10\%$ RH; photophase: 14 h). Means ($\pm$SEM) followed by the same letter are significantly different ($F = 13.59$; df = 3, 19; $P < 0.05$, Tukey test).

ance (artificial egg with at least one parasitoid egg) shown by this strain (90%) when compared to the other strains (100%).

These strains also differed on the survivorship of their immature stages when developing on an artificial diet. Strain L2 showed the higher survivorship, despite the low parasitization of artificial eggs. Strain L1 had the lowest larval survival, but the higher pupal survival (Figura 2).

The success in exploiting a host during the immature stages of *Trichogramma* is genetically determined and the differences in

Whether this genetic variability among parasitoid populations of a single species implies in distinct nutritional requirements should be better investigated. However, this factor must be considered for the development of an artificial diet for the *in vitro* production of parasitoids, when this system is to be used in the control of many pests on different agroecosystems. In such case, would be necessary to develop one artificial diet for each parasitoid species or strains, since the selection of effective strains to control the target pest in a specific crop system is one of the
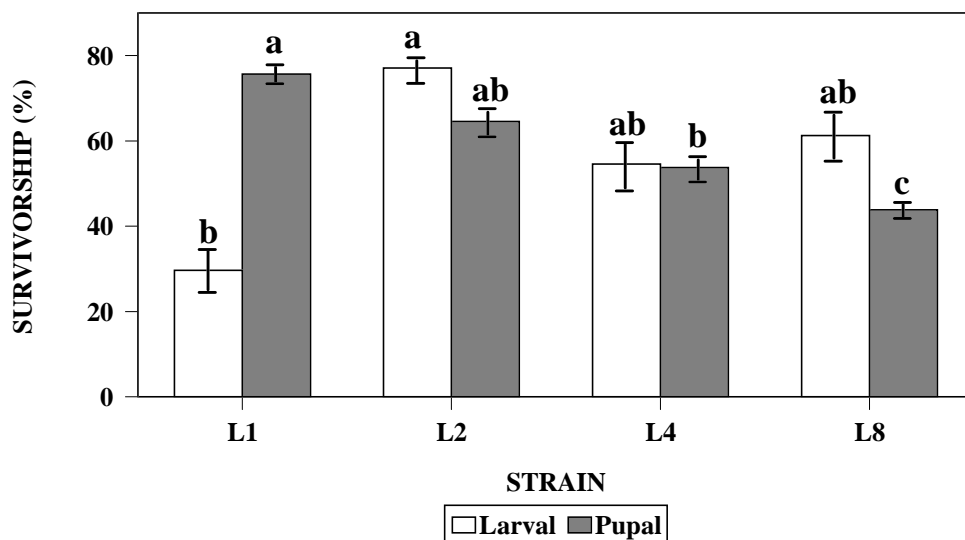
Figure 2. Survivorship (%) of the immature stages of different strains of *T. pretiosum* when developing on artificial eggs (temperature: $25 \pm 1^{\circ}$C; $60 \pm 10\%$ RH; photophase: 14 h). Means ($\pm$SEM) within an immature stage followed by different letters are significantly different (larval stage: $F = 3.59$; df = 3, 19; $P < 0.05$; pupal stage: $F = 8.97$; df = 3, 19; $P < 0.05$, Student Newman-Keuls Method).

most important factor involved in the success of using *Trichogramma* (Hassan 1994, 1997).

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