BIOLOGICAL CONTROL

Effect of the Age of the Pupal Holotissue on the Nutritional Quality of Artificial Diets for *Trichogramma* spp. (Hymenoptera: Trichogrammatidae)

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Efeito da Idade do Holotecido Pupal na Qualidade Nutricional de Dietas Artificiais para *Trichogramma* spp. (Hymenoptera: Trichogrammatidae)

RESUMO – Testou-se a qualidade nutricional de dietas artificiais contendo holotecidos pupais de *Diatraea saccharalis* (Fabr.) (Lep.: Crambidae), coletados de pupas em três estágios de desenvolvimento (1-2, 3-4 e 5-6 dias de desenvolvimento), para a criação *in vitro* de *Trichogramma galloi* Zucchi e *T. pretiosum* Riley (Hym.: Trichogrammatidae). Os holotecidos pupais foram adicionados aos demais componentes da dieta, gema de ovo, soro fetal bovino, hidrolisado de lactoalbumina e anticontaminantes, e oferecidos aos parasitóides em ovos artificiais. A qualidade dessas dietas para o desenvolvimento de ambos parasitóides foi avaliada através da aceitação e parasitismo dos ovos artificiais, sobrevivência larval e pupal, tamanho, capacidade de parasitismo e longevidade dos adultos emergidos dessas dietas, assim como a presença de deformações nesses adultos. A dieta composta de holotecidos de pupas com 5-6 dias de desenvolvimento não permitiu o crescimento larval de ambas as espécies, sendo que essa dieta também reduziu a aceitação e/ou parasitismo de ovos artificiais por *T. galloi* e *T. pretiosum*.

PALAVRAS-CHAVE: Insecta, hospedeiro artificial, técnicas de criação, criação *in vitro*.

ABSTRACT - The nutritional quality of artificial diets composed of pupal holotissues of *Diatraea saccharalis* (Fabr.) (Lep.: Crambidae) from three different age classes (1-2, 3-4 and 5-6 days old) were tested for rearing *Trichogramma galloi* Zucchi and *T. pretiosum* Riley (Hym.: Trichogrammatidae) *in vitro*. Pupal holotissues were added to egg yolk, bovine fetal serum, lactoalbumine hydrolysate and preservatives, and offered to the parasitoids into artificial eggs. The quality of these diets in supporting the development of both parasitoids was evaluated by assessing the acceptance and parasitism of the artificial host eggs, larval and pupal survival, size, parasitism capacity and longevity of the *in vitro*-reared females, and the presence of deformed adults. Diets

composed of 5-6 d old pupal holotissues did not support the larval development of both parasitoids as well as reduced the acceptance and/or parasitism of artificial eggs by *T. galloi* and *T. pretiosum*. The factors affecting the nutritional quality of pupal holotissues collected from different developmental stages are discussed.

KEY WORDS: Insecta, diet suitability, artificial host, rearing techniques, *in vitro* rearing.

The worldwide success on the use of species of *Trichogramma* to control pests on vegetable crops and forest systems evoked the development of new techniques for the mass production of these parasitoids. The *in vivo* rearing techniques (eggs of factitious hosts) of *Trichogramma* are still under investigation (Bigler 1994, Hassan 1997, Parra 1997), and new approaches for the production of parasitoids on artificial diets (*in vitro* rearing) are also being tested (Grenier *et al.* 1994).

In vitro rearing techniques of parasitoids were initially used to study the nutritional requirements and physiology, as well as their physiological interactions with their hosts (Cônsoli & Parra 1997a). However, few years later the first report on the development of an egg parasitoid in artificial conditions (Hoffman *et al.* 1975), the suitability of this technique to mass produce parasitoids was demonstrated by Chinese researchers (Guan *et al.* 1978). The successful development of *Trichogramma* spp. and *Anastatus japonicus* Ash. (Hym., Eupelmidae) on artificial hosts (Li 1992) and the possible use of less expensive mass rearing systems of parasitoids started to be investigated all over the world (see Cônsoli & Parra 1997a for review).

The first attempt to rear a parasitoid *in vitro* in Brazil was reported by Parra & Cônsoli (1992) who obtained full development of *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae). Improved results were lately attained by using more adequate artificial diets for *T. pretiosum* and *Trichogramma galloi* Zucchi (Cônsoli & Parra 1996a, 1997b). Regardless these results, development of diets that would fit the nutritional requirements of these parasitoids should be accomplished to allow the production of insects with biological performance similar to the *in vivo*-reared ones (Cônsoli & Parra 1996b).

The artificial diets developed to rear *Trichogramma in vitro* in Brazil were basically composed of hemolymph or holotissues of other insects as the major component. Despite the broad use of insect components (hemolymph or holotissues) in artificial diets for parasitic species, there are few data concerning the effects of the host age on the nutritional quality of the artificial diets.

Therefore, the nutritional quality of artificial diets composed of holotissues of *Diatraea saccharalis* (Fabr.) (Lep., Crambidae) collected from pupae of different ages on the *in vitro* development of *T. galloi* and *T. pretiosum* was evaluated.

Material and Methods

Insect Rearing. Colonies of *T. galloi* (strain "Debrasa – lab"; host: *D. saccharalis*; crop: sugarcane; local: Brasilândia/MS) and *T. pretiosum* [strain 2; host: *Helicoverpa zea* (Boddie); crop: corn; local: Jaguariúna/SP] were reared apart on eggs of the factitious host, *Anagasta kuehniella* (Zeller) (Lep., Pyralidae), at controlled conditions (temperature: 25±1°C; 60±10% RH; photophase: 14 h) (Stein & Parra 1987). The factitious host was produced on artificial diets composed of

wheat flour (97%) + brewer yeast (3%) or wheat flour (60%) + corn flour (40%) (Parra 1997). Pupae of *D. saccharalis* were obtained from insects reared on a diet based on white corn (*sugary*) and wheat germ (Parra & Mihsfeldt 1992), following the rearing techniques described by Parra (1998), at the same controlled conditions already mentioned.

Insect Extract Collection. Insect holotissues were collected from *D. saccharalis* pupae of three different age classes (1-2, 3-4 and 5-6 days-old). The melanization of the hemolymph was avoided by inactivating the pheno- loxidases by heat treating the pupae between 60°C and 62°C during 15 min. They were then surface sterilized in a 2% sodium hypochlo- ride solution (10 min), washed twice in sterile distilled water, and transferred to a laminar flow hood (Cônsoli & Parra 1997a). Pupal holotissues were collected into sterile vials by squeezing the pupae inside a 30 ml sterile disposable syringe. These vials were then centrifuged at 2,000 rpm during 1-2 min, and only the intermediate phase was used with both the supernatant and the precipitate being discarded.

After centrifugation the holotissues collected were filtered through a series of filters, and finally sterilized through a 0.22 mm Millipore. They were added to the other components of the artificial diet, comprised of 65% pupal holotissues, 18% egg yolk, 8.5% bovine fetal serum, 8.5% lactoalbumine hydrolysate and 0.3% (w/v) preservatives (0.15% streptomycin sulphate and 0.15% amphotericin B) (Cônsoli 1997).

The three artificial diets obtained by using holotissues collected from pupae of different ages were encapsulated in artificial eggs and offered to parasitoid females (Cônsoli & Parra 1999). Plastic membranes (9-10 µm thick, high-density polyethylene) containing 64 artificial eggs each were UV-sterilized inside a laminar flow hood and filled with 1 ml of diet/artificial egg. Artificial eggs were offered for parasitization in a proportion of six females to one artificial egg (Cônsoli & Parra 1999). All females were removed after 24 h and the artificial eggs were kept under controlled conditions (temperature: 25±1°C; 60±10% RH; photophase: 14 h) and the development of the parasitoids was recorded daily. Each treatment was replicated five times and each plastic membrane, with 64 artificial eggs, was considered as one replication.

The acceptance of the artificial host eggs (percentage of artificial eggs in which at least one egg was laid), parasitization (number of eggs laid/artificial egg), larval and pupal survivorship, parasitization capacity and longevity of the *in vitro*-reared females were evaluated. The size of females (estimated by measuring the length of the hind-tibia) and the presence of adult abnormalities were evaluated as well.

The evaluation of the acceptance and parasitization of the artificial host was assessed immediately after parasitization. The number of egg laid/artificial egg was determined by counting the eggs in 16 out of 64 artificial eggs in each replicate

The parasitization capacity (number of eggs parasitized/female) of the *in vitro*-reared females was assessed on eggs of the factitious host, *A. kuehniella*. Twenty-five newly emerged females from each treatment were isolated in glass vials (8.0 x 2.5 cm), honey fed and offered 100 to 120 UV-killed eggs of *A. kuehniella* daily (Stein & Parra 1987).

Differences between treatments were determined by ANOVA and treatments were compared by the Student-Newman-Keuls Method or Tukey's test whenever significant differences were found.

Results

Artificial diets composed of pupal holotissues collected from pupae of *D. saccharalis* at different ages did not have the same nutritional quality to promote *in vitro* development of both parasitoids. No difference was found in the acceptance of artificial hosts by *T. galloi*, although it laid more eggs in artificial hosts filled with diets in which 1-2 day-old holotissues were used (Fig. 1). The diet composed of holotissues collected from

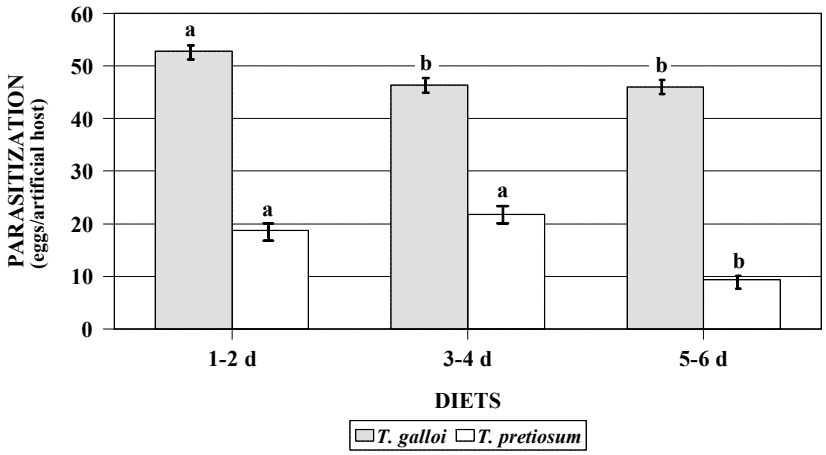

Figure 1. Parasitization (number of eggs/artificial egg) of artificial eggs by *T. galloi* and *T. pretiosum*. Eggs were filled with artificial diets based on pupal holotissues of different ages of *D. saccharalis* (temperature: 25±1°C; 60±10% RH; photophase 14 h). Bars followed by the same letter are not significantly different by using the Student-Newman-Keuls Method ($P < 0.05$) (*T. galloi*: $F = 92.09$; $df = 2, 14$; $P < 0.05$; *T. pretiosum*: $F = 11.36$; $df = 2, 14$; $P < 0.05$). Vertical lines indicate the standard errors.

5-6 day-old pupae did not allow the larval development of this species (Fig. 2). Yet, pupal survivorship was also improved when *T. galloi* was reared in the diet containing 1-2 day-old holotissues (Fig. 2).

The development of *T. pretiosum* was also affected by the age of the pupal holotissues used in the diet. However, the effects of these diets on *T. pretiosum* were different from that on *T. galloi*. No significant difference was found on the larval and pupal survivorship of this species on artificial diets composed of 1-2 or 3-4 day-old pupal holotissues (Fig. 2). Oviposition by *T. pretiosum* was affected only when artificial eggs filled with 5-6 day-old pupal holotissues-based diet were offered (Fig. 1). Moreover, acceptance of the artificial eggs filled with this diet was also reduced. In this case, females of *T. pretiosum* did not lay eggs in 8% of the artificial hosts while 100% of the eggs with all other diets were accepted.

Despite the negative effects of the age of the host holotissues on the survivorship of these parasitoids, *in vitro*-reared females showed the same parasitization capacity and longevity when offered eggs of *A. kuehniella* (Table 1). Differences in the size of adults were found only for females of *T. pretiosum* reared on the diet made of holotissues from 3-4 day-old pupae (Table 1).

It was found 10% to 15% of adult malformations in all diets tested for both parasitoid species. Oversized abdomen or absence of wing distension were the most common malformations, as also observed by several other species of *Trichogramma* (Li 1992, Dahlan & Gordh 1998).

Discussion

These results show the importance of se-

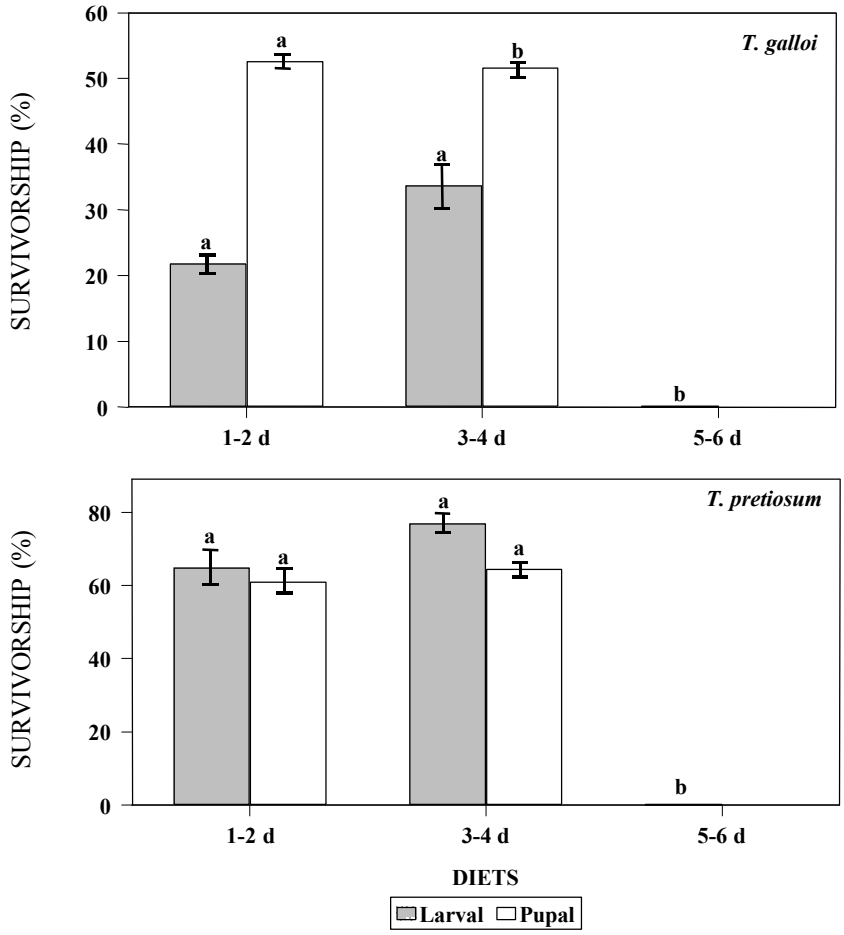

Figure 2. Larval and pupal survivorship (%) of *T. galloi* and *T. pretiosum* reared on artificial diets based on holotissues of pupae of different ages of *D. saccharalis* (temperature: 25±1ºC; 60±10% RH; photophase 14 h). Means followed by the same letter are not significantly different by using the Student-Newman-Keuls Method ($P < 0.05$) (*T. galloi*: larval survivorship: $F = 1.74$; $df = 2, 14$; $P > 0.05$; pupal survivorship: $F = 40.73$; $df = 1, 9$; $P < 0.05$; *T. pretiosum*: larval survivorship: $F = 69.04$; $df = 2, 14$; $P < 0.05$; pupal survivorship: $F = 0.294$; $df = 1, 9$; $P > 0.05$). Vertical lines indicate the standard errors.

Table 1. Parasitization capacity (number of *A. kuehniella* eggs parasitized/female), longevity and length of *T. galloi* and *T. pretiosum* females reared on artificial diets based on holotissues collected from *D. saccharalis* pupae at different ages (temperature: 25±1°C; 60±10% RH; photophase 14h).

	Diet Age of the pupae (d)	Parasitization (x±se)	Longevity (days) (x±se)	Hind-tibia length (μm) (x±se)
T. galloi[1]	1-2	40.9±3.2a	3.3±0.1a	152.1±3.3a
	3-4	53.2±13.4a	3.6±0.2a	157.0±3.3a
	5-6	-[3]	-	-
T. pretiosum[2]	1-2	101.9±12.1a	10.3±0.9a	148.8±4.9a
	3-4	131.4±16.2a	12.7±1.1a	168.2±3.7 b
	5-6	-[3]	-	-

[1]Means followed by the same letter are not significantly different by using the Tukey test ($P < 0.05$) (Parasitization: $F = 0.888$; df = 1, 46; $P > 0.05$; Longevity: $F = 0.963$; df = 1, 46; $P > 0.05$; Hind-tibia size: $F = 1.054$; df = 1, 39, $P > 0.05$).
[2]Means followed by the same letter are not significantly different by using the Tukey test ($P < 0.05$) (Parasitization: $F = 1.441$; df = 1, 46; $P > 0.05$; Longevity: $F = 1.693$; df = 1, 46; $P > 0.05$; Hind-tibia size: $F = 3.503$; df = 1, 39, $P < 0.05$).
[3]No complete development was achieved by using the artificial diet based on holotissues of 5-6 days-old pupae of *D. saccharalis*.

lecting the most adequate age of the pupal development for collection of holotissues to be used in the composition of artificial diets for rearing *T. galloi* and *T. pretiosum.*

Similar results were found with an other parasitoid, *Venturia canescens* (Gravenhorst) (Hym.: Ichneumonidae). Holotissues collected from *Galleria mellonella* L. (Lep., Pyralidae) of different ages also affected the *in vitro* development of this species (Nakahara *et al.* 1997). Diets containing holotissues from 1-2 day-old pupae showed the best growth promoting ability. Further studies revealed that a high molecular weight protein (lipophorin) was the molecule stimulating growth and pupation of this parasitoid in the artificial medium (Nakahara *et al.* 1999).

Differences in the nutritional quality of pupal holotissues from pupae of different ages are expected since the pupa undergoes very strong changes on its chemical composition during the metamorphosis. In fact, these changes begin during the last larval instar when storage proteins from the fat body are transformed into free proteins in the hemolymph of insects (major hemolymph proteins). The concentration of these proteins is higher in the hemolymph of the pupae than in the hemolymph of the larvae, making also part of the cuticle of the insects. They are also taken from the hemolymph by the ovaries and are found in a high concentration in the egg of insects, representing 30% to 35% of all of its soluble proteins (Kunkel & Nordin 1985, Plantevin *et al.* 1987, Kanost *et al.* 1990). Yet, the newly molted pupae are basically composed of a very high amount of less complex proteins and peptides since all the tissues from the larval stage are being broken down by histolysis. After a period of development they undergo the histogenesis of new tissues of the adult stage, in which more complex proteins

are synthesized. These changes during metamorphosis are represented by high variations in the contents of RNA, DNA, amino acids, peptides and proteins (Tate & Wimer 1977, Kobayashi & Kawase 1978, Srinivasan & Kesavan 1979). All these changes could explain why both parasitoids showed a better development when holotissues from young pupae where used as an insect derivative component in the artificial diet. Holotissues from young pupae can be more easily digested and absorbed by the immatures parasitoids than the holotissues from older pupae. Well-developed tissues, as found in older pupae may have a poor nutritional quality and may also require a more specific enzymatic complex if compared with the less complex nutritional broth represented by the holotissues of young pupae.

The high levels of uric acid (a waste product of the nitrogenous catabolism in terrestrial insects) at the end of the pupal stage (Lafont & Pennetier 1975, Cochran 1985) could be another factor affecting the immature development in artificial diets composed of 5-6 day-old holotissues. However, the absence of larval development in these diets is more likely related to the hormonal changes during the pupal stage (Nijhout 1998). The levels of ecdysone increase during the pupal development, reaching a peak close to adult eclosion (Nijhout 1998). The high levels of this hormone in diets based on 5-6 day-old holotissues (*D. saccharalis* will eclose at the seventh day) may have induced premature larval mortality in both parasitoids.

The feasibility of *in vitro* rearing systems in which the artificial diet is based on insect-derived components is extremely dependent on the availability of low-cost insects. *D. saccharalis* appears to be a promising species as an alternative source of holotissues while a holidic diet is not developed. *D. saccharalis* is mass produced as a host of *Cotesia flavipes* (Cameron) (Hym.: Braconidae), a larval endoparasitoid successfully used in biological control programs in sugarcane fields in Brazil (Macedo *et al.* 1983). The technology to rear koinobiont endoparasitoids *in vitro* from egg laying to adult emergence is not yet available, and the natural/factitious hosts are still required for rearing such species. In this case, *T. galloi* would be produced on artificial diets by using the same host reared for the production of *C. flavipes*. This association would be of great advantage, since *T. galloi* is also a promising biological control agent of the sugarcane borer, and can be used with success in areas where *C. flavipes* showed low efficiency (Botelho *et al.* 1999).

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